reach non-enrolled school age children and awareness in the community if targeting 2020 to control both schistosomiasis and soil-transmitted helminths in Ethiopia.

500

TO INTEGRATE OR NOT TO INTEGRATE? DEVELOPING AN EVIDENCE-BASED TOOL FOR NEGLECTED TROPICAL DISEASE CONTROL

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While evidence from clinical and modeling studies have demonstrated the potential benefit of integrating vertical disease programs, few evidence-based tools exist to assist decision makers in evaluating and comparing approaches for integration of control measures, to determine the most impactful and cost-effective approaches for their setting. We have created an application available for mobile devices or on the web, with a simple user interface, to support on-the-ground decision-making for integrating disease control programs or their components, given local conditions and practical constraints. The model upon which the tool is built provides predictive analysis for the effectiveness of integration of schistosomiasis and malaria control, two common parasitic diseases with extensive geographical and epidemiological overlap, and which result in significant morbidity and mortality in affected regions. Working with data from countries across sub-Saharan Africa and the Middle East, we present here a proof of principle for the use of our tool in providing guidance on how to optimize integration of vertical disease control programs.

501

SURGICAL MANAGEMENT OF MORBIDITY DUE TO LYMPHATIC FILARIASIS: HYDROCELE SURGERY IN HEALTH DISTRICT HOSPITALS IN MALI

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Mali is committed to national lymphatic filariasis (LF) elimination by 2020. The program has two main components: interrupting transmission of LF through mass drug administration and managing morbidity and preventing disability. Mali has achieved great progress toward LF elimination: to date, 31 of the 63 health districts (HDs) have reached the criteria to stop MDA. However, improvements in morbidity management and disability prevention are more difficult to achieve. Interventions for the surgical management of hydrocele and lymphedema management were introduced in 2012. These interventions are very demanding by the patients because of social impacts in their daily live. From 2014-15, the national program, with the support of HKI with funding from the End Fund, performed surgical treatment of 369 LF patients in 16 HDs. General practitioners performing surgery were trained on hydrocele case management and hydrocele cases were identified at the community level. Surgeons obtained informed consent prior to the surgical procedure and maintained patient records. A descriptive analysis to show the impact of hydrocele management was performed on 175 patients who were included in this analysis based on the completeness of the patient record. The median age of patients was 52 years and 75% (132/175) were married. The majority of patients 82.3% (144/175) did not report postoperative complications. The median duration of hospitalization was 4 days (ranging 2-55 days). Results showed that 32.6% (57/175) patients reported a considerable positive impact on their work and 44.6% (78/175) reported improved sex lives. Of those patients operated, 77% (54/70) indicated satisfaction after surgery and noted improvement in their daily lives. Dissatisfied patients had developed postoperative secondary infections that were managed with supportive therapy. This study shows that the management of hydrocele surgery can be done in a district hospital setting in Mali without major complications and achieve general patient satisfaction. Consequently, the LF morbidity management and disability prevention project will be expanded to other HDs.

502

ACHIEVING THE ENDGAME: IMPROVING INTEGRATED CASE SEARCHES FOR GUINEA WORM DISEASE AND TRACHOMA TO ACHIEVE ERADICATION AND ELIMINATION TARGETS

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Guinea worm disease and trachoma are both neglected tropical diseases (NTDs) slated for eradication and elimination as a public health problem, respectively, by the World Health Organization. In 2010, The Carter Center, the Ghana Ministry of Health and local partners, carried out integrated case searches for rumors of possible cases of Guinea worm disease and persons with trachomatous trichiasis (TT), the end stage of trachoma. These case searches were conducted to meet eradication and elimination targets. The first series of case searches were carried out in four districts, which were searched community to community, with patients referred to a centralized surgical facility. This method of searching did not adequately cover the target population and resulted in low surgical uptake. The second series of case searches, which were conducted house-to-house with patients being offered immediate investigation of suspected Guinea worm disease cases and TT surgical care either in the home or an adjacent primary care facility. This method resulted in higher surgical uptake. The house to house immediate resolution approach was also shown to be more cost effective. The cost to investigate suspected cases of both diseases in the house to house immediate response approach was about USD$13.99 per case examined, compared to a cost of $19.78 per case examined in the community with referral to surgical facilities approach. A review of the two approaches showed significant cost differences and favorable outcomes to the house to house immediate response approach. This approach should be considered an option for disease "end game" where case searches are required to meet targets. This approach should be considered in an integration fashion with other NTDs to maximize cost saving and efficient use of resources.

503

ARE WE REACHING EVERYONE AS WE MOVE FROM CONTROL TO ELIMINATION OF NTDS: FINDINGS FROM AN INTEGRATED TREATMENT COVERAGE SURVEY IN NORTHERN NIGERIA

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Countries like Nigeria have developed and are implementing programmes for integrated control of Neglected Tropical Diseases (NTDs). Sightsavers is leading consortium of partners in scale-up for elimination of NTDs in northern Nigeria. However, little data are available to verify reported coverages of previously conducted Mass Drug Administration (MDA). The purpose of this presentation is to validate the reported coverage from Community Drug Distributors (CDD) through integrated post-MDA

coverage survey in Kaduna, Katsina and Niger states. This also highlights differences by drug type, persons with disabilities (PWD) and reasons for non-compliance. A population based survey using two-stage cluster sampling methods was conducted to verify the proportion of individuals who ingested the drugs during last rounds of MDA. Washington Group (WG) questions were integrated into the questionnaire. Since not all drugs were distributed at the same time, to reduce recall bias, drug samples were shown to participants during the survey. A total of 7,688 persons were enrolled from six Local Government Arears (LGAs) in three states. Overall, the therapeutic coverages in survey population were lower than those reported by CDDs. 58.6% (95% CI 56.0-61.2) and 68.7% (95% CI 66.1-71.3) swallowed Mectizan/Albendazole in Kubau and Soba LGAs of Kaduna state respectively. Two LGAs in Katsina had coverage of 54.9% and 50.4% (95% CI 52.3-57.4 and 47.6-52.9) while the reported coverage was 18% and 102% respectively. Niger had survey coverage of 66.2% (95% CI 64.9-70.4) in one LGA while they reported 32%. Large proportions of eligible persons with severe disability were missed during MDA for all drugs. Among eligible population in Kaduna, 3.5% of PWD reported ingesting Mectizan/Albendazole, while 96.5% were non-disabled. Similar lower coverages were seen in other two states. Despite the intervention being community driven, more efforts are required to ensure adequate and equitable coverages are reached, and maintained at WHO levels for elimination to be attained. These reported levels should be verified so that appropriate measures are taken to improve coverage.

504

THE IMPACT OF MASS DRUG ADMINISTRATION ON HOOKWORM AND SCHISTOSOMIASIS IN LOFA COUNTY, LIBERIA BEFORE AND AFTER THE OUTBREAK OF EBOLA VIRUS DISEASE

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Lofa County was an epicenter of the 2014 Ebola virus disease (EVD) outbreak, and an extensive hand washing and hygiene campaign was launched in the summer of 2014 that lasted about 18 months. We are comparing the impact of annual (1x per year) vs semiannual (2x) mass drug administration (MDA) with ivermectin, albendazole and praziquantel on lymphatic filariasis, onchocerciasis, soil transmitted helminths (STH), and schistosomiasis in 32 villages. This study focuses on results for STH and schistosomiasis. Between 1,850 and 2,678 subjects aged 5 years and older were screened each year by duplicate Kato Katz stool exams. Rates and intensities of ascariasis and trichuriasis were low at baseline in 2012, but hookworm and *Schistosoma mansoni* infections were rampant with prevalence rates of 61% and 88% and geometric mean egg counts of 232 and 213 epg, respectively. Prevalence rates in the spring of 2014 following MDA were essentially unchanged, but egg counts for hookworm and *S. mansoni* were reduced from baseline by 51% and 31%, respectively. Ebola prematurely ended our survey in 2014 and prevented MDA for 1 year, but the epidemic brought extensive changes in hygiene in the study communities. MDA was reintroduced in April 2015, and the communities were re-tested in the spring of 2016. At that time the hookworm prevalence rate was only 27% (a 45% reduction from 2014), and with only 47 epg (a 59% reduction from 2014). In contrast, the reductions in *S. mansoni* prevalence (10%) and intensity (20%) during this time interval were unimpressive. These results suggest that local elimination of hookworm may be easier to achieve with MDA than local elimination of schistosomiasis. We also suspect that the dramatic reduction in hookworm after the EVD outbreak was due to improved hygienic practices that reduced reinfection rates following MDA. The EVD outbreak provided a unique opportunity to study the additive impact of hygiene on top of MDA.

505

FINDINGS FROM A SITUATIONAL ANALYSIS FOR INTEGRATED COMMUNITY CASE MANAGEMENT IN RURAL HEALTH ZONES OF HAUT-KATANGA IN THE DEMOCRATIC REPUBLIC OF THE CONGO

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Though rich with resources, the population of Katanga province in the Democratic Republic of Congo (DRC) is primarily rural, poor and harbors some of the highest child mortality rates in country. The province also has the lowest ratio of health care workers (HCWs) to inhabitants, particularly nurses (4 per 10,000 persons), and health coverage is very low: only 25% have access to health facilities (HFs) within 5 km of distance. In response, the DRC recently developed guidelines for implementation of integrated community case management (iCCM). In response, the MalariaCare partnership assessed nine health zones (HZs) in Katanga for opportunities to develop community health sites (CHS). Following national criteria - which include parameters for population density and preference for higher difficulty of access to and >5km distance from the local health center - the project found 96 eligible villages in 52 health areas (HAs) during a pre-visit screen. Following on-site assessments, MalariaCare further modified selection criteria to only include sites >10km & <30km from health center with head nurses able to supervise CHS. A final group of 53 villages in 45 HAs were ultimately identified for CHS development. Several challenges were encountered during this site selection process. For example, the average population per village site is 2,389 persons (median population is 2,003; range 912-7,400), exceeding the recommended 1,000 persons (500 per community health worker—CHW) per CHS. Additionally, the average distance between a CHS and an HF was 17.3 km [5.6-48 km], with a median of 15 km - raising challenges for adequate supervision and adequate resupply. This mapping exercise demonstrated that using reasonable CHS criteria, widespan implementation of CHW-based iCCM in this province will be challenging due to the lack of supporting health facility infrastructure. Consequently, supervision models should be developed to allow for the higher population densities and longer distances from health facilities, than were originally anticipated for implementation of iCCM in Katanga province.

506

DEVELOPING EVIDENCE BASED COMMUNICATION AND SOCIAL MOBILIZATION STRATEGIES 26MASS DRUG ADMINISTRATION

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There is a need for stronger, evidence based, social mobilization approaches and information education and communication (IEC) materials to support behavior change for mass drug administrations (MDA) in the control of neglected tropical diseases (NTDs). In order to address this problem a draft guide on how to design effective social mobilization strategies and IEC materials for MDAs was developed. Results of the study that served to guide the development of this document are presented here. The study consisted of a desk review and four rapid case studies - from national NTD programs supported by ENVISION, funded by the

U.S. Agency for International Development (USAID) and managed by RTI International. Case studies included a budget and expenditure analysis of IEC and social mobilization budget lines, information extracted from knowledge attitudes and practices (KAP) surveys, review of IEC and social mobilization materials and strategies, and in-depth interviews with key informants. The total dollar amount spent on IEC and social mobilization as a percentage of total program costs ranged from 4.2% to 11.8%. The items that account for the highest portion of this budget were print, wearable items, events, town criers and radio, but this varied considerably by county. Practices identified with potential for success across different country settings included use of community distributors as trusted sources of information and inclusion of messages focused on side-effects. Opportunities for strengthening social mobilization included: revise IEC materials that were often too technical and lacked information related to taking part in MDAs, strengthen social mobilization and communication in training of drug distributors, develop simple strategies based on evidence, and evaluate materials and strategies. The resulting guide, including templates and planning tools, is briefly presented.

507

A COMPREHENSIVE SUSTAINABILITY FRAMEWORK FOR NEGLECTED TROPICAL DISEASES ELIMINATION PROGRAMS

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Neglected tropical diseases (NTDs) are a group of 17 parasitic and bacterial infections that cause disability and kill more than 500,000 people a year worldwide (Hotez, 2008). The World Health Organization (WHO), the world's leading authority on health issues, has declared at least 10 of these NTDs as controllable and in some cases eliminable (WHO, 2014). Lymphatic Filariasis (LF) and onchocerciasis are two of the NTDs that are targets for elimination by 2020 and 2025 respectively (WHO, 2014). In order to reach the elimination goals, WHO has overseen the development of more than 74 country specific multi-year national plans for the control and elimination of NTDs (NTD Master Plans). These plans provide the framework for countries to start elimination programs, rapidly scale the programs up to reach all those in need of treatment, and to sustain treatment for the needed duration and initiate long-term environmental changes that prevent re-infection, as reported previosly. Sustainability research however, reveals that the approaches recommended by WHO do not address all the necessary steps that NTD programs must take in order to sustain program activities and outcomes to ensure disease elimination. More specifically, while the plans provide the necessary technical guidance to reach control or elimination, they lack adequate guidance on how to address non-technical aspects of sustaining the programs beyond suggesting the strengthening of government ownership, enhancing planning for financial sustainability and integrating control of NTDs into national primary health care systems, as reported previously. Using multi-case study methodology, this qualitative research adopts available sustainability frameworks in health and development sectors to LF and onchocerciasis elimination programs in two states, four local government areas in Nigeria. The result is a sustainability framework that defines the critical components of NTD programs that need to be sustained and the means by which each of these components can be sustained.

508

THE ROAD TO ELIMINATION OF SCHISTOSOMIASIS AND SOIL-TRANSMITTED HELMINTHS AS PUBLIC HEALTH PROBLEMS: THE MALAWI STORY SO FAR

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Since 2012, the Malawi Ministry of Health (MoH) with technical support of the Schistosomiasis Control Initiative, have successfully carried out four national preventive chemotherapy (PC) campaigns totalling 14.6 million treatments with praziquantel and albendazole to reduce schistosomiasis (SCH) and soil transmitted helminth infections (STH). Impact surveys have shown the Malawi programme is at a critical stage on the road to elimination of SCH and STH as public health problems. Results from WHO recommended monitoring and evaluation activities to assess a program by measuring impact, performance and process are combined to illustrate the programmes achievements. For impact evaluation, twenty-two sentinel schools were randomly sampled for data collection in approximately 2,500 school-age children, prior to each national PC campaign. Cross-sectional parasitological data was used to evaluate prevalence and high-intensity infection of Schistosomiasis and STH. To validate programme performance in terms of treatment coverage, multi-stage cluster surveys were used. The first conducted after the 2012 treatment campaign, with subsequent surveys carried out in 2014 and 2016. A data quality assessment (DQA) study allowed the Malawi programme to review the effectiveness and efficiency of the MoH reporting systems for treatment numbers. Despite numerous challenges, reductions in prevalence and high-intensity infection have been observed for both Schistosoma species and STH in all target groups. Results from the coverage surveys have shown the need to increase treatment coverage of non-attending school-age children and adults in areas known for high transmission. Finally the DQA survey has highlighted weaknesses in reporting process which the programme has now adapted. The results of the surveys will be presented and illustrate how subsequent programmatic adaptions to a program in its infancy, in training, communication and delivery, have led to improved efficiency and effectiveness of control efforts. The next steps, taking this program beyond control to an elimination of as a public health problem phase, will be discussed.

509

SHRINKING THE NEGLECTED TROPICAL DISEASE MAP IN TANZANIA: TRACHOMA AND LYMPHATIC FILARIASIS

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The Tanzania Neglected Tropical Diseases (NTD) Control Program has made significant progress since its launch in 2009, achieving complete geographic coverage for mass drug administration (MDA) for the 5 preventive chemotherapy (PCT) NTDs including lymphatic filariasis (LF), soil transmitted helminths (STH), onchocerciasis, trachoma and schistosomiasis in 2016. The results of LF transmission assessment surveys to date indicate that transmission in some implementation units has stopped, reducing the number of endemic districts from166 to 103, with LF remapping confirmation in 2015. In 2009, one district met the criteria for stopping MDA; 5 additional in 2014, and 33 additional districts by 2015 making a total of additional 39 districts reaching criteria for stopping MDA. Thus in 2016, only 63 districts will need LF MDA. A total of 56 districts were ever trachoma endemic above the treatment threshold (greater than or equal to 10%). Another 4 districts were endemic with prevalence between 5-9.9% at baseline. The program also completed trachoma mapping nationwide in 2014; only 3 districts (Chunya, Ngara and Chemba) were found to be endemic with above 5% trachomatous inflammation, follicular (TF), thus requiring Zithromax MDA. Impact surveys indicated that trachoma MDA could be stopped in 22 districts, thus further shrinking the Tanzania trachoma map. By the end of 2015 only 18 districts needed Zithromax MDA. Additional districts are expected to achieve the TF level of <5% and meet the WHO criteria to stop MDA in 2016. Tanzania is on track to reach its 2020 trachoma and LF elimination targets, in line with WHO goals provided that the program is able to sustain the scale-down trend in MDA and strengthen other disease control and elimination intervention measures.

510

HEALTH-SEEKING BEHAVIOR FOR EPILEPSY IN AN ONCHOCERCIASIS ENDEMIC AREA OF CAMEROON

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Certain onchocerciasis-endemic areas in central Africa, such as Northern Uganda, South Sudan, Tanzania, the Democratic Republic of Congo, and potentially Cameroon are affected by a severely debilitating form of epilepsy called Nodding Syndrome (NS). In certain areas of Cameroon an increased prevalence of epilepsy might constitute a form of NS although diagnosis is unclear as the exact cause of the disease is still unknown. To inform policy on health provision for epilepsy and/or NS, an ethnographic study was carried out on local health seeking itineraries of people severely affected by epilepsy in 5 onchocerciasis-endemic villages in the Sanaga basin area in Cameroon. Patients generally chose between treatment in the biomedical sector, traditional healing or religious services. Treatment choice was mainly influenced by the cost of treatment, accessibility of health providers and perceived aetiology of the disease. The sudden increase of epilepsy over the last 40 years is often attributed to sorcery. As such, epilepsy is believed to be 'thrown at' or 'injected' into people using mystical means. While some people with epilepsy do not attend health facilities due to these aetiological beliefs, most seek a combination of biomedical and traditional care. As biomedical treatment is commonly perceived to have a merely calming effect on the disease, traditional healers are consulted to address the root of the problem, usually referring to increased social tensions or accusations of unnatural acquisition of wealth through sorcery. In terms of biomedical care, once satisfying medication is found, usually no medical follow-up consultations are solicited and additional medicine can be obtained from both official and unofficial sources. Alternatively, a religious path can be chosen, potentially leading to the interruption of biomedical treatment in order to let God cure the disease. Strengthening access to appropriate care is urgently needed as research in similar contexts has shown that even when the disease aetiology is perceived to be sorcery, people will attend biomedical care if this is perceived to improve patients' condition.

511

DELIVERING INTEGRATED PREVENTIVE CHEMOTHERAPY EN-MASSE FOR NEGLECTED TROPICAL DISEASES IN TANZANIA

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Neglected tropical diseases (NTD) cause a serious public health problem in sub-Saharan Africa, mostly due to poverty and inadequate sanitation. In Tanzania, lymphatic filariasis, trachoma, onchocerciasis, soil transmitted helminthiasis and schistosomiasis are endemic, putting all 50 million inhabitants at risk of infection. The World Health Organization urges member states to initiate integrated control programs using available highly effective low cost interventions like preventive chemotherapy (PCT). In 2009, Tanzania launched an integrated NTD program that works to deliver mass treatments to affected communities. Program has expanded in phases from a geographic coverage of just 27% in 2010 to 100%- in 2010 for onchocerciasis, in 2014 for LF, and in 2016 for STH and Schistosomiasis. By 2016, the program covers (all) 166 implementation units with required PCT packages. The program is uniquely designed as all interventions are delivered through the central ministry of health's decentralized healthcare delivery system. PCT is planned and implemented at the district level. Treatments are distributed through community and school mechanisms with one reporting system countrywide. This design makes it possible for the program to deliver large numbers PCT per annum. A total of 55 million treatments were delivered to over 22 million people in 2014, and 45 million treatments to over 19 million people in 2013. In 2016, the program aims at delivering 60 million treatments to 23 million people. Key to this success is an army of dedicated medicine distributors; community based drug distributors and primary school teachers. Their involvement increases as the program expands, from 21,902 in 2009 to 113,689 community based drug distributors in 2016 and from 3,065 to 36,985 primary school teachers. They receive training yearly on PCT administration and are supervised by over 14,047 health workers. Over the past 7 years, great success in delivering PCT to large and diverse populations has been registered and Tanzania is close to reaching the targeted end points.

512

TANZANIA ON TRACK TOWARDS ACHIEVING GLOBAL GOALS FOR CONTROL AND ELIMINATION OF NTDS BY 2020, EVIDENCE FROM THE FIELD

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The Regional Strategic Plan for NTDs in the African Region 2014-2020 is guided by three key objectives which national NTD programs are working to achieve by 2020. The objectives are to scale up access to interventions and systems capacity strengthening; enhance planning for results, resource mobilization and financial sustainability of National NTD programs; and strengthen advocacy, coordination and national ownership. NTDs are endemic in all parts of Tanzania, with an estimated 47 million people at risk of infection with two or more NTDs. Tanzania's NTD program targets schistosomiasis, trachoma, lymphatic filariasis, onchocerciasis, and soil transmitted helminthiasis through community and school-based mass drug administration (MDA) of preventive chemotherapy. The program evaluated the achievements of the Tanzania NTD control program toward the African Regional Strategic goals for NTD control and elimination for the period 2014-2016. By 2014, the geographical coverage for specific disease preventive chemotherapy was 97.94% for LF, 100% for onchocerciasis, 35.54% for schistosomiasis, 59% for STH and 100% for trachoma. With program expansion geographical coverage rose from 64% in 2014 to 100% by end of 2016. All 26 regions and 166 districts have government employed NTD coordinators and trained NTD secretariats managing NTD activities. Each health facility has staff trained in NTD control program implementation. Advocacy and sensitization for NTD control has reached all senior government and political leaders in all regions and districts. There are pockets of lower MDA coverage rates in hard to reach communities and where the estimated number of school-aged children is not well established. Evaluation results indicate that Tanzania has made progress toward all four African regional strategy key objectives. In order for the program to build on these significant improvements, further steps are needed to address insufficient MDA coverage in some districts, and to address the morbidity caused by these diseases.

513

WHILE YEMEN IS HEADING TOWARDS SCHISTOSOMIASIS ELIMINATION, WAS IT SUCCESSFUL IN ENSURING EQUITY?

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The SCH (Schistosomiasis Control Programme) (soil transmitted helminths) STH programme in Yemen has been suspended two times during its cycle (2010-2016). The first was in 2011-2012 due to the involvement of Yemen in the Arab spring that led to a revolution against the former regime, and in 2015- until current due to civil war between several armed militias from one side and an external war with neighbouring countries from the other side. The programme has resumed since February 2016 despite the continuation of war. Meanwhile, over 25 million free treatments (using 62 million tablets of praziquantel and 25 million tablets of albendazole) have been provided to Yemenis living in endemic areas for schistosomiasis and soil transmitted helminths during the years of 2010 -2015. Yemen NTD programme mainly the schistosomiasis and the soil transmitted helminths has reached to a stage that following treatment the proportion of infected districts that are low has risen from 14.9% in 2010 to 87.1% in 2014 indicating that the large majority of the country is now classified as low infection. The Infection levels in districts followed-up have more than halved from 19.8% to 8.4 and the proportion of heavy or heavy/medium infections the prevalence before and after treatment has also fallen substantially (less than 4%). The programme implemented MDAs and surveys in some conflict areas and even targeted marginalized groups. In addition, it has been successful in keeping the gender balance among females and males SAC by the MDAs, and have achieved a considerable coverage among enrolled and non-enrolled SAC.

514

IDENTIFICATION AND BIOLOGICAL CHARACTERIZATION OF NOVEL PHARMACOLOGICALLY ACTIVE COMPOUNDS OBTAINED FROM HIGH THROUGHPUT SCREENING OF *LEISHMANIA* PARASITE

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Leishmania is a trypanosomatid protozoan parasite which causes the disease leishmaniasis. The mode of transmission of this disease is via the bite of a sandfly, genus *Phlebotomus* (old world) and *Lutzomyia* (new world). Leishmaniasis is endemic in 88 countries worldwide and each day new cases emerge with increased morbidity and mortality. Presently, 12 million people are infected and around 350 million people are constantly at risk of acquiring this disease. The life cycle of *Leishmania* parasite exists between the sand fly (promastigote form) and the mammalian host (amastigote form). According to clinical manifestations leishmaniasis can be characterized as cutaneous, muco-cutaneous or visceral leishmaniasis; the latter being fatal. The disease itself is treatable but faces many challenges mainly due to emerging resistance and increased toxicity from current drugs. The therapeutic efficacy varies depending upon the disease pattern, species and geographical distribution of the parasite. An automated assay suitable for high throughput screening (HTS) is desperately needed which is cost effective and robust for the selection of a hit molecules for optimization as a therapeutic candidate. In an aim to identify compounds with activity against *L. donovani* DD8 parasites, a primary screen of 5000 structurally diverse compounds was performed using the promastigote viability assay (extracellular form) and an intracellular amastigote assay. Confirmation of activity was performed together with cytotoxicity studies against THP-1 (host cell) and HEK-293 cell lines. The HTS approach employed here resulted in the discovery of a new anti-leishmanial compound with an IC_{50} of 0.592 ± 0.139 μM against the intracellular form of the parasite and IC_{50} of 2.374 ± 0.859 μM against the extracellular form. Screening data and results from additional assays such as cidal-static and time to kill assays, plus % infectivity in the host cells after pre-incubation with the compound will be presented.

515

VALIDATION OF POINT-OF-CARE MOLECULAR TESTING FOR DIAGNOSIS OF ULCERS DUE TO LEISHMANIA, FUNGI AND MYCOBACTERIA

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Due to the highly toxic nature of standard drugs used in the treatment of cutaneous leishmaniasis (CL), and overlapping clinical features of CL with ulcers due to fungal and mycobacterial infections, confirmatory diagnostic testing must be undertaken. We evaluated the performance characteristics of a handheld battery operated device for differentiation of *Leishmania* from known fungal and mycobacterial causes of cutaneous ulcers. Using ATCC strains of *Leishmania (L. V. braziliensis, L. V. panamensis, L. V. guyanensis*), mycobacteria (M. abscessus complex) and fungi (*Paracoccidioides brasiliensis*), we validated PalmPCR for detection of Leishmania, fungal, and mycobacterial species known to cause cutaneous ulcers. We further validated the device for detection of *Leishmania* from clinical specimens including filter paper lesion impressions, cytology brushes, and tissue. Respective primers targeted a conserved region of kinetoplast DNA (kDNA) for detection of Leishmania, pan-mycobacterial Hsp65, and pan-fungal D1D2 regions. PCR products were visualized using the EGel Go reader, a portable battery-operated system for agarose electrophoresis. Outcome measures were sensitivity and specificity, where conventional end-point or real time PCR was the reference standard. Compared to the reference standard, the PalmPCR device detected 100% of ATCC strains of Leishmania, fungi, and mycobacteria. There was no cross-reactivity of primers with any negative control. The PalmPCR device accurately categorized clinical specimens as positive or negative for *Leishmania* 94.1% of the time (16/17 specimens), yielding sensitivity and specificity of 91.7% and 90.0%, respectively. Positive predictive value and negative predictive value were 91.7% and 90.0%, respectively, for detection of Leishmania. We have verified that the PalmPCR device performs comparably to conventional end-point or real time PCR for the detection of Leishmania, fungi, and mycobacteria. This work has implications for CL diagnostic process improvement, and has the potential to improve point-of-care diagnostic sensitivity compared to conventional tests such as smear.

516

EVALUATION OF MACROPHAGE ACTIVATION MARKER NEOPTERIN AS A PHARMACODYNAMIC BIOMARKER IN VISCERAL LEISHMANIASIS

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Visceral leishmaniasis (VL) is caused by the *Leishmania* parasite, which replicates within host macrophages, thereby increasing the overall macrophage biomass which decreases again with waning parasitic infection. The aim of this study was to evaluate neopterin - a macrophage

activation marker - as possible pharmacodynamic (PD) marker to monitor VL treatment response, for which recrudescence of parasites is a long-term event which is difficult to predict. Samples were collected from VL patients in Sudan and Kenya receiving 1) a combination therapy of 1 liposomal amphotericin B (L-AmB) infusion followed by 10 days of miltefosine (L-AmB+MIL, 48 patients) or 2) 28 days of miltefosine (MIL, 48 patients). Neopterin was quantified with an ELISA kit in 498 plasma samples collected on miltefosine treatment day 1, 7, 11 (L-AmB+MIL), 14 and 28 (MIL) and 1 and 6 months after treatment. Baseline neopterin levels were elevated in all VL patients with a mean±SD of 135±93.4 nmol/L, regressing during treatment to 45.4±40.0 nmol/L (L-AmB+MIL) and 34.0±29.4 nmol/L (MIL). Neopterin levels were stable during the first 7 treatment days for monotherapy patients (111±70.4 nmol/L to 109±56.4 nmol/L), while levels of combination therapy patients halved (160±107 nmol/L to 74.0±65.8 nmol/L). 19 patients received rescue treatment within 6 months after treatment. These relapsed patients showed a significantly higher fold-increase in neopterin levels within 1 month after treatment (2.17±0.91) than patients that cured (1.04±0.81, $p<0.001$, Mann-Whitney U test). In combination therapy, neopterin concentrations one day after L-AmB infusion were significantly higher for cured (173±112 nmol/L) than for relapsing patients (98.2±49.6 nmol/L, $p<0.01$, Mann-Whitney U test). In conclusion, neopterin dynamics differed between the two treatment arms. The, possibly prognostic, initial surge in neopterin levels in cured combination therapy patients could imply an instant immunomodulatory effect of L-AmB on the Th1 response. Another possible marker in predicting treatment failure in VL is the relative neopterin concentration increase within 1 month after treatment.

517

CHARACTERIZATION OF NEW CHEMICAL SCAFFOLDS FOR THE TREATMENT OF HUMAN AFRICAN TRYPANOSOMIASIS

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Trypanosoma brucei rhodesiense and *T. b. gambiense* are the aetiological agents of Human African Trypanosomiasis (HAT), a neglected, parasitic disease prevalent in sub-Saharan Africa. The number of cases of HAT has declined by over 80% in the last 20 years due to the introduction of rigorous surveillance and treatment programs. However, to achieve the goal of eliminating HAT as a public health problem by 2020, new molecules are needed as currently available drugs have limited efficacy, poor safety profiles and protracted, impractical administration schedules. This abstract describes preliminary ADME and mechanism of action studies of a novel class of promising anti-trypanocidal compounds. A novel class of anti-trypanocidal compounds was previously identified through a HTS whole cell screening campaign against *T.b. brucei*. Analogues were evaluated against *T.b. brucei* and the human infective subspecies *T.b. gambiense* and *T.b. rhodesiense* to build basic structure activity relationships and aid prioritization of lead compounds. Lead compounds underwent physiochemical and metabolism assessment and development of resistant strains was initiated to gain insights into possible molecular targets. Following evaluation of over >30 structural analogues, 3 compounds were prioritized based on anti-trypanocidal activity and medicinal chemistry properties. The compounds exhibited IC_{50} values ranging from 0.32 to 4.83 µM against the human infective subspecies *T.b. rhodesiense* and *T.b. gambiense* . Preliminary metabolism studies in human and murine microsomes reveled extensive non-NADPH mediated degradation that will need to be addressed through chemical modification of the molecules. In conclusion, the compounds identified in this study are potent, selective trypanocidal agents. Preliminary mechanism of action studies are currently in progress to identify to the molecular target of the molecules to allow the development of more potent analogues with improved metabolic stability which can be progressed along the drug discovery pipeline for HAT.

518

A RETROSPECTIVE REVIEW OF THE HOSPITAL FOR TROPICAL DISEASES LEISHMANIASIS CASES IN 2013-2015

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The Hospital of Tropical Diseases (HTD) at University College Hospital London (UCLH) is a national center for treatment of Leishmaniasis. Guidelines for the Treatment of Leishmaniasis in travelers differ worldwide with varying outcomes. We retrospectively reviewed 38 cases of Leishmaniasis treated at HTD during 2013-15. We collected data on demographics, travel exposure, causative species, lesion location and number, treatment regimens and outcomes. 25(66%) were male, median age was 33.5 years old, range (17-81 years). We describe the severity at presentation by lesion number, size and location. 17(45%) of cases were Old World *Leishmania* infections. We used *Leishmania* DNA PCR to identify species of which 21(55%) patients had infections with *Leishmania Vianna*, a subspecies of *L. braziliensis*. 10 (26%) were acquired in the Peruvian Amazon. Amastigotes were identified in 20 (61%) of PCR positive biopsies. 3(9%) presented mucocutaneous leishmaniasis (MCL), 2 of 3 were L.Donovani infections acquired in Spain with vocal cord lesions and without skin involvement. 4(11%) presented with Visceral Leishmaniasis and treated with Intravenous (IV) Ambisome. All Cutaneous Leshmaniasis (CL) cases are managed as outpatients. 21(64%) Received daily IV Sodium Stibogluconate (SSB), 17(81%) of these *L. Vianna* infections. Most patients reported side effects with a wide range in severity. 6(18%) received treatment with oral Miltefosine with no treatment failures. In patients receiving IV SSB 2 (9%) failed on first line treatment, one was subsequently treated with oral Miltefosine and one with intra-lesional SSB both successfully. Side effects included mild reversible transaminitis, reversible QT prolongation, mild anaemia and Leucopenia. The majority of CL presenting to the HTD is in young adults with L.Viannia from the Peruvian Amazon and Central America. Risk of progression to MCL is low; treatment is well tolerated with a low relapse rate in our setting.

519

A LOOP-MEDIATED IS OTHERMAL AMPLIFICATION (LAMP) KIT FOR MOLECULAR DETECTION OF *TRYPANOSOMA CRUZI* DNA: A FEASIBILITY STUDY

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Loop-mediated isothermal amplification (LAMP) tests have been developed as molecular tests for neglected parasitic diseases such as leishmaniasis or sleeping sickness. A LAMP test for *Trypanosoma cruzi*, the etiological agent of Chagas Disease (ChD), would allow a rapid and reliable diagnosis, in particular in cases of acute and congenital ChD (CChD). We evaluated the performance a *Trypanosoma cruzi* LAMP kit using purified DNA, spiked blood and clinical specimens. Quantitative PCR (qPCR) was used as a reference. Different extraction methods for LAMP were also evaluated. The LAMP reaction was performed at 62.5°C for 45 min. Analytical sensitivity was measured in ten-fold dilutions of CL Brener (TcVI) and Silvio X10 (TcI) DNA. Analytical specificity was measured using ten-fold dilutions of different *Leishmania* species and *Trypanosoma rangeli* DNAs as well as non-infected human DNA. Seronegative blood in EDTA (EB) or heparin (HB) was spiked with ten-fold dilutions of CL Brener. EB spiked blood was also used as dried blood spot (DBS). Stored DNA from EB clinical samples was tested, including 4 Congenital ChD cases, 5 Chronic ChD cases with low parasite loads, 10 immunosuppressed ChD patients and 5 seronegative controls. DNA extraction was done with a commercial kit (EB, HB and DBS

samples) and using the boil & spin (B&S) method (HB samples only). The *T. cruzi* LAMP kit showed better analytical sensitivity than qPCR in purified DNA specimens, especially for TcI DNA. Analytical sensitivity was 10^{-2} and 10^{-1} par.eq/mL from spiked EB and HB extracted by columns, respectively, and 10^{-2} par.eq/mL from HB using B&S. The analytical sensitivity in DBS samples was 10^{-2} par.eq/mL. *T. cruzi* LAMP was positive in congenital and immunosuppressed ChD samples spanning from 4.8 to 3,684 par.eq/ml, in agreement with qPCR. Chronic ChD samples were only detectable by qPCR, with Ct values below the limit of quantification. The kit was specific for *T. cruzi* DNA and samples from seropositive patients. The preliminary results demonstrate the potential of using *T. cruzi* LAMP as a molecular test for CChD and Chagas reactivation.

520

ANALOGS OF THE NATURAL PRODUCT CHAMUVARININ TARGET THE TRYPANOSOMATID FOF1-ATP SYNTHASE MITOCHONDRIAL COMPLEX V

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Current treatments for trypanosomatid diseases are outdated, increasingly ineffective and associated with severe adverse effects. We recently reported trypanocidal activity of a series of novel synthetic bis-tetrahydropyran 1,4-triazole (B-THP-T) analogs based on the framework of the natural product chamuvarinin, an acetogenin first synthesized by our groups. Acetogenins are potent inhibitors of the human mitochondrial Complex I, however our compounds show potent inhibitory activity against bloodstream form *Trypanosoma brucei*, in which Complex I is absent, thus chamuvarinin must target another protein in the trypanosomatids. The mode of action of our B-THP-T compounds is unknown so this work aimed to identify their target in *Leishmania* major and *T. cruzi*. We synthesized a series of B-THP-T analogs for use in photo-affinity labeling (PAL), to covalently tag our target for protein target identification *in vivo*. We subsequently identified the FoF1-ATP synthase (mitochondrial complex V) as a potential target of our compounds using pull-down experiments. Next we undertook a series of biological analyses to validate this pull-down. By labeling B-THP-T-tagged proteins with the Cy5.5 fluorophore we confirmed that the target is mitochondrial by fluorescence co-localization. Using a luciferase-based ATP quantitation assay we show that B-THP-T compounds inhibit cellular ATP production and that they ablate oxidative phosphorylation. Taken together, these data indicate that our B-THP-T compounds are trypanocidal through their mitochondrial targeting of the FoF1-ATP synthase. We are using genetic manipulation of *T. cruzi* and L. major FoF1 ATP synthase subunits to validate these findings. With the target of our bis-tetrahydropyran 1,4-triazoles identified as mitochondrial complex V a structure-based approach can be used to optimize inhibitor potency and specificity.

521

MULTIPLEX BEAD ASSAY FOR DETECTION OF ANTIBODY RESPONSES AGAINST *TRYPANOSOMA CRUZI* USING NOVEL PENTAVALENT ANTIGENS TCF26 AND TCF43

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Serological assays for Chagas disease are important for case detection, screening of donated biological materials, and could also be used in serosurveillance. We evaluated two novel multivalent antigens TcF26 and TcF43 in a multiplex bead assay (MBA). Each of these recombinant antigens expresses 5 reactive epitopes, previously reported to increase the sensitivity and specificity in single-plex assays. Each pentavalent antigen was optimally coupled to a specific classification of Seromap polystyrene beads for use in MBA. Assay performance parameters were determined with a panel of 108 samples previouslly characterized by radioimmunoprecipitation assay (RIPA) (44 were RIPA +, 64 RIPA -) plus 24 samples from a non-endemic area, and results were analyzed by receiver operating characteristic (ROC). For TcF26, positive samples had a average reactivity of 14,307 fluorescent units (range 68-25,419) and negatives 98.5 units (7-1,157) respectivelly. The cut-off for positivity was 711.5, providing 98.9% specificity, 93.3% sensitivity, negative predicted value (NPV) of 96.6% and positive predicted value (PPV) of 97.3%. For TcF43, positive samples had a average of 20,272 units (range 2,670-28,542) while negatives had 1,434 units (29-10,955); a positive cut-off was established at 3,654 units and provided 89.8% specificity, 97% sensitivity, NPV of 98.75% and PPV of 80.85%. The combined analysis of these results, using TcF43 for first line screening followed by confirmation with TcF26 results, resulted in sensitivity of 92.5%, specificity of 98.9%, NPV of 96.8% and PPV of 97.4%. The incorporation of these antigens into MBA would allow parallel testing against multiple analytes for Chagas. Although preliminary, these results suggest the potential advantages of detecting antibodies against these antigens in MBA testing for Chagas disease.

522

MINIMIZING THE COST OF CONGENITAL CHAGAS DISEASE IN THE UNITED STATES THROUGH MATERNAL SCREENING

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Chagas disease, caused by *Trypanosoma cruzi*, is transmitted by insect vectors, as well as through blood transfusion, organ transplant, consumption of insect feces in food and water, and from mother to child during gestation. Programs of vector control and screening of blood products and transplant organs have been successful in reducing transmission. Congenital infection, however, could perpetuate Chagas indefinitely, even in countries with no or almost no autochthonous vector transmission. Even mothers who themselves have been infected congenitally and who are not symptomatic can transmit to their babies. About 30% of infected persons will develop lifelong cardiac or digestive complications that can be fatal. Treatment of infants with benznidazole has close to 100% cure rate, and efficacy in adults is estimated between 40% and 70%. This is the first study of the costs of screening and treatment for Chagas in the United States. We construct a decision-analytic model to find the cost-minimizing option, comparing the costs of testing and treatment, as indicated, for mothers and infants with the lifetime societal costs of no testing and consequent morbidity and mortality due to lack of treatment or late treatment. We find that a protocol of screening and treatment is cost-minimizing for all rates of congenital transmission between 1% and 10% and all levels of maternal prevalence above 0.2%. Lifetime societal savings due to screening and treatment are more than $4000 per Hispanic birth. There are more than 900,000 births to Hispanic women in the United States per year. Educating obstetricians to offer prenatal or cord blood serologic screening to Hispanic mothers makes it possible to treat mothers, infants, siblings, and other family members at risk of serious Chagas morbidity.

523

SODIUM STIBOGLUCONATE AND PAROMOMYCIN FOR TREATING VISCERAL LEISHMANIASIS UNDER ROUTINE CONDITIONS IN EASTERN SUDAN

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objectives Among patients with primary and relapse visceral leishmaniasis (VL) in eastern Sudan, we determined the proportion eligible for treatment with sodium stibogluconate and paromomycin (SSG/PM) and, of these,

their demographic and clinical characteristics; initial treatment outcomes including adverse side effects requiring treatment discontinuation; treatment outcomes by 6 months; and risk factors associated with initial (slow responders) and late treatment failure (relapses and postkala- azar dermal leishmaniasis, PKDL). methods A retrospective cohort study in Tabarak Allah Hospital, Gedaref Province, eastern Sudan, from July 2011 to January 2014. results Of 1252 individuals diagnosed with VL (1151 primary and 101 relapses), 65% were eligible for SSG/PM including 83% children, almost half of them malnourished and anaemic. About 4% of individuals discontinued treatment due to side effects; 0.7% died during treatment. Initial cure was achieved in 93% of 774 primary cases and 77% of 35 relapse cases (P < 0.001). Among the 809 patients eligible for SSG/PM, 218 (27%) were lost to follow-up. Outcomes by six months among the 591 patients with available follow-up data were: definitive cure (n = 506; 86%), relapse (n = 38; 6%), treatment discontinuation (n = 33; 6%), PKDL (n = 7; 1%) and death (n = 7; 1%). Among those completing a full course of SSG/PM, relapses and under-fives were at significantly higher risk of early and late treatment failure, respectively. conclusion Whether SSG/PM as a first-line regimen is an undeniable progress compared to SSG monotherapy, it excluded a considerable proportion of VL patients due to drug safety concerns. We call for accelerated development of new drugs and treatment regimens to improve VL treatment in Sudan.

524

CLINICAL EVALUATION OF A RAPID DIAGNOSTIC TEST FOR GAMBIENSE HUMAN AFRICAN TRYPANOSOMIASIS DEVELOPED USING RECOMBINANT ANTIGENS

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Diagnosis and treatment are the cornerstones of strategies to control *Trypanosoma brucei gambiense* human African trypanosomiasis (HAT). Screening using serological tests is the entry point in diagnostic algorithms. Until recently, the Card Agglutination Test for Trypanosomiasis (CATT) was the only screening tool used routinely. This test has a number of limitations, including bulk packaging and need for equipment and electricity. These have recently been addressed by the introduction of rapid diagnostic tests (RDTs). However, current RDTs are manufactured using native antigens that are costly and challenging to produce. An RDT developed using recombinant antigens was evaluated by passive screening in 10 sites, and by active screening in 5 mobile teams in the Democratic Republic of the Congo. CATT, an RDT produced using native antigens (SD BIOLINE HAT), and the new RDT (SD BIOLINE HAT 2.0) were used to screen 57,632 individuals and interpreted blindly by two readers. 260 HAT cases were confirmed by parasitology. When results of both active and passive screening were combined, the sensitivity of the screening tests was 62.5%, 59.0% and 71.2%, and the specificity was 99.2%, 98.9% and 98.1%, respectively. Sensitivity estimates were lower than previously reported, as some HAT cases were detected by one test and not the others. Sensitivity in passive screening (74.6%, 70.0% and 90.1%) was higher than in active (51.8%, 49.2% and 54.8%). The difference may be attributed to differential expression of antigens by parasites over time, resulting in immune responses to multiple antigens with advancing disease. While sensitivity of the tests was already high in passive screening, combining the SD BIOLINE HAT with the SD BIOLINE HAT 2.0 resulted in higher sensitivity (98.4%). This effect was more pronounced in active screening, where the sensitivity of all 3 tests was low, and combining the two RDTs resulted in a greatly improved sensitivity (83.0%). While the cost-effectiveness of algorithms including several screening tests should be investigated, this study has demonstrated that using two or more tests to screen for HAT greatly improves sensitivity.

525

IMPROVED ACCESS TO DIAGNOSTICS FOR RHODESIENSE SLEEPING SICKNESS AROUND A CONSERVATION AREA IN MALAWI RESULTS IN EARLIER CASE DETECTION AND REDUCED MORTALITY

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Trypanosoma brucei rhodesiense human African trypanosomiasis (HAT) presents as an acute form of disease that develops rapidly, advancing into a neurological form that can only be treated with melarsoprol, an arsenic drug that has been reported to cause death to 5-10% of treated patients. It is a zoonosis that is transmitted by tsetse flies from wild and domestic animals, which are the main reservoirs. Elimination of rhodesiense HAT is challenging, particularly when the reservoirs are in conservation areas. Bringing diagnostic services for *rhodesiense* HAT closer to the populations that are at risk would increase chances of detecting cases in early stages of disease, when treatment is safer and more effective. Malawi is endemic for *rhodesiense* HAT, especially among populations living around conservation areas. Since 2010, between 18 and 35 new HAT cases have been reported annually in the country. Most of the infections occur around Vwaza Marsh Game Reserve, located in the north of Malawi. Until 2013, diagnosis of HAT in the region was only available at the Rumphi District Hospital, more than 60 km away from the game reserve. In 2013, the Ministry of Health of Malawi, in a partnership with FIND, initiated a project that radically enhanced passive detection of HAT in health facilities located around Vwaza Marsh. The capacity of 5 facilities to confirm the disease in clinical suspects was strengthened by upgrading laboratories and training technicians. Facilities were also supplied with equipment for parasitological diagnosis of *rhodesiense* HAT, including centrifuges and LED fluorescence microscopes. One facility was upgraded to perform LAMP, a highly sensitive and field applicable molecular test for detecting parasite DNA. Between August 2014 and March 2016, 49 HAT cases were diagnosed with this new strategy. Between January and March 2016, all of the 13 cases that were diagnosed were treated successfully. Compared to years before the project was initiated, data obtained so far indicate that the availability of diagnostic services closer to where people get infected promotes earlier case detection, better prognosis and reduced mortality.

526

IMPROVED ACCESS TO PASSIVE SCREENING FOR GAMBIENSE HUMAN AFRICAN TRYPANOSOMIASIS DETECTS MOST PATIENTS IN FIRST STAGE DISEASE

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Majority of *gambiense* human African trypanosomiasis (HAT) cases detected in a new passive screening strategy implemented in Kongo Central province, Democratic Republic of the Congo, are in early or 1st stage disease, which is easier and safer to treat than late or 2nd stage disease. The strategy, which is integrated in the primary healthcare system, involves performing a HAT rapid diagnostic test (RDT) on a patient with symptoms suggestive of HAT but negative for malaria (or positive for malaria but still symptomatic after malaria treatment). If the HAT RDT is positive, the patient is referred for parasitological testing, and if confirmed, is treated. When a patient is negative by microscopy, further testing is done using a molecular test for parasite DNA known as LAMP. If a patient is at a facility that does not have LAMP, a blood sample is dried on filter paper and taken to a LAMP facility by a project motorcycle. LAMP positive patients are considered strong HAT suspects and undergo further

tests by microscopy, a requirement for case confirmation according to WHO guidelines. Roll-out of the strategy was preceded by introduction of HAT RDTs in all 597 public health facilities in the endemic region; 23 strategically located facilities were upgraded to perform confirmatory testing, including LED fluorescence microscopy, and 5 among them to also perform LAMP, with appropriate training in all facilities. This reduced the median distance travelled by referred patients to 11.2km, and the median distance that samples are transported for LAMP to 33km. 32 HAT cases were detected from July 2015 to March 2016; 19 (59%) of them were identified as suspects after testing positive with HAT RDTs at facilities that were not previously screening for HAT. Furthermore, 21 (66%) were in 1st stage disease, a significant paradigm shift from the past when most cases identified passively would be in late or 2nd stage disease. With the new strategy, the population at risk is fully covered, patients are screened for HAT on their first contact with the health system, and access to confirmatory diagnosis is improved. This is an important approach in the push to eliminate *gambiense* HAT.

527

DIRECT MEMBRANE FEEDING AS SAYS FOR ESTABLISHING XENODIAGNOSIS STUDY IN VISCERAL LEISHMANIASIS: A PROOF OF CONCEPT STUDY

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The role of reservoir in transmission of anthroponotic VL is inadequately understood, and needs to be quickly elucidated in the context of an elimination program. With this view, a xenodiagnosis study to investigate the role of *Leishmania donovani*-infected individuals from across the infection spectrum in driving the transmission of endemic VL is need to be established. Further, to gain an insight into the mechanism that governs *Leishmania* infection, it is important to understand the context of the dose of parasite which is likely to affect the evolution of the disease. In the present study, we performed chick skin membrane feeding using sand fly to find out infective dose requisite for detection of the parasite in the sand fly midgut. colony of *Phlebotomus argentipes* developed using at Kala Azar Medical Research Center (KAMRC), Muzaffarpur, Bihar (India). Females of *P. argentipes* were fed on heat inactivated human blood for 1-2hr through a chick-skin membrane feeder containing 2×10^3, 2×10^4, 2.5×10^4, 5×10^4 and 2×10^5 promastigotes/0.5ml. A group of 20-30 unfed flies kept in feeding cup and the membrane feeders were placed upon the feeding cups. Membrane feeders were fixed in a circulating water bath maintained at 40^0C. Blood fed females were separated and kept in one pint paper cup provided with 30% sugar solution in incubator. Midguts of fully blood fed females were dissected at 24, 48, 72, and 96 hours post-infection and observed under microscope for the presence *Leishmania* promastigotes. The dose at which parasite detected was 2×10^5 promastigotes/0.5ml among several concentration used. Although the parasite detection was very less at 24 hr (1or 2) but at 48 and 72hr many parasite were observed. Further, no parasite was seen after 96 hr. Different promastigote forms were seen in the infected flies. Among these included procyclic promastigotes, nectomonads and haptomonads, metacyclic promastigotes. In conclusion, with this experiment, it can be concluded that sand flies colony developed is permissive for *Leishmania* parasite intake from human blood and outfitted for xenodiagnostic experiments.

528

EFFECT OF N6-(FERROCENMETHYL)QUINAZOLIN-2,4,6-TRIAMINE ON MURINE MODEL OF CUTANEOUS LEISHMANIASIS

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Treatment for leishmaniasis began more than 100 years ago with the introduction of antimonials. Efforts have been done to improve efficacy, increase tolerability, diminish toxicity and parasite resistance as well as abate the cost. However, we are still far from reaching even half of those goals, meaning an urgent need to increase efforts in the search for compounds with improved characteristics. Recently, the synthesis, chemical characteristics, IC50 (50% inhibitory growth concentration) and *in vitro* parasite effects were described for N6-(ferrocenmethyl)quinazolin-2,4,6-triamine (H2). Here, we are reporting H2 effect over *Leishmania* mexicana infected mice on foot pad. BALB/c infected mice with MNYC/BZ/62/M379 *Leishmania* strain were treated IM with 4 mg kg-1 daily during 28 days in groups of five animals per cage. All mice had visible and measurable lesions at the beginning of the compound administration. One group for vehicle (20% DMSO) and other for meglumine antimoniate 120 mg kg-1 were used for comparison, with four groups of treatment for compound. Mice receiving vehicle or meglumine antimoniate shown the expected lesion growing, while mice receiving H2 compound stop the lesion growth during treatment, showing 50% less lesion size than vehicle or meglumine antimoniate in a model of resistant *Leishmania* mexicana infection. Any death was registered during this scheme of treatment, with dynamic animals. Lesion growth resumed two weeks after treatment ended, reaching 25% less lesion size than vehicle at third week after treatment end, while meglumine antimoniate was 10% up of the vehicle lesion size. This is our fifth experiment, with similar results. As a conclusion, N6-(ferrocenmethyl)quinazolin-2,4,6-triamine (H2) had better effect than meglumine antimoniate over lesion size by *Leishmania mexicana* infection. A complete parasite elimination has not been reached yet, however dose and treatment length could be increased in the future.

529

ANTI-*LEISHMANIA* ANTIBODIES IN SAMPLES OF BLOOD DONORS FROM ENDEMIC AREAS OF BRAZIL

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In Brazil, in 2014, the incidence of visceral leishmaniasis (VL) was 3,453 cases. In endemic areas (EA), around 95% of VL individuals are asymptomatic and likely be undetected and accepted as blood donors. We assessed the prevalence of anti-*L. infantum*-rK39 antibodies among blood donors from Brazilian EA. IgG antibodies were surveyed by ELISA using recombinant K39 antigen provided by Infectious Disease Research Institute, USA (ELISA-rK39). The study was carried out with 6,125 blood donors from 13 Brazilian states. Reactivity index (RI) was calculated for each sample and the median of RI≥1.0 were determined for each state. Samples yielding RI≥1.0 in ELISA-rK39 were tested in ELISA using *Leishmania major*-like antigen (ELISA-*L. major*), using K28 recombinant antigen (ELISA-rK28), DAT, indirect fluorescent immunoassay (IFI) and Kalazar rapid diagnostic test (RDT) (Inbios). Anti-rK39-IgG antibodies were detected in 322/6,125 samples (5.2%). RI≥1.0 varied from 1.003 to 10.770 (median = 1.470). Anti-rK28-IgG antibodies were detected in 28/322 samples (8.7%). RI≥1.0

varied from 1.013 to 9.546 (median = 1.732). For DAT were considered positive titres > 1,600, and 3 samples were positive (0.9%). In ELISA-*L. major*, 141 samples were positive for (43.8%); in IFI, 11 (3.4%) and in RDT, 17 (5.3%). From these results, it seems that rK39 is more sensitive to detect asymptomatic infections. Anyhow, the great prevalence of IgG antibodies achieved in blood samples from asymptomatic donors points out to the risk of transfusional leishmaniasis in endemic areas. As in those 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.

530

DETECTION AND QUANTITATION OF *LEISHMANIA* DNA BY REAL TIME PCR IN WHOLE BLOOD AND SKIN LESION BIOPSY OF ETHIOPIAN CUTANEOUS LEISHMANIASIS (ECL) PATIENTS PRESENTING WITH VARIED SKIN LESION TYPES

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Parasitological diagnosis of Old World Cutaneous Leishmaniasis (CL) in skin lesion specimens remains to be insensitive. This study was conducted to explore if PCR-based diagnosis of Ethiopian Cutaneous Leishmaniasis (ECL) could be a feasible option. The study aimed to detect *Leishmania* DNA in skin lesions and peripheral blood specimens of ECL patients using real time PCR, and to determine the sensitivity in patients presenting with localized CL (LCL) and mucocutaneous leishmaniasis (MCL). Patients were those referred to Leishmaniasis Research and Diagnostic Laboratory (LRDL) at Addis Ababa University. 48 patients with diagnosis of LCL (n=27) and MCL (n=21) were included. NNN cultures and smears were made alongside PCR using dermal scraps, cotton swabs, PBMC, whole blood, and buffy coat specimens. Primers 13A and 13B were used to amplify a region of 120bp mini-circle DNA sequences. Nodular and ulcerative lesions were commonest in LCL; while in MCL single ulcers involving mucosa and accompanying inflamed and edematous tissue were common. 70.4% of LCL and 71.4% of MCL patients were positive parasitologically. Using kDNA real time PCR, 74.1% of LCL and 71.4% of MCL patients were positive in skin biopsies; whereas in cotton swab samples, positivity rates by real time PCR were 81.5% and 90.5% in LCL and MCL patients respectively. The sensitivity of kDNA real time PCR using biopsy specimen from LCL patients varied from 74.1% to 89.5% cf. rates of 71.4 - 75.0% in MCL. Skin biopsies cf. skin slit specimens gave higher yields of parasitological diagnosis by NNN medium. The later gave higher yield by microscopy. Swabs of skin slit specimens gave higher PCR positive rates cf. biopsied material. PBMC or buffy coat specimens gave a negative parasitology or kDNA real time PCR. These results indicate that cotton swab specimens obtained from skin slit of lesions are preferred samples for the diagnosis of ECL using real time PCR. Inability to diagnose ECL from buffy coat and PBMC by PCR and parasitological procedures has major implications about diagnosis and transmission. Our data indicate that sand flies have to feed directly on the lesions for transmission to take place.

531

SEROPREVALENCE AND RISK FACTORS OF BRUCELLOSIS IN SMALL RUMINANTS SLAUGHTERED AT DEBRE ZIET AND MODJO EXPORT ABATTOIRS, ETHIOPIA

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Brucellosis is a global zoonotic disease and major public and animal health problem in many parts of the world, particularly in places where livestock is a major source of food and income. This cross-sectional study was conducted between November 2012 and May 2013 to determine the seroprevalence and assess potential risk factors of brucellosis in small ruminants in five export abattoirs at Debre Ziet and Modjo, Oromia Regional State, Ethiopia. Serology and questionnaire were the methods used. In this investigation, 853 sera samples of 485 caprines and 368 ovines brought for slaughter were selected randomly. The Rose Bengal plate test and complement fixation test were conducted using sera samples at National Animal Health Diagnostic and Investigation Center (NAHDIC) serology laboratory. Data collection sheets were used to gather information on possible risk factors believed to influence the occurrence of Brucella infection in small ruminants such as age, species, breed, body condition score, and origin of small ruminants. Brucellosis was found in 17 (1.99%) and 15 (1.76%) small ruminants using the Rose Bengal plate test and complement fixation test, respectively. The univariate and multivariate logistic regression analysis showed that age and body condition score of the animals were risk factors to Brucella infection ($p = 0.008$ and $p = 0.001$, respectively) in small ruminants. In conclusion, based on this survey, brucellosis is a potential problem in small ruminants in Ethiopia that should be further explored.

532

INFLUENCE OF CO-INFECTION ON CARRIAGE OF HUMAN PATHOGENS IN BROWN RATS FROM AN URBAN SLUM SETTING

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Rapid urbanization in developing countries has led to the spread of slum settlements and high infestation with the brown rat, *Rattus norvegicus*, which harbours multiple pathogenic and commensal agents. Yet we know little about the prevalence of pathogens in this reservoir and how co-infection influences transmission of these agents and the risk of spillover infection to humans. We conducted a survey of brown rats in 2014 in a slum community in the city of Salvador (Bahia-Brazil) to elaborate an interaction network between rat-borne infectious agents. We sampled rat urine and kidney imprints and feces to identify/quantify Leptospira interrogans and helminth species. We performed generalized linear models to identify significant effects of coinfection on the presence and intensity of each helminth species and L. interrogans controlling for environmental factors and the demographics and body condition of rats. Final models were combined to build the interaction network. We trapped a total of 299 rats of which 71% and 39% were carriers of the human pathogens *Leptospira interrogans* and the nematode *Angiostrongylus cantonensis*, respectively. We identified 10 other helminths among rats; the most prevalent were *Strongyloides* sp. (97%) and *Nippostrongylus brasiliensis* (41%), of which the latter was present at higher intensity with higher intensities of both Strongyloides sp. and *L. interrogans*. The intensity of *A. cantonensis* was also higher when rats had higher intensities of *Strongyloides* sp.. However no influence of *L. interrogans* was found. Moreover, the prevalence of *A. cantonensis* was negatively associated with co-parasitism with *N. brasiliensis* and *Heterakis spumosa.* Our findings confirm that rats carry zoonotic pathogens, such as *A. cantonensis* (the causal agent of eosinophilic meningitis in humans), which are under-recognized and expanding causes of human disease in urban slum settings. Furthermore, coinfection with parasites may significantly modify carriage of human pathogens in rats and contribute to variation in the transmission risk between regions.

533

FIELD OBSERVATIONAL STUDY EVALUATING THE SPILLOVER OF ANTIBIOTIC-RESISTANT BACTERIA BETWEEN DIFFERENT VARIETIES OF CHICKENS AND RURAL IN NORTHERN ECUADOR

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The twentieth century industrialization of animal husbandry has resulted in dramatic increases in animal production. It has also led to departures from traditional agricultural techniques including the pervasive and widespread administration of antimicrobial agents. Antibiotics are administered in animal production facilities for both prophylactic and therapeutic purposes. Over-exposure of growth promotion antibiotics has resulted in the worldwide emergence of antibiotic-resistant bacterial strains in most species of livestock and poultry. Such strains are increasingly spreading from large animal production facilities into human populations, which presents a pressing public health concern. Both physicians and veterinarians are faced with a declining supply of effective antibiotics. In Ecuador, small scale farming operations in rural communities often prescribes high amounts of antibiotics for poultry raised for meat consumption known as "production chickens" (e.g. broilers). In contrast, free-ranging local varieties of household chickens receive almost no antibiotics and are important as potential bridge hosts introducing antibiotic-resistant strains from production chickens to humans. Through a cross-sectional village-scale approach, we analyze the relationships between antibiotic-resistant prevalence between household and production chickens along with a subset of humans living near household chickens. For each household, we evaluate the prevalence of antibiotic-resistant *Escherichia coli* in household chickens and the spatial relationship to the nearest production chicken coop. This investigation is relevant to many other tropical countries where development agencies commonly introduce production chickens as means of supporting micro-development. There is a pressing concern to better understand how antibiotic-resistant bacteria spread from conventional to non-conventional animal breeds and these impacts on surrounding human populations.

534

EXAMINING RATS IN THE MARSHALL ISLANDS AS A POSSIBLE ANIMAL RESERVOIR FOR *MYCOBACTERIUM TUBERCULOSIS* AND ATYPICAL MYCOBACTERIA

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The Marshall Islands have some of the highest rates in the world for both Mycobacterium tuberculosis and Atypical mycobacteria infection. The Marshall Islands and specifically Majuro General Hospital, in the capital, have significant problems with rat infestations and rodent control. To investigate the possibility of an animal reservoir for these diverse groups of Mycobacteria a total of ten rats from two areas in the hospital were captured in live traps. Five rats were captured in and around the nurses station of the intensive care unit. The other five rats were captured in the storage area near the surgical ward. More rats could have been captured, however hospital staff sometimes turned the rats loose or tripped the traps closed during the night. All of the captured rats were drowned in fresh water the the surgical pathology department, washed and then autopsied for evidence of granulomas and lymphadenopathy. The heart and lungs were then harvested and saved in formalin containers. Histologic sections of the heart and lungs showed no evidence of granulomas and acid fast staining was negative for mycobacterial organisms. Three of the rats appeared well nourished, but ill. None of the rats had lymphadenopathy or grossly evident liver disease. However, nine out of ten of the rats showed evidence of chronic lymphocytic bronchitis in a pattern that would be significant in human patients. A control group of two rats was trapped. One from the mountains of New Mexico and one rat from the area of China town near the medical school. Both rats were processed in the same manner. These rats showed no evidence of bronchitis, although the rat from New Mexico did have several foci of coccidioidomycosis. This is only intended to be a preliminary study. It was not possible to culture the lungs for infectious agents and the urine and feces of the rats was not examined during this study. Some hospital staff were concerned that other animal reservoirs might be present such as feral dogs, cats and domesticated pigs. Further studies might include wild dogs or local pigs as well as larger numbers of rats analyzed with more sensitive technology such and the Genexpert and lung cultures.

535

MODELING ENVIRONMENTAL TRANSMISSION OF ZOONOSIS IN MULTISPECIES SYSTEMS

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Animals harbor important pathogens that impact both livestock and human health. In addition, the increased use of transmission models to study animal disease has highlighted the need to consider the host-pathogen interaction in the context of environmental and ecosystem drivers of infection transmission. We developed a novel use of disease transmission models to study the environmental transmission of zoonotic disease in multispecies herding systems characteristic of many pastoral economies in the emerging world. Specifically, we examine the role of diversity on pathogen persistence demonstrating that, depending on environmental factors and the mechanisms of host species interaction, diversity can have differing effects on the transmission dynamics. Our results extend prior work of the role of diversity in pathogen persistence by providing a mechanistic understanding of how environmental processes can impact modes of transmission. These models will not only help inform public health interventions but could be crucial for conservation decisions in areas characterized by livestock-wildlife interphases.

536

RISK FACTORS FOR ACUTE HUMAN BRUCELLOSIS IN NORTHERN TANZANIA

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Brucellosis is an important zoonotic cause of febrile illness with chronic sequelae, but little is known about transmission pathways in sub-Saharan Africa hampering the design of control programs. We conducted a prospective cohort study of acute human brucellosis in northern Tanzania. We enrolled pediatric and adult patients with fever from two referral

hospitals in Moshi, Tanzania. We administered a standardized risk factor questionnaire and performed *Brucella* microagglutination testing on acute and convalescent serum. Cases were patients who had either a four-fold rise in titer between paired serum samples or a single reciprocal titer ≥160. Controls had titers <20 in both serum samples. We calculated odds ratios (OR) for individual behaviors and combined behaviors to form exposure scales to livestock and livestock body fluids. Of 1,446 patients enrolled from February 2012 through September 2014, we identified 50 (3.5%) acute brucellosis cases and 512 controls. Remaining participants either had titers >20 - <160 or supplied only acute serum. Median (interquartile range) age of cases was 31 (23, 40) years, 33 (66.0%) of 50 were female, and 20 (43.5%) of 46 were from rural areas. On bivariate analysis, increasing age was associated with brucellosis (OR 1.1 per year, p<0.01). Birthing goats (OR 8.8, p=0.01) or livestock (OR 6.2, p=0.02); consuming raw livestock blood (OR 2.7, p=0.03); exposure to goat (OR 4.0, p<0.01) or pig blood (OR 3.7, p<0.01); feeding cattle (OR 3.9, p<0.01), goats (OR 2.8, p<0.01), or pigs (OR 7.0, p<0.01); and cleaning waste of cattle (OR 4.1, p<0.01) or pigs (OR 5.6, p<0.01) were associated with brucellosis. Brucellosis was associated with high levels of contact with livestock measured by the exposure scales (OR 3.2, p<0.05). No association was found between raw milk consumption and acute disease. We found that livestock contact was an important risk factor for brucellosis in northern Tanzania, and that risk varied both by type of livestock contact and by livestock species. Many of the risk factors identified are behaviors potentially modifiable by targeted interventions.

537

INFLUENZA A VIRUS AMONG PIG AND DUCK POPULATIONS IN RURAL BACKYARDS IN GUATEMALA, 2013-2014

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The human-animal interface may favor the emergence of novel influenza A virus (IAV) strains that might pose a threat to human and animal health. We conducted monthly cross-sectional surveys of backyard pigs and ducks from November 2013-October 2014 on the Pacific coast of Guatemala where IAV was isolated from migratory waterfowl. We collected nasal swabs (pigs), tracheal and cloacal swabs (ducks), and blood from both species. Households with each of the species were selected randomly based on an expected IAV prevalence of 10%. One of every two pigs and 1/7 ducks were selected by convenience within each household; animals could be re-tested in different months. We tested swabs for IAV RNA by rRT-PCR targeting the universal IAV matrix gene, and subtyped the matrix positive samples by rRT-PCR targeting the 2009 pandemic N1 gene. We tested sera to detect exposure to IAV using a commercial ELISA kit, and subtyped ELISA positive sera from ducks by HI against a H5N3 low pathogenic strain isolated from waterfowl. An average of 32 households with pigs and 46 households with ducks were sampled monthly. We collected a total of 669 samples from pigs and 1090 samples from ducks. We detected 7/669 (1%; CI 95%: 0.4-2) rRT-PCR positive pig samples; none tested positive for pandemic N1. Only 1/1090 (0.1%; CI 95%: 0.1-0.5) of tracheal swabs from ducks tested positive by rRT-PCR (cloacal results pending). We detected antibodies against IAV in 85/669 (13%; CI 95%: 10-16) of sera from pigs and 98/1090 (9%; CI 95%:7-11) from ducks. Among seropositive ducks, 1/98 (1%; CI 95%: 1-6) were reactive to the H5N3 virus. HI results may suggest exposure of ducks to IAV from wild waterfowl, but isolation and sequencing of IAV strains from positive swab samples to identify IAV viruses circulating in pig and duck populations is pending. We found low percentages of current IAV infections in these backyard animals. Serologic results are limited by potential re-testing of individuals and knowing the animal age, but suggest low exposure to IAV. Interspecies transmission of IAV and the implementation of an integrated surveillance of IAV at this setting, where multiple hosts interact, should be evaluated.

538

LEPTOSPIRA SEROPREVALENCE AND RISK FACTORS IN HEALTH CENTER PATIENTS IN HOIMA DISTRICT, WESTERN UGANDA

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The burden of human leptospirosis in Uganda is unknown, but is suspected to comprise a significant portion of undifferentiated febrile illnesses. The study objectives were to estimate the prevalence of *Leptospira* antibodies and risk factors for seropositivity in humans visiting two health centers in Hoima District, Uganda. 359 patients were recruited at the Kikuube and Kigorobya Health Center IV's during March and April 2014. Every non-pregnant adult over the age of 18 presenting to the health center, either as a patient or as a caregiver, was eligible. Interviews were conducted by clinical officers and a blood sample was taken by lab technicians. Exposure variables included demographics, exposure to animals, past medical history and domestic environment. Sera were tested by the Microscopic Agglutination Test (MAT) using eight Leptospira serovars from different serogroups. 126 study participants (35.0%, 95% CI 30.2-40.3%) were seropositive (MAT titer >= 100) against any serovar. The highest prevalence (19.8%, 95% CI 15.9-24.4%) was against *L. borgpetersenii* sv Nigeria (serogroup Pyrogenes) with 71 seropositive cases. The prevalence of probable leptospirosis (MAT titer >=800) was 1.9% (95% CI 0.9-4.2%) and was uniquely related to serovar Nigeria. Probable leptospirosis was statistically significantly associated with self-reported malaria in the past year (p=0.048). The few participants who reported having skinned cattle in the two weeks prior to their blood sample (n=6) had a relative risk of 2.6 (p=0.036) for seropositivity against any serovar. This is to our knowledge the first study of the prevalence of antibodies to *Leptospira* serovars in humans in Uganda. The seropositivity to leptospirosis and the specific seroprevalence of 20% against L. borgpetersenii sv Nigeria suggests high exposure in this population. Individuals participating in cattle skinning may also be at higher risk for exposure. Overall, leptospirosis may represent an underappreciated burden of illness in Hoima, Uganda.

539

IMPACT OF REV1 LIVESTOCK VACCINATION ON THE RISK OF HUMAN BRUCELLOSIS IN AZERBAIJAN

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Brucellosis is one the most common and widely spread zoonotic diseases in the world. Effective control of the disease is, in general, focused on reducing or eliminating brucellosis in livestock through vaccination. However, despite the availability of effective control measures, brucellosis continues to pose a public health risk in both developed and developing countries. The objective of our study was to evaluate the impact of Rev1 small ruminant brucellosis vaccination on the risk of human brucellosis in Azerbaijan. We used monthly time-series data on human brucellosis cases in Azerbaijan before and after a Rev1 small ruminant vaccination campaign

(1995-2013). We used an interrupted time-series framework to estimate the relative and absolute indirect effect of the livestock vaccination policy on human risk. Incident risk was calucated for age groups before and after vaccination. To identify spatial variations in temporal reporting trends, we used the scan statistic. Additionally, we mapped temporal trajectories of human brucellosis incidence, by administrative district, using a spatially lagged incidence rate in a segmented regression model. Our results showed a post vaccination decline in human brucellosis of ~1% per month. Overall, human brucellosis was reduced by 34% by 2013. Risk reduction was greatest in the 20-29 age group. Despite national declines in human brucellosis, we identified spatial changes in the case distribution characterized by a geographic expansion and an increasing incidence among districts clustered in the south-east, compared to a decrease elsewhere in the country. In conclusion, our findings support the human health benefits of livestock vaccination. However, we found spatial variation in the impact of the vaccination program that can be used to target future efforts. Our findings highlight the use of integrating spatial and quasi-experimental techniques to evaluate the progress of public health interventions.

540

CLIMATE CHANGE AND VECTOR-BORNE DISEASES: USE OF PARTICIPATORY EPIDEMIOLOGY TO INVESTIGATE EXPERIENCES IN VULNERABLE, CATTLE-KEEPING HOUSEHOLDS IN TANZANIA

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Climate change is predicted to increase incidence of vector-borne diseases (VBDs) in humans, however little is known about the impact on animal VBDs in vulnerable areas where livestock are a primary livelihood strategy. In the absence of historical data with which to examine the inter-relation between climate and disease, participatory epidemiological (PE) methods were used with Maasai pastoralists of Monduli district, Northern Tanzania to establish local observations on two major VBDs of cattle, namely East Coast Fever (ECF) and Animal Trypanasomiasis (AT). Data were collected between November 2014 and January 2015 in ten randomly selected villages of arid and semi-arid lands involving gender segregated groups (10 men groups and 9 women groups). Matrix scoring for both men and women groups confirmed that Maasai easily recognise these VBDs. ECF and AT ranked amongst the top five most important cattle diseases in the district with strong agreement between informant groups (Kendall's W = 0.399 for men and 0.451 for women; $p < 0.01$). All groups associated ECF with the wet season or directly after rainy season while AT was more variable throughout the year, with more cases reported in dry seasons. Likewise, different villages reported seasonal differences in occurrence of disease vectors (*Rhipicephalus appendiculutus* and Tsetse flies). Comparing 2014 to 1984, participant groups consistently reported declines in rainfall, vegetation cover and quality pasture, as well as increases in severe drought. Experiences with ECF/AT and vector abundance between these time periods was more variable across villages, and likely relates to changes in climate and animal management practices over the last 30 years. This baseline study is the first to document the inter-relation between climate and cattle diseases from the pastoralist perspective. Preliminary analyses reveal a complex interplay between human, animal and environmental factors, understanding of which is urgently required to devise approaches to mitigate effects of climate change in vulnerable areas.

541

ONE HEALTH WORLDWIDE: EMERGING INFECTIOUS DISEASES, GLOBAL HEALTH VETERINARIANS, AND ZOONOTIC SURVEILLANCE

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What do zoos have to do with Global Health? How are veterinarians engaging in pandemic prevention? Do non-zoonotic veterinary pathogens impact human health? Given that most zoonotic pathogens are from wildlife, surveillance of pathogens in wildlife is garnering more attention from domestic and international health organizations. The National Zoological Park's Smithsonian Global Health Program (SGHP) is a team of wildlife veterinarians, veterinary pathologists, biologists, physicians, and other public health professionals tasked with investigating and combatting emerging diseases worldwide. One Health ventures that SGHP is invested in include: human-animal interface surveillance (through USAID|PREDICT), xenosurveillance for MERS coronavirus and Zika virus, and emerging ulcerative dermatitides in African megafauna. SGHP engages in international collaboration and capacity building of diagnostic and public health infrastructure in developing tropical countries. Veterinarians form an integral part of this corps of experts, and their unique and often overlooked understanding of the interactions between humans and wildlife make them an essential presence on the front lines of emerging zoonoses detection and prevention. Although much has been devoted to understanding the human consequences of well-known zoonotic outbreaks, such as the Ebola virus, very little focus has been placed on poorly studied zoonoses or non-zoonotic wildlife diseases and the potential risk they present to human health. This has led to the emergence of serious threats from previously "benign" diseases such as Zika virus. There is even evidence that non-zoonotic animal pathogens may play a role in the severity and transmissibility of zoonotic diseases, as when the competency of certain arbovirus vectors increases as a result of co-infection with animal-borne filariasis. It is the mission of multidisciplinary One Health groups such as SGHP to tackle these challenges from all angles and combat pandemic threats. Collaboration with other health professionals is a vital component of ongoing integrated strategies in improving health outcomes globally.

542

SEROPREVALENCE AND THE RISK FACTORS ASSOCIATED WITH TOXOPLASMOSIS IN WOMEN RECEIVED ANTENATAL CONSULTATION (ANC) AND DOMESTIC CARNIVORES IN DAKAR

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Toxoplasmosis is a cosmopolitan zoonosis caused by *Toxoplasma gondii* manifested by fetal loss mainly in sheep and humans. The identified definitive host is the cat while all domestic mammals such as wild birds and humans are intermediate hosts. Disease sporadic pace, its modes of transmission are manifold. The object of this study was to evaluate the seroprevalence of toxoplasmosis and the risk factors in pregnant women and domestic carnivores in Dakar. For this, blood samples of 100 pregnant women, 141 cats and 120 dogs were collected and sera were analyzed. Regarding the acquisition of risk factors, a questionnaire was associated with each sample. Two agglutination series tests showed that the women surveyed were infested at 50 ± 9.8% for toxoplasmosis. Multivariate analysis showed that 43% of these women have had an abortion and of those, 53% were positive for toxoplasmosis. After analysis of risk factors, professional status and milk consumption predispose women to be contaminated ($p < 0.05$ and odds ratio> 1) with toxoplasmosis. For carnivores, the agglutination test shows that the infestation is higher for

definitive hosts (cats 55.37 ± 9%) compared to dogs (43.97 ± 8%). These high prevalence confirms that this parasite is prevalent in an endemic form in the region especially with the close cohabitation between women and cats. They must motivate the strengthening and systematization of screening tests during pregnancy.

543

UPDATE Q FEVER STATUS FROM THE RUMINANT PLACENTAS, THAILAND 2014-2015

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Q fever, caused by *Coxiella burnetii* is a zoonotic disease, found worldwide. The ruminants such as cows, buffalo, goats, and sheep are reservoirs. The most common clinical sign in livestock is abortion, but infected adult animals almost never show clinical signs. The main transmission route is by inhalation in humans, and possibly animals as well. As ruminant placenta is occasionally sold in wet markets in Thailand for human consumption, at-risk people include the farmers, veterinarians, animal husbandry workers, placenta-merchants, and cooks, and potentially the general public. Human Q Fever cases were reported in Thailand. We aimed to know the Q fever status in ruminants in Thailand. Approximately 300 samples of the cotyledon part of ruminant placenta were collected by convenience sampling from each region. The samples were grossly normal, though some were retained placentas. The samples were extracted for DNA and tested with the real-time polymerase chain reaction (PCR) technique, which targets the IS1111. The tests were run in the Thai governmental veterinary laboratory system, comprised of the National Institute of Animal Health (the central region), and seven Veterinary Research and Development Centers (regional labs). The positive percentage of results, by region in Thailand was 63.84 (203/318) in the east, 50.9 (170/334) in the upper north, 45.62 (151/331) in the west, 38.82 (125/322) in the central, 24.77 (81/327) in the lower north-east, 19.73 (59/299) in the upper north-east, 8.46 (28/331) in the lower north, and 7.51 (16/213) in the south region. The total percentage for the whole country was 32.46 (833/2475). Q fever occurred in ruminants in Thailand without showing abortions, as occasionally seen in other countries. Good sanitation such as disinfection, wearing personal protective equipment, including gloves, masks, and boots, especially during the parturition time, and routinely cleaning the animal house should be done by the farmers, and veterinarians. The placenta-merchants and cooks should wear gloves when handling raw placenta and thoroughly cook placenta if served for human consumption.

544

DOCUMENTING THE SEROPREVALENCE OF ZOONOTIC AND VECTOR BORNE DISEASES IN RURAL NICARAGUA

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Rural communities, particularly in low and middle income nations, are at high risk of zoonotic and vector borne diseases. The rural poor in these parts of the world are disproportionately affected, but the extent of which is largely undescribed, in part due to limitations on surveillance capacity in resource constrained settings. In Nicaragua, the tropical climate is particularly conducive to transmission of mosquito borne viruses, such as Dengue, Chikungunya, and Zika, and frequent close contact with domestic and other animals in rural communities facilitates transmission of zoonotic pathogens. In order to understand the extent to which infections like these are circulating within the region, we undertook a cross-sectional study to estimate the seroprevalence of infectious diseases among agricultural workers in the Pacific lowlands of Nicaragua. Workers of a large sugar estate underwent routine, annual occupational health screenings from October 2015-June 2016. Age and sex of workers were available. From June-October 2015, blood was also collected from domestic animals in the same region. Serum from humans and animals were available for testing and were subjected to ELISA assay for detection of antibodies to various pathogens (ELISA for IgM or IgG and and MAT for *Leptospira* reactivity). Workers were mostly male (89%) and young (median age 29yrs). In humans, we detected a high prevalence of antibodies to *Leptospira* (33%) and also detected antibodies to hantavirus (6%) and *Trypanosoma cruzi* (0.5%). Dogs had evidence of leptospirosis (12%) and *T. cruzi* (5%). Testing for seroprevalence of other infections, including Dengue, Chikungunya, West Nile, and Zika are ongoing. Our data suggests that zoonotic and vector borne pathogens are likely established and endemic, provides evidence that hantavirus may be a threat, and documents the presence of Chagas disease in this region of Nicaragua. This new data can inform public health and veterinary practices and suggest areas for targeted surveillance and intervention strategies.

545

SURVEILLANCE OF AMOEBIC KERATITIS-CAUSING *ACANTHAMOEBAE* FOR BACTERIAL ENDOSYMBIONTS

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Acanthamoebae are causative pathogens of several infections including amoebic keratitis (AK), a potentially blinding eye infection. Acanthamoebae isolated from the environment and from corneas of patients with AK are known to harbor bacterial endosymbionts belonging to chlamydiales, rickettsiales, or legionellales. Acanthamoebae harboring Chlamydia-like endosymbionts have demonstrated enhanced production of cytopathic effect on fibroblast monolayers, suggesting that endosymbionts may increase virulence. We sought to illuminate the potential bacterial endosymbionts present in clinical isolates of acanthamoebae identified at our reference parasitology laboratory. Isolates of *Acanthamoeba* spp. obtained from our biobank of surplus, anonymized, corneal scrapings from 2012-2015 were screened for endosymbionts by PCR. Isolated DNA was amplified in PCR reactions with 3 separate primer pairs,detecting bacteria belonging to orders chlamydiales, rickettsiales, or legionellales. 3 primer pairs specific to the 18s rRNA gene of *Acanthamoeba* spp. were used for amplification of *Acanthamoeba* DNA. Sanger sequencing of PCR products was performed, followed by BLAST analysis for sequence homology and species identification. We screened

27 clinical isolates of *Acanthamoeba* spp. for organisms known to act as endosymbionts as described above. Five strains of *Acanthamoeba* (19%) from corneal scrapings were found to contain bacterial DNA belonging to legionellales (2 *A. polyphaga* (40%), others not speciated). 3 of the clinical isolates (11%) contained members of the rickettsiales (2 *A.castellani* (66%), 1 A. palestinensis (33%)). One strain (4%, not speciated) contained a member of the chlamydiales, and sequencing revealed this to be *Neochlamydia hartmannellae*. The remaining isolates of *Acanthamoeba* may contain other endosymbionts undetectable by our assays. Organisms known to act as bacterial endosymbionts are prevalent in corneal scrapings containing AK-causing *Acanthamoeba*. Whether these potential endosymbionts contribute to virulence is unknown, but offers a potential avenue for investigation of novel therapeutics in AK.

546

OPTIMIZED METHODS OF GENOMIC DNA EXTRACTION AND MYCOPLASMA DECONTAMINATION FROM DIFFERENT SPECIES OF PATHOGENIC AMOEBAE

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Free-living amoebae (FLA) are generally regarded as innocuous soil organisms; however, a handful of species are capable of causing human disease. *Naegleria fowleri*, *Acanthamoeba* spp. and *Balamuthia mandrillaris* are recognized as etiologic agents of amoebic encephalitis. Acanthamoeba are also responsible for the sight-threatening infection amoebic keratitis, particularly among contact lens wearers. A rate-limiting step in genomic analyses of pathogenic FLA is DNA extraction. Many commercially available DNA isolation kits are useful for PCR but fail to provide high yields of pure genomic DNA for molecular studies. We examined different in-house methods for the capacity to extract FLA DNA. We found that an optimized phenol-chloroform-based purification method was applicable to different species of amoebae when compared to a commercial kit. While similar to other standard methods of extraction, this modified procedure resulted in DNA of high yield and quality as it likely takes into consideration the high amounts of carbohydrates and nucleases found in amoebic cells. Mycoplasma contamination is also of great concern in cultures of pathogenic amoebae, especially in strains propagated in mammalian cell lines. We examined different methods of decontamination of Balamuthia cultures harboring mycoplasma that were resistant to commonly used antibiotics. The methods were based on the rapid treatment of amoebic cysts with non-amoebicidal levels of detergents and/or acids to eliminate the contaminating bacteria as determined by PCR. Our non-antibiotic-based approach was effective in curing cultures of Balamuthia from mycoplasma without affecting viability of the amoebae. Taken together, the use of reliable methods of DNA extraction combined with simple and economic procedures of bacterial decontamination offers an effective approach to produce genomic DNA from pathogenic amoebae on a large scale for a range of molecular genetic analyses.

547

DIAGNOSTIC ACCURACY OF AMEBIC COLITIS BY COLONOSCOPY

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Amebiasis, caused by *Entamoeba histolytica*, is rapidly increasing as a sexually transmitted infection in Japan. It has been also reported that asymptomatic amoebic colitis incidentally diagnosed by colonoscopy recently increased. Although etiologic diagnosis of intestinal ulcer mostly relies on histological examination of biopsy specimen, it is still unclear whether the method has enough sensitivity to rule out amoebic colitis. We collected samples from suspected cases of amoebic colitis by colonoscopy. Microscopic examination, culture and PCR of aspirated fluid, as well as histological examination of biopsy specimen, were performed. Diagnosis of amoebic colitis was made when either test was positive. Of the 23 suspected cases, amoebic colitis was diagnosed in 13 cases (57%). The proportions of co-infection with HIV, HBV, HCV, and syphilis were 54%, 54%, 8% and 39%, respectively. At the colonoscopy, 10 patients (77%) had clinical symptoms, including diarrhea (62%), abdominal pain (23%), bloody stool (15%), fever (15%), and nausea/vomiting (15%), whereas 3 (23%) were completely asymptomatic. One case accompanied amoebic liver abscess. The sensitivities of microscopy, culture, PCR and histopathology were 69%, 23%, 67% and 46%, respectively. The proportion of cases with abdominal symptom was statistically higher in histological negative than that in histological positive (p 0.04). Three clinical isolates, including 2 isolates from asymptomatic cases, were stably passaged as a xenic culture. Either diagnostic tool including histological examination used in this study doesn't have enough sensitivity (23-69%) for the diagnosis of amoebic colitis by colonoscopy, indicating that combination of multiple methods be needed for the accurate diagnosis. Also, we established 3 clinical isolates from aspirated fluids of the lesions. We will perform genetic and phenotypic analyses of these clinical isolates in order to elucidate the pathogenesis of amoebic colitis, especially asymptomatic chronic infection.

548

GUT MICROBIOME CHANGE PRIOR TO THE ONSET OF AMEBIASIS

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Amebiasis is one of the causes of severe diarrhea in young infants <5 years of age in low and middle income countries. In our previous work we observed that the composition of the microbiota was markedly different in ≤ 2 year old children experiencing amebic diarrhea compared to those asymptomatically colonized. In particular, a high level of the pathobiont *Prevotella copri* was associated with symptomatic amebiasis. We postulated that either gut dysbiosis and the domination of the microbiota by *P. copri* increased the probability that an *Entamoeba histolytica* infection would be symptomatic or the resistance of *P. copri* to host-derived reactive oxygen allowed it expand after the amebic parasite caused gut inflammation and diarrhea. To distinguish between these two possibilities, we examined the 16S microbiome profile in 16 surveillance specimens collected and stored in the study biobank 89 ± 77 days prior to the occurrence of *E. histolytica* positive diarrhea. In our preliminary analysis we have observed a stronger *P. copri* signal in the samples preceding disease. This seems to indicate that a preexisting *P. copri* expansion dispose children to symptomatic disease and the previous result was not a consequence of *E. histolytica* infection. We also observed that even although *P. copri* was higher in diarrheal cases than the amount detected in *E. histolytica* positive surveillance samples the onset of diarrhea actually led to an expansion of the *Enterobacteria* at the expense of both *P. copri* and *Bifidobacteriales* species which dominate the pre- diarrhea microbiome in these children. The Shannon diversity index was as expected lower in pre-diarrheal and diarrheal samples compared to samples from 2-year old controls p<0.05. Additional studies are planned to determine the significance of these results.

549

GENOMIC AND TRANSCRIPTOMIC COMPARISON OF CLINICAL AND NON-CLINICAL STRAINS OF "BRAIN-EATING" AMEBA *NAEGLERIA FOWLERI*

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The "brain-eating" free-living ameba, *Naegleria fowleri*, causes rare, but severe brain infection, known as primary amebic meningoencephalitis (PAM) that is almost always fatal. The ameba is found globally in warm fresh waters, hot springs, and waterparks. Recently, *N. fowleri* has been found to colonize public tap water systems in US linking the death of a young child. This coincides with an observed geographical spread in PAM cases to the colder Northern States. A major issue affecting our ability to investigate and prevent PAM infections is the ubiquitous nature of this pathogen. It is unclear why PAM cases are so rare while people are likely to be routinely exposed to *N. fowleri*. Variability in the ameba virulence may explain this. However, a genotyping system capable of categorizing *N. fowleri* strains based on their virulence does not exist. The existing genotyping categorizes the US strains of *N. fowleri* into only three broad genotypes. Thus, this genotyping is also not ideal for linking exposures with cases. Here, we performed Illumina Hiseq to do whole genome sequencing of 38 *N. fowleri* strains (30 from clinical cases and 8 from environmental samples) representing all three genotypes. We also performed RNAseq in a subset of *N. fowleri* strains to identify gene expression differences between the clinical and environmental strains. The *de novo* assembly of mitochondrial genome shows that it is ~49.5 KB in size. Preliminary analysis identifies SNPs in the mitochondrial genomes, some of which are genotype-specific and some are unique ("private") to a particular strain. The private SNPs can be utilized to develop a more discriminatory genotyping system, which will allow source tracking in clinical cases, and conduct molecular epidemiological surveys. The *de novo* assembly of nuclear genome of *N. fowleri* shows that it is ~27.5 MB in size, and contained in <600 contigs with a G-C content of ~34%. The only reference genome of *N. fowleri* available publicly is dispersed in >1100 contigs suggesting a superiority of our assembly. Analysis of nuclear genome and transcriptomic data is currently ongoing. Key findings will be presented in the ASTMH Meeting.

550

IMPACT OF NOVEL *ENTAMOEBA* SPECIES ON DIARRHEAL INFECTIONS IN SOUTH AFRICA

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South Africa has been significantly impacted by diarrheal infections. The purpose of our study is to identify the etiology, including novel species and burden of parasitic diarrheal disease in South Africa. We collected diarrheal and non- diarrheal stool samples from the rural and urban communities of South Africa. DNA was extracted and a diagnostic taqman qPCR assay capable of identifying protozoan parasites (*Entamoeba histolytica*, *Cryptosporidium* and *Giardia*) was performed. In both locations approximately half of the samples were qPCR positive: 105 (49%) from rural areas; *Cryptosporidium* (24%) *E. histolytica* (3%) and *Giardia* (22%) and 113 (42%) from the urban areas; *Cryptosporidium* (11%), *E. histolytica* (9%) and *Giardia* (22%). We further identified *Entamoeba* species using a new assay with increased sensitivity. Our main finding was the presence of *E. bangladeshi* in eight different stool samples. This was an interesting finding as *E. bangladeshi* has not been previously reported outside Bangladesh. Consistent with previous findings, *E. moshkovskii* was not found in these populations and as predicted *E. histolytica* and, non-pathogenic *E. dispar* positive samples were also identified. Novel *Entamoeba spp* have been identified in different endemic regions of the world but have not been studied in S. African populations. We are currently performing additional analysis on the unspeciated *Entamoeba* positive samples as a part of a systematic approach to identify novel clinically relevant species. Our preliminary results suggest that novel *Entamoeba* species may potentially exist in the South African population.

551

WHOLE GENOME SEQUENCING OF *CYCLOSPORA CAYETANENSIS* OOCYSTS PURIFIED FROM HUMAN STOOL SAMPLES

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Cyclospora cayetanensis is a coccidian parasite that causes cyclosporiasis, an intestinal infection characterized by acute diarrhea in humans. Since the 1990s, CDC and local public health departments have conducted numerous investigations of cyclosporiasis outbreaks. These investigations are currently hampered by the lack of molecular epidemiological tools. In order to improve outbreak response, we aim to develop a subtyping method that would allow linkage of cases to clusters and to implicated food sources. Attempts to propagate *Cyclospora in vitro* and *in vivo* have so far been unsuccessful, so stool samples from infected humans are the only available source for this parasite. Here, we describe the optimization of methods to purify *Cyclospora* oocysts from stool, extract genomic DNA and prepare next generation sequencing libraries from ultra-low quantities of DNA. Using these methods, genomic sequences from 10 geographically distinct *C. cayetanensis* samples were obtained by Illumina sequencing. The genome is approximately 44.5 Mbp with a GC content of 51.9%. Preliminary genomic comparisons indicated an overall low intra-species genomic variation. More detailed sequence comparisons are now in progress to develop SNP profiling of annotated regions of the genomes for epidemiological purposes, and to identify subtyping markers that can assist in future outbreak investigations. A public Bioproject database in NCBI is being created to host whole genome assemblies as part of this work.

552

GENOME-WIDE SEARCH TO IDENTIFY IMMUNODOMINANT *BABESIA MICROTI* ANTIGENS FOR DIAGNOSTICS AND VACCINATION

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Babesiosis, caused by intraerythrocytic protozoan of the genus Babesia is transmitted by Ixodid ticks but also through blood transfusion. The highest prevalence of both tick and transfusion-transmitted infection occurs in the United States where *Babesia microti* infection is endemic in the Northeast and upper Midwest. Clinical manifestations range from asymptomatic infection to fulminant disease that could be fatal. There is no FDA-licensed vaccine to ameliorate parasite burden and clinical disease and no laboratory test for diagnosis of acute infection or for screening of blood donors. In spite of the recent availability of the full genome sequence of *B. microti*, there is a scarcity of well-defined, immunodominant *B. microti* antigens for development of diagnostic assays for blood donor screening or for vaccine efficacy studies. By applying a combination of genomics data mining and screening of whole-genome-fragment-phage-display libraries expressing the open reading frames of *B. microti* on immune sera from *B. microti* infected patients, we have identified over 50 immuno-dominant antigens of unknown biological function. Twenty-four of the highest reactivity *B. microti* antigens have been produced as recombinant protein in *E. coli*. Of these, 12 antigens displayed a strong EIA reactivity with sera

from *B. microti* infected individuals from endemic areas in Connecticut suggesting their potential as diagnostic antigens. Bioinformatics analyses and studies on their biochemical and cellular characterization as well as their value as diagnostic vaccine antigens are in progress.

553

GAMMA IRRADIATED SOLUBLE EXTRACTS OF *TOXOPLASMA GONDII* TACHYZOITES INDUCED BETTER HUMORAL AND CELLULAR IMMUNE RESPONSE DUE TO PREFERENTIAL UPTAKE BY APCS SCAVENGER RECEPTORS

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Toxoplasmosis occurs in one-third of the adult world population, without adequate vaccines and causing disease in fetus or specific groups. Aside to sterilizing effect, gamma radiation acts on antigens inducing enhanced antisera production against snake venoms or cell and humoral response to recombinant leprosy proteins. Gamma radiation affects proteins directly or indirectly in water by action of oxidant radicals from water radiolysis. Early reports showed gamma irradiated crotoxin had enhanced uptake by macrophages, limited by scavenger receptors competitors, as probucol. Irradiated tachyzoites induced adequate immune response with protection, attributed to mitotic death and DNA damage. Irradiated proteins could take a part in this process and we study the immune response induced by gamma irradiated soluble extracts of *Toxoplasma gondii* tachyzoites, using mice immunized with native proteins as controls. Mice immunized with irradiated extracts without adjuvants showed significant protection after challenge with ME-49 ($p<0.05$) and RH ($p<0.0001$) strains compared to controls. There are increased specific and high avidity IgG production ($p<0.05$) when compare d to controls group. By flow cytometry and *in vitro* culture, spleens of mice immunized irradiated extract presented increased proliferation of CD4+, CD8+ and B cells and IFN-γ production as compared to controls. J774 cells had increased uptake of biotinylated irradiated extracts as compared to the uptake of native extract ($p<0.05$), due to longer and continuous uptake. All these data points to an alternative and effective uptake and immune processing of irradiated *T. gondii* extracts, probably due to specific receptor of oxidized proteins as scavenger receptors, resulting in enhanced immunity. This data also implies that irradiated proteins could be involved in the protection induced by irradiated parasites. Use of antigen gamma radiation can be a simple process to enhance vaccine efficiency, avoiding the use of adjuvants.

554

PHYLOGENETIC ANALYSIS OF *BLASTOCYSTIS* SPP. ISOLATES IN CLINICAL STOOL SAMPLES FROM BRAZIL

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Blastocystis spp. is an organism described as enteroparasite protozoan, commonly found in stool samples from humans. Several subtypes have been described in humans, but pathogenic potential and aspects epidemiological are still controversial. The aim of the present study was to investigate *Blastocystis* subtypes (STs) from patients of Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (HC/FMUSP), Brazil. *Blastocystis* spp. positive stool samples diagnosed in Section of Parasitology of Central Laboratory (HC-FMUSP) were used for DNA isolation. Polymerase chain reaction (PCR) was performed using specific primers targeting the small subunit of rRNA gene. Direct DNA sequencing of PCR products was performed, and the DNA sequences were aligned and compared to other sequences present in GenBank database. Phylogenetic analysis was inferred using the Neighbor-Joining method by MEGA6 software. Additionally, *Blastocystis* STs were identified by determining the exact match or closest similarity against all known *Blastocystis* STs using www.pubmlst.org/blastocystis. Four STs were identified: ST1 (16.0%), ST2 (8.0%), ST3 (68.0%) and ST6 (8.0%). Allele nos. 34 and 36 were the most frequent haplotypes. The present study is one of the few that generates STs data from human population in Brazil, confirming the absence of ST4. Another important finding is the presence of ST6, rarely detected in human isolates. Subtype prevalence involving human samples may contribute to the monitoring of infection transmission of *Blastocystis* spp in endemic areas, and in future, explain any pathogenic aspects related to distinct subtypes.

555

DISTRIBUTION AND HUMAN-INFECTIVE POTENTIAL OF *CRYPTOSPORIDIUM*, *GIARDIA DUODENALIS* AND *ENTEROCYTOZOON BIENEUSI* GENOTYPES IN STORM OVERFLOW AND WASTEWATER IN SHANGHAI, CHINA

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Few data are available on the distributions of *Cryptosporidium*, *Giardia duodenalis*, and *Enterocytozoon bieneusi* genotypes and subtypes in storm overflow from urban areas. In the present study, 40 overflow samples were collected from two pump stations during July-September in 2012 and 2014 in Shanghai, China, with 40 raw wastewater samples from the same stations as controls. They were analyzed by using PCR for *Cryptosporidium* spp. (targeting the small subunit rRNA gene), *G. duodenalis* (targeting the triosphosphate isomerase, β-giardin, and glutamate dehydrogenase genes), and *E. bieneusi* (targeting the ribosomal internal transcribed spacer). Genotypes of these pathogens were identified by sequence analysis of PCR products. Samples that contained *C. hominis*, *C. parvum*, *C. viatorum*, *C. ubiquitum*, and *C. meleagridis* were further subtyped by sequence analyses of the 60-kDa glycoprotein gene. *C. hominis*, *C. parvum*, *C. ubiquitum* and *C. viatorum* were the dominant *Cryptosporidium* species. *C. baileyi*, *C. muris*, and *C. meleagridis* were also found in both wastewater and overflow samples. The *C. hominis* and *C. parvum* subtypes were common ones previously found in humans in China, but *C. ubiquitum* belonged to two novel subtype families and *C. viatorum* was found in China for the first time. There were eight Group 1 *E. bieneusi* genotypes in wastewater and storm overflow with genotype D as the dominant one. For *G. duodenalis*, subassemblage AII was the dominant genotype in these samples. There were no significant differences in the distribution of *Cryptosporidium* species and *E. bieneusi* and *G. duodenalis* genotypes between wastewater and overflow samples. These results reaffirm that storm overflow is potentially a significant contamination source of human pathogens in surface water and more attention should be paid to its roles in environmental transport of waterborne pathogens.

556

DETECTION OF *CYCLOSPORA CAYETANENSIS* IN FOOD AND CLINICAL SAMPLES USING A GELIFIED REAL-TIME PCR AS SAY

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Cyclospora cayetanensis is a coccidian parasite associated with numerous foodborne outbreaks. Current diagnostic detection of *C. cayetanensis* relies on microscopy, albeit this technique does not identify the parasite to the species level. PCR methods in general are more complex than microscopy and most protocols require multiple steps to set up reactions. The gelified PCR technology allows the development of ready-to-use PCR assays that minimize labor and quality issues. By using a pre-mixed, pre-loaded and gelified reagents the execution of PCR tests is reduced to a few very simple steps; i.e., (i) addition of DNA template to pre-mixed, pre-aliquoted gelified reagents, (ii) mixing of content, and (iii) centrifugation and placement into the real-time PCR thermal cycler for execution of PCR run. We developed a streamlined real-time gelified PCR method to detect *C. cayetanensis* to the species level in foods and clinical samples. The assay was designed with an internal amplification quality control to monitor amplification, ensure adequate quality of the DNA preparations, and troubleshoot technical glitches. The evaluation of the method in food samples were performed with cilantro spiked with different concentrations of *C. cayetanensis* oocysts. Preliminary results indicated that this assay can detect approximately 10 oocysts of *C. cayetanensis* seeded in 25g of cilantro. The sensitivity and specificity of the technique was evaluated using a total of 38 human stool specimens, of which 34 were microscopically positive for *Cyclospora* sp, collected during outbreak investigations in the U.S. No false-positive or false negative results were obtained. The gelified assay was also evaluated regarding its stability at different temperatures, including room temperature, and 30°C. The results from these experiments revealed that the assay may be stable for up to 2 months when pre-loaded vessels with the gelified qPCR mix were stored at 30°C and 4 months at room temperature.

557

AMIXICILE: A POTENTIAL ALTERNATIVE TREATMENT FOR TRICHOMONIASIS

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Trichomoniasis is a sexually transmitted infection in humans caused by *Trichomonas vaginalis*. This parasitic infection is the leading causative agent of vaginitis in women and urethritis in men worldwide. Currently, metronidazole is the most common drug therapy for this infection, but resistance is becoming increasingly prevalent. Amixicile is a novel inhibitor of pyruvate:ferrodoxin oxidoreductase in anaerobic parasites. This study compared the efficacy of a newly synthesized drug, amixicile, to that of both metronidazole and the synthetic precursor of amixicile, nitazoxanide, in inhibiting or killing *T. vaginalis in vitro*. One standard strain from ATCC and six unidentified patient-isolated strains of the *T. vaginalis* parasite were included in the study. The three drug treatments were compared, each at concentrations varying from 1.56 - 200µM. Under anaerobic conditions *in vitro*, the minimum inhibitory concentrations were determined for each of the three drugs against the seven *T. vaginalis* strains tested. The MIC for metronidazole, nitazoxanide, and amixicile were approximately 12.5µM, 100µM, and 6.25µM, respectively. To determine whether the inhibitory effect was reversible, antibiotic-exposed isolates were again placed in fresh culture media and incubated for 48 hours. Amixicile appeared to have the greatest efficacy in clearing the parasite at low drug concentrations. These sensitivity tests show amixicile's potential in serving as an alternative to metronidazole in the treatment of the *T. vaginalis* infection in humans. Further testing is required to confirm the effect of amixicile in more clinical isolates and *in vivo*.

558

DRUG RESISTANCE IN *BABESIA* PARASITES THAT INFECT HUMANS

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In our previous work screening compounds against *Babesia in vitro*, we found that atovaquone was the most potent available agent against this parasite of growing significance in human health. We then studied atovaquone resistance in the parasite *in vitro* using continuous drug pressure on *B. divergens* cultures. In our mixed culture base pair changes resulted in M64V/I amino acid changes. These amino acid changes were associated with an increase in IC50 to atovaquone. Sequence analysis of the cytochrome b gene revealed that these mutations are likely in the Qo region and correlate with the M133V mutation described in *Plasmodium*. We then compared these *in vitro* results to those from patients with smear positivity for *B. microti* over several transmission seasons. In most samples we found a wild type cytochrome b gene. In one patient with documented chronic disease we identified a base pair change leading to an amino acid change adjacent to the conserved PEWY region, a Y202C amino acid change that correlated with the described Y268C mutation seen in *Plasmodium*. As the number of immunosuppressed patients that are at risk of *B. microti* infection increases, there is concern for worsening treatment failures. We propose that more formal studies of partner drugs and novel agents should be done given the current limited options to treat *B. microti* infections.

559

A RAPID MOLECULAR TEST TO DIAGNOSE *TOXOPLASMA GONDII* IN MICE AND HUMAN SAMPLES

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Toxoplasmosis is a parasitic disease caused by *Toxoplasma gondii*. This infection is prevalent in humans and animals worldwide. It has been estimated that one-third of the world population has been exposed to this parasite. It is acquired by ingesting tissue cysts from undercooked or raw meat, consuming food or drink water contaminated with oocysts shed by felids, or by accidentally ingesting oocysts from the environment. Although the course of the primary infection is usually subclinical and the vast majority of infected human populations remain asymptomatic, the infection can cause significant morbidity and mortality in certain groups. The symptoms include encephalitis, chorioretinitis, congenital infection and neonatal mortality. Current diagnosis is based on detection of Toxoplasma-specific IgM and IgG. In this study, we established and optimized a diagnostic test using Recombinase Polymerase Amplification (RPA) assay for the molecular diagnosis of *T. gondii* in Lima. . The RPA assay does not require a thermocycler or other specialized equipment and can be adapted to lateral flow detection, so it may be performed on the field. For identification and amplification by RPA, we selected a gene fragment within a gene (B1) that was conserved across *T. gondii* strains. The limit of the RPA assays that we performed has a sensibility of detection

of 0.5 parasites (about 50 fg of DNA), while the conventional PCR detects about 1 parasite (100 fg of DNA). We also analyzed eight samples (all of them were given a positive diagnosis for Toxoplasmosis), five were positive by RPA and only three were positive by conventional PCR. The specificity was 100 % and did not detect other parasites like *Trypanosoma cruzi, Cryptosporidium, Cyclospora, Leishmania* and *Plasmodium*, Therefore, we observe that this assay can be applied to human samples for the diagnosis of the infection. The sensitivity of our methodology is 63% (5/8) and that of conventional PCR is 38% (3/8), The RPA assay can be further improved by combining it with lateral flow test. These results are very encouraging and suggest that RPA could be used as a novel molecular diagnosis technique for *T. gondii* infection.

560

IMMUNOBLOT FOR DIFFERENTIATION BETWEEN ACUTE AND CHRONIC INFECTION OF *TOXOPLASMA GONDII* USING A MURINE MODEL

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Toxoplasmosis is caused by the obligate intracellular parasitic protozoan, *Toxoplasma gondii*. This infectious agent is generally foodborne and enters the host through ingestion of raw or undercooked meat derived from infected animals. Usually the acute toxoplasmosis occurs in immunosuppressed patients, while most of the people remain in the chronic phase without symptoms. Current diagnostic tests are not able to differentiate between acute and chronic infection. In this study we validated the response against *T. gondii* antigens in an experimental mouse model of Toxoplasmosis in two stages of infection. Five female Swiss Webster mice were infected orally with cyst of *T. gondii* ME49 strain and four mice were inoculated with saline solution (control group). Blood was collected 8, 15, 30 and 60 days post-infection. The mouse brain infections were confirmed by conventional PCR and by optical microscopy. The immunoblot was performed using the tachyzoite lysate proteins of RH strain as antigen, and IgM and IgG as detection antibodies. For the IgM immunoblot we identified three immunogenic proteins with low molecular weight (25, 30 and 39 kDa); these bands appeared within 8 and 15 days post infection and gradually disappeared until 60 days. For the IgG immunoblot we observed the same antigenic bands and also the specific recognition of a 28 kDa protein. The band corresponding to this protein was observed after 30 days (chronic stage) post-infection, and afterwards its intensity increased progressively. We can also detect other high molecular weight immunogenic proteins. The detection of these proteins by immunoblotting might be useful to estimate the stage and development of infection in the diagnosis of the toxoplasmosis disease.

561

STANDARDIZATION OF AN ON-BEAD SANDWICH ELISA FOR THE DETECTION OF *TOXOPLASMA GONDII* ANTIGEN SAG1

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Toxoplasmosis is a disease caused by *Toxoplasma gondii*. Infected adults generally develop a chronic infection and remains asymptomatic. However, if a person becomes immunosuppressed, the infection can be reactivated and cause severe damage in different tissues, mainly leading to a diffuse encephalopathy. The diagnosis of this disease is principally done by the detection of antibodies in blood; nonetheless, IgG antibodies can persist live long in immunocompetent toxoplasma-infected individuals, making it difficult to differentiate between a recent and an older infection. On the other hand, the antibody production can be impaired in immunocompromised patients. For these reasons, it is important to design new diagnostic tests that can detect circulating antigens (CAg). Although some tests have been already developed, it is necessary to create more easy, simple and accurate approaches for the detection of CAg. The use of microparticles provides a large surface-to-volume ratio that facilitates detection, stability, and manipulation. The aim of this study is to standardize an on-bead sandwich ELISA for the detection of the main surface antigen of *T. gondii* (SAG1 or P30). In order to do this, we used magnetic microparticles Dynabeads-M270 Epoxy and got a good performance using a concentration of 0.05 mg. The standardization process was done using Total Lysate Antigen from parasite culture of the RH strain. The optical density (OD) of the positive control was 2.6 times the OD of the negative control and the minimum amount of SAG1 that could be detected was 100 ng (considering that it accounts for 3 to 5% of the total *T. gondii* protein). We processed 7 mice serum samples (4 were infected with RH strain and 3 with ME49 strain). The ODs of the samples from RH-infected mice were 4 to 7 times higher than the ODs of the negative control. However, the ODs of the serum samples from the ME49-infected mice were very similar to negative controls. The future direction of this project is to perform this assay using other types of microparticles and new commercially available antibodies in order to increase the sensibility and specificity in the toxoplasmosis diagnosis.

562

URBAN PIGEONS (*COLUMBA LIVIA DOMESTICA*) AS SOURCE OF ENVIRONMENTAL SPREAD OF *CRYPTOSPORIDIUM* WITH ZOONOTIC POTENTIAL

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Urban pigeons, also known as domestic pigeons or Rock Pigeons (*Columba livia domestica*), are birds of Columbiformes order quite common in many urban centers, living in close contact with humans. Three hundred and eleven pigeons were captured with appropriate cages found at home and in human's peridomicile in the state of Rio de Janeiro, Brazil. The bottom of the cages was protected by a plastic, to facilitate the collection of feces; after the birds defecated they were released. Microscopic diagnosis was performed to verify the presence of *Cryptosporidium* oocysts using the technique of centrifugal flotation in sugar solution. In positive samples a DNA extraction was performed, followed by PCR technique for the 18S target gene. All samples obtained from the Nested-PCR reaction were stained and observed on agarose gel and subsequently purified. After this procedure they were sequenced at BLAST platform, and a search of sequences obtained was performed to determine their identities and possible similarities and homologies with previously deposited species in GenBank '. Phylogenetic analyzes were performed using the MEGA 6 software. Of a total of 387 fecal samples 16.31% (54/311) were positive in microscopy, and of these, 5,68% (22/387) were sequenced and identified two species *C. meleagridis* and *C. baylei*. Species *C. meleagridis* is worrying in terms of public health, because the pigeons are easily adapted to human environment, whether for the abundant supply of shelter, lack of predators, lots of food available; in addition, *C. meleagridis* is the third most prevalent species in humans.

563

GENETIC DIVERSITY AND TRANSMISSION DYNAMICS OF *CRYPTOSPORIDIUM* PARASITES IN CATTLE OF SOUTHERN GHANA

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The coccidian parasite *Cryptosporidium* causes enteric disease in human beings, domestic animals and wild vertebrates. Infection is often self-limiting in immunocompetent individuals, but could be severe and chronic in persons with compromised immune system. Studies have shown a strong association between human cryptosporidiosis and contact with potentially infected cattle, particularly pre-weaned calves. Whereas studies on cryptosporidiosis in Ghana have focused on microscopy identification of the pathogen in immunocompromised people, limited information exists on the genetic composition of this zoonotic parasite species. The study reported in this paper investigated the genetic diversity of *Cryptosporidium* parasite across different age groups of cattle from the southern part of Ghana. Stool samples were collected from cattle and were processed by formol-ether concentration. After morphological identification of the oocysts, genomic DNA was extracted from the concentrate, followed by polymerase chain reaction assay to identify the species of the parasites. PCR positive samples were subjected to Restriction Fragment Length Polymorphism analysis using SspI for species diagnosis or VspI for genotyping of *C. parvum*. Out of the 90 stool samples analysed so far, 11 were positive for *C. parvum*. Further analysis are ongoing to ascertain if the strain is similar to those in human beings.

564

PATHOGENICITY OF *DIENTAMOEBA FRAGILIS*

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Dientamoeba fragilis is a trichomonad protozoan which is commonly reported throughout the world. Despite its initial discovery over 100 years ago remarkably little is known about this parasite. The parasites life cycle and mode of transmission are poorly defined, and controversy surrounds the pathogenic potential of this organism. This talk will highlight the latest clinical studies, animal studies and the recent publication of the *D. fragilis* transcriptome from which several potential markers of pathogenicity were described. Molecular detection of the parasite will also be discussed.

565

COMPARISON OF TWO RECOMBINANT ANTIGENS FOR DIAGNOSIS OF CHRONIC HUMAN FASCIOLIASIS

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Fascioliasis has been recognized as an emerging/reemerging zoonotic disease with an estimated prevalence of up to 17 million people infected and 180 million at risk for infection worldwide. In the United States, fascioliasis should be considered in immigrants, refugees or travelers with eosinophilia. Public health laboratories need a simple and reliable method for diagnosis of fascioliasis to identify and treat cases. The recognized laboratory test of choice for diagnosis of fascioliasis is detection of disease specific antibodies, most commonly using excretory-secretory antigens for detection of IgG antibodies. Recently, recombinant proteins such as FhSAP2 and Fh-CLP-1, have been used in an ELISA based format to detect IgG antibodies. To develop a better assay that could be used for diagnostic and surveillance, we used the GST-FhSAP2 recombinant antigen and Fh-CLP-1 to develop Western blot (WB) to detect *Fasciola hepatica* total IgG antibodies. We evaluated the assays using well-characterized sera from persons with or without fascioliasis. The sensitivity and specificity of GST-FhSAP2 and FhCLP1 WB were similar at 94% and 98% and 100% and 99%, respectively. For the multiplex immunoblot, the sensitivity and specificity were 100% and 98%. Although the defined positive sample size is small, the study supports the previous study results. In conclusion, the GST-FhSAP2 and FhCLP1 antigens perform well in immunoblot format separately or combined and can be readily adopted by public health and commercial reference laboratories for clinical diagnosis of *F. hepatica* infection in refugees, immigrants, and travelers with eosinophilia in the United States.

566

APICAL SODIUM-DEPENDENT BILE ACID TRANSPORTER OF *CLONORCHIS SINENSIS*: 3D STRUCTURE AND FUNCTIONALITY

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Apical sodium-dependent bile acid transporter (ASBT, SLC10A2) plays a key role in the bile acid recycling. Bile acid uptake of ASBT is electrophysiologically coupled with co-transport of sodium ion. When *Clonorchis sinensis* survive in the bile duct, an extreme environment of bile juice, *C. sinensis* ASBT (CsASBT) could serve as an important contributor in its bile-taxis and survival. In this respect, we report here a homology modeling and molecular docking of CsASBT as a drug target. Its complete coding sequence was 1,641 bp long and encoded a polypeptide of 546 amino residues. Inward-facing (IF) and outward-facing (OF) conformations of CsASBT 3D model were generated by homology modeling using the crystal structures of *Neisseria meningitidis* (PDB ID: 3zuy) and *Yersinia frederiksenii* (PDB ID: 4n7x) as templates. The modeled structures were further refined and verified for higher reliability. IF- and OF-CsASBT were built in region of 185-492 aa and 189-489 aa, respectively, whereas remaining region was predicted to be disordered and showed few homologues in the trematodes. Similar to the ASBTs, CsASBT was predicted to have 10 transmembrane domains (TM) divided into two groups: a core group formed with TM3-5 and 8-10; a panel group formed with TM1, 2, 6 and 7. TM1-5 and TM6-10 were structurally homologous but oppositely oriented, thus producing an internal twofold pseudosymmetry. IF-CsASBT had three sodium binding sites. One binding site was coordinated by TM4b, TM5 and TM9a, and the other by TM3, TM4 and TM9. A third binding site was predicted to be coordinated by TM4a. Taurocholate binding pocket was located in an intracellular cavity. Structure-based virtual screening was carried out using a reliable IF- and OF-CsASBT model. Inhibitors, not working on human ASBT, were selected through a pharmacophore-based filtering. Taken together, we report the refined models of CsASBT important for further study on function of the ASBTs and on structure-based drug design targeting the ASBTs.

567

OPISTHORCHIS FELINEUS HEMOZOIN DEPRESSES INTEGRIN CELL SURFACE EXPRESSION ON CHOLANGIOCYTES

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Infection with the fish borne liver fluke *Opisthorchis felineus* is common in the Eastern Europe (Ukraine, European part of Russia), Northern Asia (Siberia) and Central Asia (Northern Kazakhstan). The pathophysiology of the liver and bile duct tract due to parasitism coincides with the presence

of adult worms in the bile ducts, implicating worm excretory-secretory products acting on cholangiocytes, the epithelial cells lining the biliary tree. Excretory-secretory products of *O. felineus* include hemozoin, a byproduct of digestion of ingested host blood, which sequesters the toxic heme moiety. Hemozoin release from *O. felineus* accumulates in ectasia in the bile ducts -- hemozoin 'knobs'. We investigated the spread of hemozoin in the host circulation during distant organs during acute and chronic opisthorchiasis infection, and also the influence of hemozoin on cell growth. Using spectrophotometry and luminometry, we detected highest concentration of hemozoin in the bile ducts. Hemozoin was present in the parenchyma of the liver of hamsters during acute and chronic opisthorchiasis felinea, but not detected n heart, spleen, lung and muscles. The human cholangiocyte cell line H69 was exposed to hemozoin (O.F. hemozoin) isolated from bile ducts of hamsters, as well to synthetic hemozoin, and cell growth monitored in real time (xCELLigence System, Acea). O.F hemozoin lead to the changes of the adhesion characteristics of cells that dramatically decreased the cell index. By multiparametric flow cytometry, we found that both O.F and synthetic hemozoin depressed integrin beta-1, integrin beta-5 at the surface of H69 cells, and O.F hemozoin lead to the increasing of CD49a, an integrin alpha subunit. These findings indicated that liver fluke hemozoin induced raid and marked changes in the growth and adherence of cholangiocytes, findings that warrant further investigation.

568

HYDROLOGICAL IMPACTS ON DISEASE TRANSMISSION OF *OPISTHORCHIS VIVERRINI* IN THE LAWA LAKE COMPLEX: A MODELLING PERSPECTIVE

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Several stages of *Opisthorchis viverrini*'s transmission cycle are mediated by water: from contaminated feces to snail as an egg, from snail to fish as cercariae, and from fish to definitive host as the infected fish is caught and consumed. All three of these processes are dependent on hydraulic connectivity, which changes seasonally in the floodplain ecosystem around the Lawa Lake complex in Khon Kaen Province, Thailand, our study site of interest. The prevailing theory, with limited empirical evidence to support, is that there are very limited circumstances and geographical areas in which these processes occur. Biology, parasitology, and ecology research inform us of some guiding assumptions that provide a tentative picture of how and where transmission is occurring in the environment. However, an exhaustive hydrologic model has never been developed and integrated into a transmission framework in order to explain the liver fluke development cycle. To that end, the development of a site-specific hydrodynamic model to understand connectivity between Lawa Lake, the Chi River, and surrounding wetlands is key to helping us identify "hot zones" of transmission, understand the scale of these transmission processes, and interpret historical and present data about infection levels in snails, fish, and humans. The model outputs flow vectors that elucidate the relationship between the villages around the lake, the scale of transmission, and proximity to susceptible habitats for snails and fish. The full model integrates the hydrologic model outputs as time-varying parameters into the disease model to describe transmission patterns. Results generated can be used to inform sustainable environmental control strategies in northeast Thailand and across Southeast Asia to reduce parasite and subsequent cancer burden.

569

OPISTHORCHIS VIVERRINI INFECTION EXACERBATES THE SEVERITY OF DIABETIC LIVER INJURY AND NON-ALCOHOLIC FATTY LIVER DISEASE

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The highest co-prevalence between infection with *Opisthorchis viverrini* and diabetes mellitus (DM) is found in the northeastern Thailand. Inflammatory responses that lead to hepatobiliary disease is seen during opisthorchiasis. Moreover, non-alcoholic fatty liver disease (NAFLD) and diabetic liver injury caused by DM lead to the majority burden of liver disease at large. However, the association among opisthorchiasis, DM and hepatobiliary disease have not been clarified. The aim of these studies was to investigate the effect of *O. viverrini* infection on the development and progression of DM, NAFLD and diabetic liver injury in models using hamsters and cell lines. An experiment was carried out on four groups of hamsters: (1) normal control (NC), (2) chronic opisthorchiasis (OV), (3) NC and HFD fed with 10% fructose in drinking water for short-term (one month) and long-term (four months) (HF), and (4) *O. viverrini* infection at 4 months and followed by HFD fed with 10% fructose in drinking water for one and four month(s) (OVHF). Hamsters were euthanized at designated time points at five and eight months following infection. The fasting blood glucose levels of all experimental groups did not differ significantly. Intriguingly, the homeostatic model assessment of insulin resistance (HOMA-IR) in short-term HF treatment of group 3 was higher than other groups at the same time point. However, the HOMA-IR of long-term HF treatment of group 3 was not different from OVHF group but higher than OV group at the same time point. Histological features of hamster livers revealed that the highest amount of lipid droplet was found in short-term HF treatment of OVHF group. In addition, we observed that excretory/secretory products of *O. viverrini* suppressed the growth of HepG2 cell line by dose-dependent manner when monitored under real-time monitoring system. These findings indicate the effect of *O. viverrini* on the improvement of insulin sensitivity. Infection with *O. viverrini* might inhibit regeneration of hepatocytes and increase the severity of diabetic liver injury, and hence chronic opisthorchiasis might represent a risk factor for NAFLD.

570

DEVELOPMENT OF A NOVEL METHOD FOR *OPISTHORCHIS VIVERRINI* DNA DETECTION IN URINE BY PCR ASSAY

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Opisthorchis viverrini infects 9 million people worldwide and is endemic in Thailand, Laos, Vietnam and Cambodia. Treatment is the most effective tool we have to reduce the transmission and burden of disease while increasing the quality of life for persons infected. In order to effectively treat, we must diagnose those who are infected. Microscopy is the current diagnostic standard for *O. viverrini* through the identification of eggs in stool but is hampered by under diagnosis and misclassification. Our goal is to develop a protocol for *O. viverrini* DNA detection in urine for future diagnostic purpose. We developed and validated an extraction technique to isolate *O. viverrini* DNA from urine using BioMatrix Resin by adapting a method recently developed for *Schistosoma mansoni*. PCR, using *O. viverrini* -specific primers (pOV-6) was performed and optimized to detect the isolated DNA. 5ml of clean negative human urine was spiked

with 3500 ng of *O. viverrini* DNA and a serial dilution was performed to determine the detection limit. A 5ml pooled sample of 10 positive human samples was made and we performed a serial dilution to determine the profile of O.viverrini DNA by PCR. The PCR using pOV-6 primers on DNA extracted from urine worked successfully in spiked samples and human samples. The extraction method resulted in the retention of 33% of the original DNA amount. The detection limit of *O. viverrini* DNA in urine is 0.077 pg. The pooled samples showed DNA presence at a 10-25% dilution but not at higher or lower concentrations. This is the first report of the detection of *Opisthorchis viverrini* DNA in urine. Urine is easier to obtain from humans than feces or blood samples because of cost, time, and intrusiveness. Overall we were able to achieve the detection of *O. viverrini* DNA in urine, which prompts potential investigation as to why the DNA is there and allows for further develop a sensitive and specific diagnostic assay for opisthorchiasis with the goal of reducing morbidity and mortality.

571

MOLECULAR CHARACTERIZATION OF THE LARVAL PHASE OF *SCHISTOSOMA MANSONI* IN *BIOMPHALARIA GLABRATA* MOLLUSKS UNDER EXPERIMENTAL CONDITIONS

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Schistosomiasis mansoni affects approximately 207 million people in the world. In Brazil, a major goal of the Control Program for this parasitosis is to reduce the risk of geographic expansion. The main intermediate host of *Schistosoma mansoni* is *Biomphalaria glabrata*. The detection of larval stages in intermediate hosts is an important challenge to public health once it can indicate early natural infections rates. The objective of this study is to standardize the detection of *S. mansoni* from primary sporocysts developed in *B. glabrata* mollusks tissue artificially infected with Belo Horizonte *S. mansoni* lineage, using the Polymerase Chain Reaction (cPCR), a Two Sequential PCR-amplification (Re-PCR) and TaqMan® Real-Time PCR system (qPCR). Twenty *B. glabrata* mollusks were infected with thirty miracidia obtained from a laboratorial cycle of *S. mansoni*. For *S. mansoni* DNA extraction, after thirty days, four daughter-sporocysts were collected. Moreover, the head-foot portion was removed from four additional specimens of *B. glabrata*. The nucleic acid was extracted using the DNeasy Blood and Tissue Kit (Quiagen). Extracted DNA was quantified using Nanodrop and amplified with the three molecular techniques cited above, using primers that amplify 121bp *S. mansoni* DNA tandem sequences. The amplification product was detected in agarose gel 2%. The extraction process of daughter-sporocysts yielded around 6.1 ng/µL of *S. mansoni* DNA. The amplification products of cPCR were faint, but the results were better after Re-PCR and qPCR, which showed a mean of 20.07 Cycle Treshold (Ct). *S. mansoni* DNA extraction from complete head-foot portion yielded an average of 487.5ng/µL, with good results in cPCR, Re-PCR and qPCR that pointed a Ct average of 15.92. *S. mansoni* sporocyst molecular detection from the head-foot portion demonstrated a high sensibility and specificity. Therefore, it could represent a new tool in early characterization of snail susceptibility to *S. mansoni* in natural conditions and assist in the control of this parasitosis.

572

IDENTIFICATION OF GENES TARGET OF REGULATION BY MAPKS IN *SCHISTOSOMA MANSONI*

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Eukaryotic protein kinases (ePKs) are important for the regulation of several cellular functions. It is known that 252 ePKs are encoded by the *Schistosoma mansoni* genome, which corresponds to 2% of the predicted proteome. However, only 24 ePKs have experimental functional evidence. The family of mitogen-activated protein kinase (MAPKs) influences various biological activities and are widely studied as drug targets. Our group identified MAPKs orthologs in *S. mansoni* by *in silico* analyses and demonstrated by functional studies that MAPKs (SmCaMK2, SmJNK, SmERK1, SmERK2 and Smp38) are involved in parasite development, reproduction and survival and may therefore be considered potential targets for the development of new drugs. In this study, we aim to contribute to the experimental characterization of ePKs by the identification of specific genes regulated by MAPKs pathways. To elucidate these target genes, the five selected genes were knocked down by RNA interference in schistosomula and RNA-Seq analysis of treated parasites were performed, including three biological replicates. For all genes selected, we observed approximately 75% reduction on transcript levels, except for SmERK-2. RNA-Seq libraries were then prepared with RNA derived from knockdown parasites according to the *Truseq stranded mRNA Library Prep* protocols and were sequenced on *Illumina HiSeq 2500* platform. We generated 27 paired-end libraries that generated 100 bp reads. Sequences were aligned to the latest *S. mansoni* reference genome (version 5.0) and differentially expressed genes were identified by comparing each MAPK knockdown against control parasites (treated with unspecific GFP-dsRNA). We also checked the occurrence of potential off-targets by using GFP or mCherry dsRNA as unspecific controls. RNA-seq analyzes were performed comparing schistosomules treated with these unspecific dsRNAs and schistosomules untreated. This work will allow a better understanding of these signaling pathways, helping to elucidate the functional roles of MAPKs, as well as assisting in the future development of therapeutic intervention tools for schistosomiasis control.

573

HISTONE MODIFYING ENZYMES ARE POTENTIAL THERAPEUTIC TARGETS AGAINST SCHISTOSOMIASIS AS IT IS ESSENTIAL TO VIABILITY AND REPRODUCTION

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Schistosomiasis is the second most prevalent parasitic disease in the world. The treatment rely on a single drug, Praziquantel, and due to the identification of drug resistant parasites, the development of new chemotherapy against schistosomiasis is required. Histone modifying enzymes (HMEs) play a central role in regulating chromatin epigenetic modifications and are implicated as therapeutic targets in various diseases. In this work, we employed RNA interference to validate 1 histone deacetylases (SmHDAC8), 5 demethylases (HDM) and 10 methyltranferases (HMT) as drug targets in *Schistosoma mansoni*, from those, 4 were chosen for experimental validation. Additionally, specific inhibitors developed by the A-PARADDISE consortium were used to interrogate HMEs as drug targets against *S. mansoni*. To elucidate the roles of HMEs, schistosomula were exposed to dsRNAs, injected in mice and evidenced that SmHDAC8 is important to parasite development and survival. Additionally, HDAC8,

PRMT3 and KDM1/KDM2, seem to be associated in egg production since infected mice had significantly lower egg burdens and female worms presented underdeveloped ovaries. For the inhibitors screening, schistosomula were exposed to 300 inhibitors and parasite viability was assessed by measurement of lactate produced in the medium and by propidium iodide staining. These inhibitors were also tested on adult worms, in which parasites mobility were evaluated using the WormAssay software. In the lactate and propidium iodide assays, 60 and 76 active inhibitors were identified, respectively. Using the WormAssay, 76 compounds were active in male worms, 101 were active in female worms, from those, 54 were active in both genders. Also, the IC50 was established for the active compounds and cytotoxicity was tested in mice fibroblast cells. These results indicate that HMEs are essential to parasite viability, oviposition and/or development of reproductive system, confirming its potential as drug targets. In addition some inhibitors seem to be potential candidates for drugs against schistosomiasis.

574

CLONING AND CHARACTERIZATION OF A *SCHISTOSOMA JAPONICUM* AQUAGLYCEROPORIN THAT FUNCTIONS IN OSMOREGULATION

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As one of the three major human pathogens that cause schistosomiasis, *Schistosoma japonicum* is the only one that is endemic in China. Despite great progress on schistosomiasis control over the past 50 years in China, *S. japonicum* transmission still occurs in certain endemic regions, which causes significant public health problems and enormous economic losses. During different life stages, parasites are able to survive dramatic osmolality changes between its vector, fresh water, and mammal host. However, the molecular mechanism of parasite osmoregulation remains unknown. To address this challenging question, we report the first cloning of an *S. japonicum* aquaglyceroporin (SjAQP) from an isolate from Jiangsu province, China. Expressing SjAQP in Xenopus oocytes facilitated the permeation of water, glycerol, and urea. The water permeability of SjAQP was inhibited by 1 mM HgCl2, 3 mM tetraethylammonium, 1 mM ZnCl2, and 1 mM CuSO4. SjAQP was constitutively expressed throughout the *S. japonicum* life cycle, including in the egg, miracidia, cercaria, and adult stages. The highest expression was detected during the infective cercaria stage. Our results suggest that SjAQP is very likely to play a role in osmoregulation throughout the *S. japonicum* life cycle, especially during cercariae transformation, which enables parasites to survive osmotic challenges.

575

THERAPEUTIC EXPLOITATION OF IPSE, A UROGENITAL PARASITE-DERIVED HOST MODULATORY PROTEIN, FOR CHEMOTHERAPY-INDUCED HEMORRHAGIC CYSTITIS

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The vast majority of urogenital schistosomiasis-infected individuals lack day-to-day hematuria, a cardinal sign of urinary tract injury. This suggests that parasite factors may help balance life cycle propagation via bladder wall penetration to allow egg passage into urine, against inducing life-threatening hemorrhagic cystitis (HC) that would preclude life cycle completion. IL-4-inducing principle from *Schistosoma mansoni* eggs (IPSE) is the most abundant egg-secreted protein of *S. mansoni*, which induces IL-4 release from basophils via binding to cell-surface IgE. IPSE also sequesters chemokines and alters host gene transcription by translocating into host nuclei. We hypothesized that the *S. haematobium* homolog of IPSE (H-IPSE) may regulate HC. Because schistosome transgenesis remains elusive, we tested host-modulatory properties of H-IPSE using a model of HC based on nitrogen mustard alkylating agents (cyclophosphamide and ifosfamide), which are used to treat cancers but often result in HC. Current options to prevent ifosfamide-induced HC using Mesna can have significant side effects. IL-4 has been shown to ameliorate HC in mice, but systemic IL-4 administration results in unacceptable morbidity. Given the IL-4-inducing properties of IPSE, we postulated that H-IPSE may ameliorate ifosfamide-induced HC. Mice were administered combinations of ifosfamide, IL-4, Mesna, anti-IL-4 antibody and H-IPSE. Readouts include bladder wet weight, histology (i.e., edema, hemorrhage), and hemoglobin content, spontaneous and evoked pain, voided urine spot assay, cytokine analysis and transcriptional profiling. We found that H-IPSE is comparable, and possibly superior, to IL-4 in suppressing ifosfamide-induced HC in mice, including associated urinary frequency and spontaneous pain behavior. Through use of anti-IL-4 antibody and a nuclear localization sequence mutant of H-IPSE, we determined that the therapeutic effect was dependent on IL-4 and nuclear localization. To our knowledge, our work is one of the first successful therapeutic exploitation of uropathogen-derived molecule in a clinically relevant bladder disease model.

576

SCHISTOSOMA HAEMATOBIUM IPSE, A CANDIDATE PRO-ONCOGENIC FACTOR

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Urogenital schistosomiasis (UGS) affects over 112 million people globally. Adult worm pairs in the pelvic venous plexus deposit eggs in the bladder. The eggs secrete antigens that induce granuloma formation, in turn provoking immunopathogenic sequelae that include urothelial hyperplasia and carcinogenesis. The IL-4-inducing principle of *Schistosoma mansoni* eggs (IPSE) is a prominent antigen released by schistosome eggs. IPSE binds immunoglobulins and chemokines, translocates into host nuclei and modulates gene transcription, and induces basophils, mast cells, and NK T-cells to release IL-4, thereby orchestrating a dominant Th2 response. Given that the IPSE gene is only found in Schistosoma, it is a candidate pro-carcinogenic factor in schistosomal bladder cancer. We hypothesize that the *Schistosoma haematobium* homolog of IPSE (H-IPSE) plays a major role in driving the inflammation-associated urothelial proliferation and bladder carcinogenesis during UGS. Recombinant H-IPSE was co-cultured with a panel of urinary bladder cell lines derived from primary and transformed tissues, representing diverse species of origin and stages of carcinogenesis: HTB-9 (Grade II human bladder carcinoma), HCV-29 (from normal human bladder urothelium) and MB49 (mouse urothelial carcinoma). Readouts included CFSE proliferation assays, cell cycle analysis, TUNEL apoptotic assays, real-time monitoring of cell migration and invasion using the xCELLigence platform, and qPCR-based transcriptional profiling. Whereas an influence of H-IPSE on proliferation and apoptosis of HTB-9 cells was not seen, it stimulated proliferation of HCV-29 and MB49 cells in a concentration-dependent manner. Moreover, cell cycle analysis showed that H-IPSE increased the proportions of MB49 cells in the S-phase. These findings indicate that H-IPSE might contribute to bladder cancer progression in the context of UGS. Ongoing work will define the mechanisms by which H-IPSE promotes *S. haematobium*-associated oncogenesis.

577

PROTON CHANNELS IN *BIOMPHALARIA GLABRATA* EMBRYONIC CELL MEMBRANES: PUTATIVE TARGET FOR *SCHISTOSOMA MANSONI* LARVAL TRANSFORMATION PROTEINS

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The *Biomphalaria glabrata* embryonic (Bge) cell line was derived from the freshwater snail *B. glabrata,* an intermediate host for *Schistosoma mansoni*, a causative agent of intestinal schistosomiasis. Bge cells share characteristics with hemocytes, the immune effector cells of *B. glabrata* snails, and therefore, were used as an *in vitro* model for the study of host cell-larval parasite interactions. This study employed the whole-cell patch clamping technique to identify the major ion channel channels in the cell's plasma membrane and to explore their possible role in host-parasite interactions. Bge cells were exposed to pH gradients that resulted in changes in proton channel current. Exposure to Zn^{2+}, a potent proton channel blocker, reduced response amplitude in Bge cells by 3-fold, further supporting the presence of proton channel activity. A series of voltage steps (-70 mV to 20 mV) applied to Bge cells evoked current responses that were significantly enhanced by *S. mansoni* larval transformation proteins (LTP), indicating an LTP-modulation of these channels. Since proton channels can play a role in the production of reactive oxygen species (ROS) in mammalian immune cells, we tested this function in Bge cells. Using the fluorescent probe 2',7'-dichlorofluorescein-diacetate (DCFH-DA) to detect intracellular ROS, we found that cells generated an ROS response that was significantly reduced by Zn^{2+}, indicating the involvement of proton channels in ROS production. LTP had no significant effect on ROS production suggesting that LTP-induced flux increases through proton channels may not extend to its modulation of the oxidative response. Lastly, immunofluorescence analyses of Bge cells and *B. glabrata* hemocytes revealed the expression of a HVCN1-like proton channel demonstrating that *B. glabrata* hemocytes also express a proton channel that may mediate ROS production. Thus, this channel could be important for the killing of larval *S. mansoni* by hemocytes of resistant *B. glabrata* snail strains.

578

CHARACTERIZATION AND FUNCTIONAL STUDIES OF SERINE/THREONINE PROTEIN PHOSPHATASE 1 (PP1) ENCODING GENES FROM *SCHISTOSOMA JAPONICUM*

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Serine/threonine protein phosphatase1 (PP1) play a wide range of physiological roles including cell cycle regulation, glycogen metabolism, contractility, morphogenesis and spermatogenesis. However, little is known about the functions of PPs in the reproductive biological processes of schistosomes. In the present study, three PP1 genes were identified in *Schistosoma japonicum*. The sequence alignments and phylogenetic analyses showed that three PP1 proteins (Sj-PP1-1, Sj-PP1-2 and Sj-PP1-3) belong to PP1 beta, PP1 gamma and PP1 alpha subfamily, respectively. RT-PCR analysis revealed that three PP1 genes were all transcribed in both sexes and throughout development. In-situ hybridization indicated that Sj-pp1 were predominantly expressed in gonad related organs such as the testis of male, the ovary and vitellarium of female as well as ootype surrounding area. RNA interference with combined three Sj-PP1 dsRNAs by soaking for 7d caused stunted growth of female and male worms. CLSM observation found distinct morphological changes in Sj-PP1 dsRNA treated female worms with significantly smaller ovaries which were dominantly occupied by immature oocytes and low maturity of vitellarium surrounding by immature vitelline cells. In addition, no significant changes were observed on males treated for 7d, except for a reduced diameter of the testicular lobes accompanied by a reduction of cell density in testes and empty seminal vesicles with prolonged RNAi by 12d. Edu corporation assay detected evident decrease of cellular mitosis activities in ovary, vitellarium of females and testis and parenchyma of males. With extension of RNAi process, remarkable reduction in egg production were observed and serious interference on paring behavior was found between female and male. These findings suggested that PP1 may function in developmental and reproductive processes of *Schistosoma japonicum*.

579

INTERACTIONS BETWEEN HOST IMMUNE STATUS AND PARASITE METABOLIC ACTIVITY IN *SCHISTOSOMA MANSONI*

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Schistosomes are obligate parasites that lack genes required for the synthesis of many lipids (i.e. cholesterol and long-chain fatty acids), and therefore rely solely on the mammalian host to supply these essential molecules. Lipid acquisition appears to be particularly important for female schistosome reproductive activity. Here we show that lipid is specifically concentrated in the ovary of the female parasite. Interestingly, reproductively inactive females, in both unisexual infections and immunodeficient hosts, exhibit reduced accumulation of lipid in the ovary, underlining the connection between lipid metabolism and parasite reproduction. Coincident with these changes in lipid accumulation, schistosomes from immune competent and immunodeficient hosts also exhibit alterations in ATP metabolism. At 6 weeks post-infection, schistosomes isolated from immunodeficient animals have significantly higher ATP content than those isolated from immune competent animals. However, at 8 weeks post-infection the situation is reversed, with schistosomes isolated from immunodeficient animals having significantly lower ATP content than those isolated from immune competent animals. These findings suggest that parasites in immunodeficient hosts are unable to synthesize sufficient ATP once egg production is underway. To explore the molecular pathways connecting parasite lipid acquisition, energy metabolism, and reproduction, we are characterizing the parasite [mTOR] and [AMPK] signaling pathways to assess their status in these different developmental states. These findings suggest that a link between host immune status and parasite energy metabolism is an important aspect of the host-schistosome relationship.

580

DUAL RNA-SEQ RESPONSES OF FIELD-DERIVED SPECIMENS OF THE AFRICAN SNAIL *BIOMPHALARIA PFEIFFERI* TO INFECTION WITH THE HUMAN PARASITE, *SCHISTOSOMA MANSONI* PROVIDE INSIGHT INTO HOST-PARASITE RELATIONSHIPS AND REPRODUCTIVE IMPLICATIONS OF PARASITISM

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Biomphalaria pfeifferi exhibits extraordinary compatibility with *Schistosoma mansoni* and likely transmits more cases of this parasite to people than any other snail species. Ironically, we know relatively little at the molecular level regarding the interactions of *B. pfeifferi* and *S. mansoni* from early-stage sporocyst transformation to the development of cercariae. To redress this shortcoming, using field-derived west Kenyan representative of schistosomes and snails, we have undertaken dual RNA-seq of three intramolluscan developmental stages (1- and 3-days post exposure and cercariae producing infections). Parasite sequences were first separated by screening against the *S. mansoni* genome. Then, a *de novo B. pfeifferi*

transcriptome was assembled from over a half billion non-*S. mansoni* paired-end reads. Transcripts were annotated using protein and nucleotide databases, including the *B. glabrata* genome database. Snail reproductive inhibitory peptides like ovipostatin and developmentally regulated albumen gland protein were up-regulated in shedding snails, suggesting that host castration is not merely a passive response to diminished energy supplies. The lack of expression of snail sex pheromones may be related to the strong self-fertilizing preferences of *B. pfeifferi*. Fibrinogen-related proteins (FREPs) showed complex patterns of responses. Distinctive profiles of expressed *S. mansoni* features were seen, including up-regulation of defense and stress response proteins. Five *S. mansoni* venom allergen-like proteins, known for host immunomodulatory functions, were highly up-regulated in shedding snails. These field-derived snails harbored several notable symbionts including *Capsaspora owczarzaki*, microsporidians, *Perkinsus*-like protists, and ectosymbionts like *Trichodina* and *Chaetogaster*. Our database provides unique insights into schistosome-snail interactions taking place in a natural transmission focus, potentially including candidate molecules amenable to manipulation to facilitate new control approaches targeting the ability of larval schistosomes to succeed in their snail hosts.

581

SEX-BIASING GENE DRIVE TO ELIMINATE SCHISTOSOMES: A PROPOSAL

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Sex-biasing gene drives ensure that nearly all offspring are of one sex in order to progressively reduce the reproductive potential of the population. One strategy confers a fitness advantage to a sex chromosome by shredding the "opposing" sex chromosome during meiosis such that the driving chromosome is preferentially inherited by offspring. We propose a population suppression drive intended to locally or globally eradicate the schistosomes. Draft versions of the genome sequences of *Schistosoma mansoni*, which causes hepato-intestinal schistosomiasis, and *S. haematobium*, the cause of urogenital schistosomiasis, are available. Because the genetics and chromosomal architecture of *S. mansoni* are better understood, we are focusing on *S. mansoni* and will follow with *S. haematobium* once comparable information is available. We propose to deploy CRISPR/Cas9- and pseudotyped retrovirus–based techniques to introduce the gene drive components into the schistosome germ line. Schistosomes are a ZW species; females are ZW, males ZZ. The Z and W are largely homologous, but there are unique regions of both. It is therefore feasible to make a female-biasing 'Z-shredder' and a male-biasing 'W-shredder'. From a fitness perspective the Z-encoded W-shredder may be superior because it can also act as a conventional drive in ZZ males. That is, in ZW females it will shred the W, thereby ensuring that offspring inherit the driving Z chromosome and are consequently male, but will also copy itself from the driving Z to the wild-type Z in heterozygous males. Both activities are advantageous and should consequently be evolutionarily favored over time, albeit opposed by any unlikely suppressors that might evolve on the W or autosome. The gene encoding ribosomal protein S4 resides on Z, is single copy, and is expected to be critical for survival, while numerous repeats on the W are suitable for evolutionarily stable shredding. We are assaying components for building drive systems and are optimistically assembling and testing a W-shredder rps4 drive; and as a safeguard, an immunizing reversal rpS4 drive. Our proposal, approach and preliminary findings will be discussed.

582

ADVANCING WATER TREATMENT FOR RESOURCE RECOVERY TO ENHANCE DISEASE MITIGATION

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Despite being a fundamental resource required for the sustainment of life, water plays an integral part in the lifecycle and transmission of many pathogens, including bacteria, viruses, and parasites. This dichotomy presents an increased risk of exposure to a wide range of pathogens for humans and animals living in regions without regular access to clean water and waste processing systems, such as flush toilets. A large number of the technologies that have been designed solely for water and waste treatment fail in the field because they are not economically viable, whether through initial costs or long-term maintenance, or they have not been designed to withstand the unique environmental or cultural challenges presented by differing regions of application. In order to eliminate water as a reservoir for disease transmission, technologies designed for water treatment need to provide effective filtration or sterilization while allowing for the recovery of valuable resources. Examples of such resources may include, but are not limited to fertilizer, harvestable energy, and chemical components that may lead to the production of larger commodities. By providing waste and/or water treatment to reduce the transmission of disease while recovering harvestable products, the cost of initial construction and maintenance on such systems can me offset by the sustainability of the system and the potential for economic stimulation through the creation of technical maintenance jobs and new trade industries. This study will review a number of currently available technologies, describe the advantages and challenges for each technology, and discuss the potential for adaptation for use in resource-limited environments.

583

PREVALENCE OF SOIL TRANSMITTED HELMINTHS IN WATER, SANITATION AND HYGIENE (WASH) SUPPORTED AND NON-SUPPORTED SCHOOLS IN OGUN STATE, NIGERIA

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Water, Sanitation, and Hygiene (WASH) interventions have been advocated as a complementary tool in the control of soil-transmitted helminths (STH) infection. We therefore assessed the prevalence of STH in WASH supported and non-supported schools in Odeda, a rural local government area of Ogun state, Nigeria. Eight schools were randomly selected across the study area; three WASH supported (S-schools) and five non-supported schools (NS-schools). Stool samples were collected from 428 pupils and screened for STH infection, followed by an assessment of school-based WASH resource using WHO recommended guide. Results showed significant differences ($p < 0.05$) in the provision of safe water and environmental hygiene for S-schools (100% and 73.3%) and NS-schools (16% and 26.9%) respectively. There exist no significant differences ($p > 0.05$) in the sanitation condition between S-schools (44.4%) compared to NS-schools (20.0%). Overall prevalence of STH infection was 33.4%, while specific prevalence of 26.2% was recorded for Hookworm, 18.2% for *Ascaris lumbricoides* and 1.6% for *Trichuris trichiura*. STH Prevalence were significantly lower ($p<0.05$) in S-schools (27.4%) compared to NS-schools (37.5%). Mean intensities of hookworm and *Trichuris trichiura* infections were higher in NS-schools than S-schools (0.6097epg and 0.4247epg for hookworm) and (0.1193epg and 0.0epg for *Trichuris trichiura*) respectively.

This study provides evidences that WASH interventions have the potentials of reducing intestinal helminthiasis burden in public primary schools and should be scaled up to include more public schools in the state.

584

WATER SUPPLY AND SANITATION CONDITIONS IN RURAL SOUTHERN MOZAMBIQUE AND ITS AS SOCIATION WITH MORBIDITY AND MORTALITY INDICATORS DURING 2012-2015

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Water, sanitation and hygiene (WASH) are major health determinants, with an estimated total disease burden of 5.7% occurring worldwide.The situation of access to safe water and sanitation facilities and its impact on morbidity and mortality in southern Mozambique remains unknown. The aim of this study is to describe the current situation of safe water supply and sanitation facilities in the Manhiça Health Research Centre (CISM) study area and evaluate its association with several morbidity and mortality indicators. We conducted a retrospective cohort study with 61,900 children living in the center study area followed up until 15 years of age during the period 2012-2015. Water and sanitation household data was obtained from the CISM demographic surveillance system in Manhiça district, an area of around 2,380km2. Clinical data for all children under 15 was obtained from CISM round-the-clock morbidity surveillance system covering pediatric outpatient and hospital admission at the Manhiça District Hospital and rural health posts. A negative binomial regression model using Wald test was performed to assess the incidence rate ratio for every morbi-mortality indicator. Preliminary data showed that 86% of the children lived at least once in a household with unimproved sanitation facilities, 27% with unimproved water source and 77% with the main water source located outside the household during 2012-2015.Unexpectedly, the incidence rate ratios to develop diarrhea for children using unimproved sanitation and water facilities were significantly protective. Only the use of rivers and lakes as water sources significantly increased the children's rate to develop diarrhea by 20%. Other morbidity indicators (malnutrition, parasitemia, anemia) did show a rate increase with the use of unimproved water and sanitation facilities. Spatial distribution and clustering for the water, sanitation and morbidity variables will be also analyzed.The possible explanation of the findings will be discussed.This analysis will help to plan evidence-based interventions to improve access to safe drinking water and sanitation in rural southern Mozambique.

585

ASSOCIATION OF HOUSEHOLD, COMMUNITY AND SCHOOL SANITATION WITH HOOKWORM INFECTIONS AMONG SCHOOL-AGED CHILDREN IN KWALE COUNTY, KENYA

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Most of our understanding of the association between sanitation and STH infection is focused separately on schools and households, and few studies simultaneously assess the relative importance of sanitation in the household, wider community, and schools. Yet, understanding the relative impact of sanitation on STH transmission between these domains is necessary to target disease control interventions for greatest impact. Here we investigate associations and interactions between hookworm infection and household, community, and school sanitation among children aged 5-14 in Kwale County, Kenya. Data were collected during a cross-sectional parasitological survey between March-May 2015 as the baseline for the TUMIKIA study. Data were available from 22,864 households randomly selected across the county using a two-stage sampling design. In each household, one member was randomly selected and invited to provide a stool sample. Sanitation was assessed using structured observations and questionnaires. Sanitation conditions for every school in Kwale County were assessed during a survey in June 2015. Records from school and household surveys were linked for each child. Generalized linear mixed models were used to estimate the association between measures of household, community, and school sanitation with presence and intensity of hookworm infection. In total, the analysis included 5,251 school-aged children in 841 villages. Overall hookworm infection prevalence was 17.4% (16.4--18.4%), while mean intensity was 156.2 epg (141.4--172.6). Household sanitation coverage was estimated, as the proportion of households with reported access to a toilet, to be 49.2% (47.9--50.6%), and village sanitation coverage had an IQR of 25.0--78.4%. Multilevel analysis revealed associations across various domains, highlighting important areas of exposure for school-aged children in endemic communities. This study contributes further to our understanding of the impact of sanitation on hookworm infection in respective domains, and provides insight into effective targeting of programs to maximize reductions in hookworm infection.

586

THE DRIVERS OF THE CHOLERA EPIDEMIC IN BAUCHI, NORTHEAST NIGERIA 2014

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On 19th February, 2014, the Nigerian Federal Ministry of Health (MOH) was alerted of an ongoing outbreak of cholera in Bauchi State, Nigeria. The State has experienced repeated cholera outbreaks almost yearly with the 2010 outbreak, recording one of the highest case burdens among the states in Nigeria. We investigated the outbreak to assess the magnitude of the outbreak and identify risk factors for transmission. We collected the line-list from the State MOH and conducted a case-control study. We collected data on demographic characteristics, hygienic practices, and on food and water consumption using a structured questionnaire. We identified 2998 cases among the 1,444,393 residents of the 4 affected Local Government areas. Overall attack rate and case fatality rate were 0.21% and 0.77% respectively. Among the 248 case-control study

participants, 113 (45.6%) were female and 135 (54.4%) were male. There were 124 cases and 124 controls. Compared to controls, cases were more likely to have been exposed to diarrhoea case (OR:6.72, 95% CI 3.87-11.75), live as an Islamic Mendicant 'almajiri' (OR:5.22, 95%CI 1.94 - 14.78), not wash hands with soap after toileting (OR:4.71, 2.59 - 8.62), not wash hands before eating (OR:3.82, 95%CI 1.13 - 14.20), be ≤25 years (OR:3.32, 95%CI 1.97 - 5.60), drink street vended locally processed cereal drink 'kunu zaki' and custard 'koko' (OR:3.28, 95%CI 1.15 - 9.31) and (OR:2.91, 95%CI 1.10-7.71) respectively. In conclusion, 'Almajiris' were found to be key players boosting the epidemic and consumption of food from street vendors was a major risk factor for the spread of the disease. Sanitary inspection officers should mobilize the 'Almajiris' to maintain clean environment around them and food hygiene. The health department should train hawkers of foods on the street on personal and food hygiene.

587

PREVALENCE OF ROTAVIRUS INFECTION OVER TIME IN RURAL, COASTAL ECUADOR

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Rotavirus is a key cause of diarrheal disease in the developing world. However, patterns of infection over time have not been well characterized. Using 10 years of population based case-control data from rural coastal Ecuador, we investigate the prevalence of rotavirus infection over time and differences by subgroup in a region that has been experiencing continuous road construction. The prevalence of rotavirus infection has steadily declined over time from 7.0% in 2003 to 1.3% in 2012. This decrease was 3.4 times faster for symptomatic infection (rates of 1.3% and .4% respectively). Rotavirus infection was highest among individuals under age 5 (OR=3.19, 95% CI: 2.58, 3.94) but all age groups except children under 1 year of age exhibited decreases in prevalence of infection over time. In contrast, younger children showed patterns characteristic of a long epidemic, with a peak infection risk of 16.7%. Despite their higher levels of exposure, household and community controls had similar prevalence of infection (p=.82), suggesting that community interactions may be important for transmitting asymptomatic rotavirus infection in our study region.

588

A RANDOMIZED CONTROLLED TRIAL OF A HOSPITAL-BASED HANDWASHING WITH SOAP AND WATER TREATMENT INTERVENTION (CHOBI7) TO REDUCE CHOLERA AMONG HOUSEHOLD CONTACTS OF CHOLERA

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Household contacts of cholera patients are at a 100 times higher risk of developing a cholera infection than the general population during the week post-presentation of the index patient at the hospital. In an effort to initiate a standard of care for highly susceptible household contacts of cholera patients, we developed a hospital-based handwashing with soap and water treatment intervention entitled CHoBI7(Cholera-Hospital-Based-Intervention-for-7-days). The CHoBI7 intervention includes: (1) a cholera prevention package containing chlorine tablets for water treatment, soapy water bottles, a handwashing station, and a sealed water vessel with cover to ensure safe water storage, and (2) a pictorial ("Chobi" in Bangla) module on handwashing with soap and water treatment delivered by a health promoter through hospital and home visits during the week post-presentation of the index cholera patient at the hospital. The efficacy of the CHoBI7 intervention was evaluated by conducting a randomized controlled trial of 219 intervention household contacts of cholera patients and 220 control contacts of patients in Dhaka, Bangladesh. Case households were followed over a one week period at 5 timepoints for clinical and environmental surveillance which included collection of rectal swab and source and stored water samples to test for the presence of *Vibrio cholerae* by bacterial culture. Five hour structured observation was also conducted to assess handwashing practices. Compared to control contacts, intervention contacts had a significant reduction in symptomatic cholera infections (OR: 0.00 (95% CI: 0, 0.623)), and a 47% reduction in overall cholera infections (OR: 0.50 (95% CI: 0.21, 1.18). Intervention households had no stored drinking water with detectable *Vibrio cholerae*, and a 14 times higher odds of handwashing with soap at key events than control households during the intervention period (OR: 14.68 (95% CI:8.32, 25.90)). In conclusion, the CHoBI7 intervention presents a promising approach for cholera control among highly susceptible household contacts of cholera patients.

589

THE ASSOCIATION BETWEEN HEAVY RAINFALL EVENTS AND DIARRHEAL DISEASE: THE INFLUENCE OF URBAN AND RURAL GEOGRAPHY

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Climate change is expected to have downstream impacts on health outcomes in the 21st century. Changes in precipitation are expected with greater contrasts between wet and dry periods and increase in extreme weather events. Heavy rainfall events (HRE) have been shown to be associated with diarrheal disease. Diarrheal disease remains an important cause of mortality amongst children under five years of age with causing over 700,000 deaths per year and is associated with long term health outcomes such as stunting. Differences in urban and rural settings could play an important role in driving the relationship between precipitation and diarrhea due to underlying differences in infrastructure and social factors. The study aims to analyze the role of urban and rural contexts in affecting the relationship between HRE and diarrheal disease. Mixed effects Poisson regression was conducted on daily case counts of diarrhea in all public hospitals and clinics from all 68 parishes in the Esmeraldas province in northwestern coastal Ecuador across 2013-14 with heavy rainfall estimates and antecedent conditions lagged up to 14 days. Average daily rainfall estimates from the TRMM 3B42 platform were used to define heavy rainfall events as daily rainfall higher than the 90th percentile and antecedent conditions as wet or dry depending on 8 week total rainfall being in the highest or lowest tertile for the respective parish. In rural areas, there was a protective effect of HRE on daily case counts of diarrhea during the wet season whereas a positive association was observed in the dry season. In urban areas there was no protective effect observed and the expected counts of diarrhea were higher in all environmental conditions analyzed when compared to rural areas in the wet season. Factors associated with urbanization, such as crowding or infrastructure, seem to dominate over climate related factors. Despite this, dry conditions appear to be highly associated with increased diarrhea in all areas. Further work is needed to elucidate the mechanistic structure of what factors are driving the differences between urban and rural areas in how rainfall is associated with diarrhea.

590

MULTI-SECTORAL COLLABORATION BETWEEN THE NTD AND WASH SECTORS: EXPERIENCE FROM UGANDA

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In order to eliminate blinding trachoma all components of the SAFE strategy (Surgery, Antibiotic, Facial cleanliness and Environmental improvement) must be in place; however, the F & E components are often given less attention and financial resources. Many national programs are eager to implement F & E activities but do not know where or how to start. In Uganda, the Ministry of Health (MOH), in partnership with the Ministry of Water and Environment, Ministry of Education and Sports, various Water, Sanitation and Hygiene (WASH) and trachoma organizations came together to develop a comprehensive F & E plan. This process involved conducting an F & E situational analysis, organizing multiple multi-sectoral meetings and reaching a consensus on program priorities. Ultimately, four main activities were identified: 1) integration of face washing and trachoma messages into existing WASH strategies and activities in trachoma endemic regions; 2) revision and dissemination of school sanitation guidelines; 3) revision and dissemination of national sanitation guidelines; and 4) development of a Social and Behavior Change Communication (SBCC) strategy to be used as part of mass media campaigns. A review committee composed of representatives from different government ministries and organizations evaluated different WASH organization's proposals and selected the best proposal for each activity. As of December 2015, three WASH/SBCC organizations have been working successfully to achieve the above four objectives. In addition to the F & E activities identified, there has been an increase in cross-collaboration between the MOH and WASH partners through the sharing of data and participation in relevant stakeholder technical meetings such as the Uganda National Sanitation Working Group and the Uganda Neglected Tropical Disease Technical Committee. The Uganda Trachoma Control Program provides an excellent example of what can be achieved when different government, public health and WASH sectors collaborate towards a common goal.

591

BURDEN OF DISEASE ATTRIBUTED TO WATER-BORNE TRANSMISSION OF SELECTED GASTROINTESTINAL PATHOGENS, AUSTRALIA 2010

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Water is an important source of infectious diseases transmission, however the burden of water-borne disease is not well characterized. We have previously published estimates of the burden of disease caused by *Campylobacter*iosis, non-typhoidal salmonellosis, cryptosporidiosis, giardiasis, and norovirus in Australia in 2010 using number of cases, number of deaths and disability adjusted life years (DALYs). Post-infectious sequelae were included in DALY estimates for *Campylobacter*iosis (irritable bowel syndrome [IBS], reactive arthritis [ReA] and Guillain-Barré syndrome]) and salmonellosis (IBS and ReA). Here we have applied regional WHO estimates of the pathogen-specific proportion of cases attributable to water-borne transmission in 2010 (point estimates and 95% uncertainly intervals [UI]) to our published point estimates of overall disease burden for these pathogens. The WHO estimates for the Western Pacific Region Stratum A (WPR A) included Australia, Brunei, Japan, New Zealand and Singapore. The proportion of cases attributed to water-borne transmission ranged from 0.01 [95%UI 0.00-0.22] for salmonellosis to 0.39 [95%UI 0.03-0.72] for cryptosporidiosis. Norovirus had the greatest number of water-borne cases (479,632 [95%UI 0-1,111,874]) followed by giardiasis (178,275 [95%UI 6,147-418,023]) and *Campylobacter*iosis (85,140 [95%UI 0-247,681]). Deaths were attributed to water-borne transmission of *Campylobacter*iosis (six [95%UI 0-17]), norovirus (four [95%UI 0-9]), and salmonellosis (one [95%UI 0-20]). The water-borne DALY burden was greatest for *Campylobacter*iosis (2,004 [95%UI 0-5,831]), giardiasis (280 [95%UI 10-658]) and norovirus (244 [95%UI 0-566]). Attribution was made at the point of human exposure and disease caused by food contaminated through exposure to dirty water was not attributed to water-borne transmission. Therefore, improvements in water quality has potential to lessen the burden of both water- and food-borne disease. These data can inform Australian guidelines relating to water used for drinking, food production and recreation.

592

DETECTING AND ENUMERATING SOIL-TRANSMITTED HELMINTH EGGS IN SOIL: NEW METHOD DEVELOPMENT AND RESULTS FROM FIELD TESTING IN BANGLADESH AND KENYA

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Globally, about 1.5 billion people are infected with at least one species of soil-transmitted helminth (STH). Soil is a critical environmental reservoir of STH, yet there is no standard method for detecting STH eggs in soil. We developed a field method for enumerating STH eggs in soil and tested it in Bangladesh and Kenya. We optimized a method, based on a US EPA method for enumerating Ascaris in biosolids, through a series of recovery efficiency experiments; we seeded soil samples with a known number of *Ascaris suum* eggs and assessed the effect of protocol modifications on egg recovery. We found the recovery efficiency increased when we used 1% 7X as a surfactant compared to 0.1% Tween 80 and two centrifuge flotation steps compared to one. Other protocol modifications, such as sample mixing and sedimentation time, did not impact recovery efficiency. Soil type affected the egg recovery efficiency; sandy samples resulted in higher recovery efficiency compared to loamy samples processed using the same method. We documented a recovery efficiency of 73% for the final optimized method. Soil samples from 100 households in Bangladesh and 100 households in Kenya were processed with the optimized method from June to November 2015. Field staff collected soil samples from the surface layer of soil directly adjacent to the doorway of the house entrance. Both the prevalence and concentration of STH eggs in soil was higher in Bangladesh than in Kenya. In our field tests, we found the prevalence of any STH egg in soil was 78% in Bangladesh and 37% in Kenya. In Bangladesh and Kenya, *Ascaris* was the most common STH (67% and 22%) followed by Trichuris (36% and 21%). We did not detect hookworm eggs in either country, suggesting the method may not be appropriate for hookworm enumeration. The median concentration of STH eggs in soil in positive samples was 0.64 eggs/g dry soil in Bangladesh and 0.15 eggs/g dry soil in Kenya. The proportion of STH eggs determined to be viable was similar in both countries (85.7% in Bangladesh and 83.6% in Kenya). This new method is feasible for detecting STH eggs in soil in low-resource settings and could be a key tool for standardizing soil STH detection globally.

593

EVALUATING BEHAVIOR CHANGE IN SINGLE AND COMBINED INTERVENTIONS OF A LARGE-SCALE WATER, SANITATION, HYGIENE AND NUTRITION INTERVENTION TRIAL (WASH BENEFITS), IN RURAL BANGLADESH

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Promoting multiple behavioral interventions together risks limiting sustained adoption of the individual behaviors. WASH Benefits, a large scale efficacy trial, randomly allocated 720 clusters of 5551 pregnant women to a control group, single interventions (water [W], sanitation [S], handwashing [H], nutrition [N]), or combined interventions (WSH and WSHN). Enabling hardware and behavior change was promoted by trained local community health promoters through periodic household visits. In samples of intervention households, we conducted monthly observations beginning 3 months after initiation of the intervention, starting November 2012 to October 2014, to monitor intervention uptake. We analyzed and compared uptake among households receiving single (W, S, H or N) versus combined (WSH & WSHN) interventions. Observed dual pit latrine uptake did not differ significantly among the arms (S: 70%, WSH: 70%, WSHN: 72%; p>0.05). A slightly higher proportion of of households in the single handwashing arm (93%) had water and soap present at the handwashing station near the latrine, compared to the combined intervention arms (WSH: 85%, WSHN: 87%; p<0.01). Detectable residual chlorine in stored water was somewhat higher in households receiving the single water intervention (76%) than combined interventions (WSH: 68%, WSHN: 67%; p<0.05). Among households who received the nutrition intervention, report of feeding lipid-based nutrient supplementation (LNS) to the child (6-24 months) was similar across the arms (N & WSHN: 84%; p>0.05). Rigorous implementation of interventions deployed at large scale achieved high levels of uptake in single and combined intervention arms. However, we found somewhat lower uptake of fully stocking handwashing stations and treating water among households receiving combined interventions compared to households receiving single intervention; uptake differences were small. High uptake of large scale combined WASH and WASHN interventions is possible in the context of an efficacy trial, though further work should assess their effectiveness under programmatic conditions.

594

WHO CAN AFFORD GPS POINTS? SCALING A VILLAGE-LEVEL WATER ACCESS INFORMATION SYSTEM IN RURAL ZAMBIA

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An ideal information system for monitoring a country's water system incorporates geocoordinates for all improved water points. However, the generation and maintenance of such an information system is extremely expensive in terms of both finances and human power. Furthermore, a database of geo-located water points alone is insufficient to determine water access of the population – the database must be matched to a population database to determine access. The Ministry of Local Government and Housing (MLGH) leveraged an established mobile-to-web information system of village-level access to sanitation to monitor village-level access to improved water points. The addition of 4 separate data elements allowed for the creation of two indicators: the percent village access of improved water and villages with improved water points that are not functioning. Using District Health Information System (DHIS2), we have seen large disparity in village-level water access within districts and have been able to utilize the information system to direct borehole drilling operations to areas most in need. Furthermore, the approximate geolocation of the water point is being made available through village geo-coordinates. Zambia is now scaling village-level water access monitoring to all rural districts. In addition to demonstrating the power of monitoring water access, we discuss challenges, solutions and opportunities in developing and sustaining nationwide village-level water access monitoring in Zambia.

595

QUANTIFYING THE ROLE OF FAILING WATER AND SANITATION INFRASTRUCTURE ON HEALTH, HEALTHCARE COSTS AND SOCIETAL WELLBEING DURING VARYING DISASTER SCENARIOS

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The United Nations Office for Disaster Risk Reduction (UNISDR) estimates that 100 million people are affected by disasters annually, while climate change remains the biggest global health threat of the 21st century. The relationship between climactic variation, environment and zoonotic and vector-borne diseases is well established. A mounting body of evidence further suggests that climate change poses a significant threat to food security, and to microbial, chemical and physical food and water safety. Climate-related food-borne illnesses are predicted to disproportionately affect poor, elderly and young populations, and to be pronounced in low resource settings, where public health infrastructure and human resource capacity is limited or fragile. We created a series of models to estimate the impact of various disaster scenarios that result in varying levels of levels and trends in the quality and scope of key water provision, sanitation and health care delivery infrastructure. A multi-level model was developed encompassing a matrix-map of the interactions between key drivers of water quality, access, provision, and scarcity. Key inputs to health care service quality, accessibility and coverage were used to determine the change in the likely consequences of any breakdowns on these key drivers. This model uses variables from four categories of data; disease incidence, critical infrastructure, climactic variables and food security, to develop estimates of changes in population wide disease burden. These results are then used, along with data on healthcare infrastructure, to estimate marginal impact on healthcare costs, and resulting impact on households, as well as the wider social burden resulting from greater disease burden within the population, such as growth and cognitive development in children and impacts on education, and lifetime earnings. Ultimately the value of this model will be in developing a 'currency' for risk that not just improves our understanding of the true costs to society of various levels of risk of major adverse events, but also allows us to measure the return on investment on early interventions.

596

A RANDOMIZED, PLACEBO-CONTROLLED, DOUBLE-BLIND PILOT STUDY OF SINGLE-DOSE HUMANIZED ANTI-IL5 ANTIBODY (RESLIZUMAB) FOR THE REDUCTION OF EOSINOPHILIA FOLLOWING DIETHYLCARBAMAZINE TREATMENT OF *LOA LOA* INFECTION

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Diethylcarbamazine citrate (DEC) treatment of loiasis is complicated by adverse reactions that are correlated with the number of circulating microfilariae (mf). The cause of these reactions is unknown, but they are accompanied by a dramatic interleukin-5 (IL-5)-dependent increase in eosinophilia and evidence of eosinophil activation. To explore the role of IL-5 driven eosinophilia on post-DEC reactions, 8 adults with parasitologically-confirmed loiasis and <5000 mf/mL blood were enrolled on a randomized, double-blind, placebo-controlled trial of the humanized anti-IL-5 antibody, reslizumab, (1.0 mg/kg IV) administered 3 to 7 days prior to initiation of DEC treatment (9 mg/kg/day for 21 days). Subjects were assessed prior to, at days 1, 2, 3, 5, 7, and 14 and months 1, 3, 6, 12, 18 and 24 post-DEC treatment. The primary endpoint was the reduction in absolute eosinophil count (AEC) during the first week of DEC treatment. Secondary outcomes included the severity of post-treatment adverse events (AE), markers of eosinophil activation, and mf clearance. Baseline characteristics were comparable between the two groups. Single dose reslizumab lowered the AEC by 77% prior to initiation of DEC therapy (vs. a 12% decrease in the placebo group, $p<0.05$). More importantly, reslizumab significantly reduced AEC in the first week of DEC treatment, with peak AEC remaining below baseline in all subjects who received reslizumab and in none of the placebo subjects. Mf clearance occurred within 2 days of initiation of DEC in all 7 mf+ subjects. Mild to moderate AEs were seen in all 8 subjects and were not significantly different between the reslizumab and placebo groups. In summary, although reslizumab was able to block peripheral eosinophilia post-DEC treatment in subjects with loiasis and had no effect on microfilarial clearance, the reduction in AEC appears to have been insufficient to prevent post-treatment AEs. Assessment of eosinophil activation and cytokine profiles is ongoing.

597

MOLECULAR DETECTION OF *ONCHOCERCA VOLVULUS* IN SKIN BIOPSIES FROM THE DEMOCRATIC REPUBLIC OF THE CONGO (DRC)

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Defining the optimal diagnostic tools for evaluating onchocerciasis elimination efforts is paramount. The sensitivity of skin skip microscopy decreases as microfiladermia is suppressed, highlighting the need for molecular tools. We assessed the ability of a pan-filarial real-time PCR with melt curve analysis (qPCR-MCA) tool to detect *Onchocerca volvulus* (OV) in residual skin snip biopsies and evaluated the performance of this tool relative to microscopy. Residual skin snip biopsies were collected from 471 people during an onchocerciasis survey in Kisangani, DRC, an area co-endemic for OV, *Mansonella* spp. (MSPP) and *Loa loa* (LL). Melting-temperature (T_m) ranges for species identification were determined using known gDNA. By qPCR-MCA, 43.5% (205) of the samples were negative, 47.5% (224) had OV, 3.8% (18) had MSPP, and 5.1% (24) had LL. An OV specific qPCR (qPCR-O150) was run as validation for all qPCR-MCA(+) samples and 25% of the qPCR-MCA(-) samples. There was 100% concordance for negative samples, and 97% concordance among positive samples: 5 were qPCR-MCA(+) but qPCR-O150(-) while 3 were qPCR-O150(+) but qPCR-MCA(+) for either LL or MSPP but not OV. Sequencing was done for 61 samples because they had a non-OV T_m or a dissociation curve suggestive of mixed infection. Of these, 15 had LL, 30 had *M. perstans* (MP), 6 had OV, and 8 had mixed template chromatograms. Overall, 43.5% of skin snips were negative, 43.7% had OV, 3.0% had LL, 3.2% had MP, and 6.6% had ≥ 1 species. The sensitivity and specificity of microscopy was 79.5% and 95.5% compared to qPCR-MCA and 80.6% and 96% compared to qPCR-O150. Skin snip microscopy was less sensitive than qPCR even in a hyper-endemic setting that received yearly ivermectin. The qPCR-MCA identified 30 cases other filariae that were sequence confirmed. Although this assay detected OV, it was not sufficiently robust to differentiate all species in mixed infections, which had to be resolved by species-specific PCRs. Nevertheless, the qPCR-MCA assay is a useful, rapid screening method able to detect OV and identify samples with mixed infections which will be invaluable for validating other diagnostic assays.

598

TOLL-LIKE RECEPTOR 2 EXPRESSION ON IMMUNE CELLS IS ELEVATED IN CURED ASYMPTOMATIC INDIVIDUALS IN LYMPHATIC FILARIASIS

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Various clinical manifestations observed in lymphatic filariasis-endemic (LF-endemic) areas may be partly due to alterations in innate and adaptive immune cell populations expressing toll-like receptor 2 (TLR2) and toll-like receptor 4 (TLR4). The endosymbiotic bacteria *Wolbachia*, have been shown to induce inflammatory responses that are mediated primarily by the toll-like receptors. The use of the mass control drugs, ivermectin and albendazole, and the macrofilaricide doxycycline introduces a new dynamic in the immune response profiles of individuals in LF-endemic areas. In a study conducted in the Western region of Ghana where therapy has been ongoing for a minimum of 5 years, whole blood from a cohort of 428 individuals comprising 15 patent, 64 latent, 98 endemic normals, 101 lymphedema and 150 previously asymptomatic infected but now uninfected ("cleared infection"), were stimulated in 96-well microtiter plates with the TLR-specific ligands PamCSK4, LPS and HKLM. The expression of TLR2 and TLR4 on innate and adaptive immune cells were measured by flow cytometry. Expression of TLR2 was highest on monocytes ($CD14^+$), a dendritic cell sub-population ($CD11c^+$int), a macrophage sub-population ($CD33^+$high) and $CD4^+$ T cells in "cleared infection" individuals. Additionally, significant differences in TLR2 expression were observed between the "cleared infection" and latent individuals ($p < 0.05$) on monocytes; "cleared infection" and lymphedema pathology individuals ($p < 0.0001$) and "cleared infection" and endemic normal individuals ($p < 0.0001$) on $CD11c^+$int; "cleared infection" and lymphedema individuals ($p < 0.0001$ and $p < 0.05$) on $CD33^+$high and $CD4^+$ T cells, respectively. However, no trend or pattern was observed in TLR4 expression on innate and adaptive immune cells in both infected and uninfected individuals, with observed levels being <4% in all cell types examined. Our findings show that therapy leads to heightened recognition of pathogen-associated molecular patterns by TLR2 on innate and adaptive immune cells, and a recovery in immune response in asymptomatically infected LF individuals who have cleared the infection.

599

IN VITRO-GENERATED AND *EX VIVO*-ISOLATED HUMAN DENDRITIC CELLS RESPOND SIMILARLY TO LIVE MICROFILARIAE OF *BRUGIA MALAYI*

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Infection by *Brugia malayi*- one of the two major species causing lymphatic filariasis in humans- has been classified by dysregulation of dendritic cells (DC) most associated with microfilarial (mf) antigen exposure. Our previous data have shown that live mf both induce apoptotic cell death and inhibit the mammalian target of rapamycin (mTOR) in human monocyte-derived DC (moDC) generated *in vitro*. The mTOR signaling pathway is an important regulator of cellular metabolism, proliferation, growth, and autophagy. Recent data suggest phenotypic and functional differences exist between *in vitro* generated moDCs and the myeloid DC (mDC) isolated directly *ex vivo* from blood that could lead to a misconstruing of the importance of our *in vitro* findings. Therefore, we sought to compare the responses of these two DC populations to live mf. Elutriated monocytes from healthy volunteers were either cultured with IL-4 and GM-CSF to generate moDCs or sorted *ex vivo* using an antibody cocktail to isolate mDCs ($CD11c^+$/HLA-DR^+/$CD14^-$/$CD16^-$/$CD1c^+$). Once generated, both cell types were exposed to either media alone, live mf, LPS (an mTOR activator), or rapamycin (mTOR inhibitor) for 1 hr. Using immunoblot analysis, we demonstrate that mf, similar to rapamycin, significantly downregulate the phosphorylation of mTOR (and its downstream effectors p70S6K, and 4EBP1 $p<0.05$) in both moDC and mDC. Interestingly, the rapamycin-like effects of mf are also observed when mf are physically separated from DC using transwells. Because cell contact is not required for mf impairment of the mTOR pathway in DC, we suggest that excretory/secretory products from live mf may mediate this effect. The similarity of these two cell types (moDC and mDC) in response to mf extends beyond mTOR inhibition to the induction of apoptotic cell death. When exposed to live mf, both cell types have a marked (~7-fold) increase in cell death as measured by propidium iodine positivity by flow cytometry. Together, these data suggest that while there are phenotypic and functional differences between moDC and mDC, with respect to responses to live mf (and mTOR inhibitors), they appear to function similarly.

600

ONCHOCERCA VOLVULUS PROTEOME-WIDE LINEAR EPITOPE SCANNING USING HIGH-DENSITY PEPTIDE MICROARRAYS AND CONFIRMATION OF IMMUNODOMINANT MOTIFS BY ELISA

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We have employed high-density peptide microarrays containing 832,709 partially overlapping 15-mer peptides covering the entire *Onchocerca volvulus* proteome, and screened serum samples of Onchocerciasis patients (n=12) and controls (n=6) for immunoreactivity. After selection of cut-offs for signal intensity and significance, approximately 2000 immunoreactive peptides were identified, of which at least 290 could be considered immunodominant. This pool of immunodominant peptides was further divided into clusters, each cluster characterized by the presence of a motif of 5 or 6 amino acids with invariant anchor points and variability around these anchors. Hence, all peptides belonging to a cluster had at least one such motif. Three of these motifs were strongly represented, e.g. motif 1 was found in 74 peptides, motif 2 in 39 peptides, and motif 3 in 37 peptides. These peptides were found in a large variety of apparently non-related OVOC proteins. For each of these three motifs, we have used at least four peptides for development of a peptide ELISA. Sensitivity and specificity for each peptide was determined using 21 plasma samples from *Onchocerca*-confirmed individuals (FR3 repository), and 187 control plasmas from non-*Onchocerca* endemic regions (healthy, and infections with HIV, HCV, Dengue, *Brugia, Wuchereria* and *Loa*). Within one cluster, the antibody reactivity against e.g. peptide 1 could be inhibited by adding e.g. peptide 2 (from a non-related gene) in solution in ELISA. Sensitivity range for peptides containing motif 1 was between 90.5 % and 100%; and specificity range was between 91.4 % and 94.1 % (results for motif 2 and 3 are pending). The immune response against these peptides was mainly IgG1, IgG3, IgM and IgE. The key amino acids responsible for the immune recognition were determined by micro-array epitope scanning. Taken together, our data demonstrate that part of the humoral immune response induced by infection with *O. volvulus* is directed against a small number of highly abundant peptide motifs present in apparently structurally nor functionally related OVOC genes.

601

THE EFFECT OF *LITOMOSOIDES SIGMODONTIS* INFECTION ON IGE-MEDIATED ANAPHYLAXIS IN SENSITIZED MICE

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IgE-mediated anaphylaxis is a life-threatening condition. Binding of IgE with its cognate antigen causes basophils and mast cells to rapidly release pre-formed inflammatory mediators. While numerous animal studies have reported that helminths can prevent allergic sensitization, only 4 studies have evaluated the effects of infection on pre-existing allergy. Since chronic helminth infection suppresses basophil and mast cell function, we hypothesized that chronic infection would protect against the clinical symptoms of anaphylaxis in previously sensitized mice. Mice were sensitized by weekly intraperitoneal (IP) injection of either ovalbumin (OVA)/alum or PBS/alum for 3 weeks. At 5 weeks, mice were infected with *Litomosoides sigmodontis* (*L.s.*), a rodent filarial parasite, or mock-infected. Ten weeks post-infection, immunological and clinical parameters were measured before and after IP challenge with OVA. In sensitized mice, chronic *L.s.* infection resulted in serum levels of OVA-specific IgE that were an average of 50% lower than those observed for mock-infected mice (6,296 vs. 13,056 pg/mL, p-value- 0.1494). Chronic infection also caused a modest reduction in OVA-specific IgG2a levels. Following challenge, serum levels of murine mast cell protease 1 were significantly lower in sensitized mice that were *L.s.*-infected as compared to mock infected (86,276.81 vs. 276,635.2 pg/mL, p- value= 0.0385). With respect to clinical symptoms, *L.s.*-infected mice that had been previously sensitized exhibited an average drop in core body temperature of 4.3 °C after 1 hour. Although less than the average 6.3°C drop observed in sensitized and mock-infected mice, the difference was not statistically significant. These results suggest that chronic helminth infection reduces allergen-specific IgE levels and allergen-driven mast cell degranulation in previously sensitized animals.

602

MIXTURE MODELING TO DETERMINE POPULATION-SPECIFIC CUTOFFS FOR IMMUNOLOGIC ASSAYS IN NEGLECTED TROPICAL DISEASE SETTINGS APPROACHING ELIMINATION

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As Neglected Tropical Disease (NTD) programs succeed and transmission intensity declines, the ability to discriminate between positive and negative antibody tests becomes increasingly challenging. Previous techniques for defining diagnostic test cutoffs are no longer sufficient as prevalence rates decline towards zero. With varying degrees of non-specific background reactivity across populations and the absence of a gold standard, an objective yet flexible approach for cutoff determination is needed. Mixture modeling allows for the probabilistic representation of subpopulations within an overall population. By fitting a mixture model to continuous data, members of the overall population can be assigned to groups (e.g., positive and negative), and the uncertainty of that classification can be calculated based on the associated conditional probabilities. These groups can be used to create an absolute cutoff and a pre-specified indeterminate range (e.g. greater than 5% uncertainty), resulting in positive, negative and indeterminate classifications. The number of groups may be specified in advance or optimized by an algorithm using model selection criteria, such as the Bayesian Information Criterion. We performed mixture modeling on standardized ELISA results from two post-treatment NTD settings. Antibody responses to a lymphatic filariasis recombinant antigen (Wb123) were analyzed via normal mixture modeling utilizing a two-group model which yielded a cutoff of 0.092 and an indeterminate range of (0.082, 0.100). This corresponded to 96.1% negative, 2.3% indeterminate and 1.5% positive results. The same methods were used to analyze responses to a recombinant onchocerciasis antigen (Ov-16), resulting in a cutoff of 0.63 (0.57, 0.68) with 96.7% negative, 1.5% indeterminate and 1.8% positive results. These results demonstrate the utility of mixture modeling as a tool to provide population-specific diagnostic cutoffs with a corresponding indeterminate group that reflects our certainty regarding the cutoff. Such an approach may benefit other neglected and infectious disease programs driving towards elimination.

603

THE LIVE ATTENUATED CHIMERIC VACCINE RWN/DEN4Δ30 IS WELL-TOLERATED AND IMMUNOGENIC IN HEALTHY FLAVIVIRUS-NAÏVE ADULT VOLUNTEERS 50-65 YEARS OF AGE

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West Nile Virus (WNV) is the leading vector-borne cause of meningoencephalitis in the U.S. Many infections are asymptomatic. Severe illness is most common in the elderly, including hepatitis and meningitis. Complications include: paralysis, coma, and death. The second largest outbreak of WNV in the U.S. occurred in 2012 (5,674 cases, including 2,873 cases of severe neurologic disease, 286 deaths). The NIH candidate vaccine is a recombinant live attenuated (LA) WNV vaccine based on the chimerization of wild type WNV (NY99 genome) with the LA dengue virus serotype 4 vaccine rDEN4Δ30. Previous vaccine studies were conducted in healthy volunteers at 103, 104 and 105. Based on the safety and immunogenicity data of previous studies, a dose of 104 was chosen. A randomized placebo controlled phase I trial was performed. 28 healthy flavivirus-naïve volunteers aged 50-65 were enrolled and randomized to receive 104 PFU of rWN/DEN4Δ30 or placebo at 0 and 6 months. Neutralizing antibody (Nab) to WNV was measured at day 28, 56, 90, and 180 following first vaccination and days 208, and 236 following second vaccination. Nab was measured to PRNT50 against WT WNV conducted in a BSL3 lab at LID. Seroconversion was defined as > 4 fold rise in Nab titer to the wild-type WNV parent virus at study day 90 post first vaccination compared with day 0. Following first dose, 3 subjects had detectable viremia with a maximum peak titer of 0.7log10 PFU/mL. No subject was viremic following second dose. 95% of vaccines seroconverted following first dose. The Geometric mean peak titer (GMT) at day 90 was 65.3, D180 was 27.63 and D360 was 34.52. Main AEs reported were similar in placebos: headache (25% vaccinees (V), 12.5% placebo (P)), Fatigue 10% (V), 37% (P), and nausea 25% (V), 12.5 (P). rWN/DEN4Δ30 was found to be well tolerated and immunogenic in adults aged 50-65. Vaccination induced a 95% seroconversion rate at day #90 after a single dose. As WN is more severe in patients over 50, these results underscore the potential use for rWN/DEN4Δ30 vaccine in this population, given the unpredictable and sporadic outbreaks of WNV.

604

ACUTE AND DELAYED MORTALITY FOLLOWING WEST NILE VIRUS INFECTION IN TEXAS

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West Nile virus (WNV) has a well characterized acute disease process; however, the long term survival and contributors of death at a population level are not well understood. In this study, we sought to investigate all-cause and cause-specific mortality following WNV infection.Our study

population consisted of 4144 Texas residents who were infected with WNV from 2002-2012. Out of this population, we identified 554 deaths (13%). Our analysis focused on both acute deaths (n=286) occurring < 90 days after infection, and delayed deaths (n=268) occurring among those who survived the first 90 days. Standardized mortality ratios (SMRs) adjusted for age, sex, and calendar year were calculated according to ICD10 chapters. We found a substantial number of reported WNV cases died within the first 90 days of infection (286 out of 4144; 7%), primarily due to their WNV illness or an unspecified infectious origin. Delayed mortality occurred in 10% (210/2113) of initially surviving patients diagnosed with West Nile Neuroinvasive disease (WNND) and in 3% (58/1742) of those diagnosed with West Nile Fever (WNF). WNND cases experienced increased risk of delayed death due to infectious (SMR: 4.7, 95%CI: 3.2-6.9) and renal causes (2.6, 1.4-4.7). We also found increased risk for all-cause mortality in those who were under 60 years of age at the time of infection (SMR 1.98, 95% CI: 1.5-2.6) but not among those over 60 years (0.99, 0.8-1.2). Cases under 60 years exhibited increased risk of delayed mortality due to renal (11.4, 4.7-27.3), infectious (5.3, 2.5-11.2), digestive (3.9, 1.9-8.1), and circulatory (2.0, 1.2-3.4) causes. We present the first population-level evidence of acute and delayed mortality among a considerable proportion of patients with a history of WNV infection. Our data provide further evidence to the literature supporting continued morbidity and mortality years after WNV infection.

605

A SINGLE MUTATION IN THE ENVELOPE PROTEIN ALTERS FLAVIVIRUS ANTIGENICITY, STABILITY AND PATHOGENESIS

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Flaviviruses include clinically significant pathogens such as West Nile (WNV), dengue (DENV), and Zika (ZIKV) viruses. Flaviviruses particles are covered with a dense array of three-domain (DI, DII, DIII) envelope (E) proteins, which are targeted by neutralizing antibodies following infection or vaccination. The structural flexibility or 'breathing' of flavivirus envelope (E) proteins allows virions to sample an ensemble of conformations at equilibrium. The molecular basis and functional consequences of virus conformational dynamics are poorly understood. By analyzing a large panel of WNV E variants, we identified mutations capable of changing the structural ensemble sampled by virions at equilibrium. Here, we describe the antigenic and biological consequences of a mutation at residue 198 (T198F) of the E DI-DII hinge. T198F displayed increased sensitivity to neutralization a monoclonal antibody targeting a poorly exposed epitope in the DII fusion loop. Increased exposure of this cryptic epitope was accompanied by changes in virus stability; following prolonged incubation in solution at physiological temperatures, the T198F mutation resulted in a 3-fold reduction in the half-life of infectious WNV. Introduction of a mutation at the analogous residue of the dengue virus E protein similarly increased accessibility of the fusion loop epitope and decreased virus stability in solution, suggesting that this residue modulates the structural ensemble sampled by distinct flaviviruses at equilibrium. Despite resulting in a 3-fold reduction in the stability of WNV in solution, the T198F mutation did not substantially impair *in vitro* replication kinetics. However, *in vivo* studies in mice revealed that the T198F mutation attenuates lethal WNV infection in a B-cell dependent manner. Overall, our study provides insight into the molecular basis and the *in vitro* and *in vivo* consequences of flavivirus breathing that has the potential to inform the design of effective vaccines and therapeutic agents.

606

AN OPTIMIZED SYNTHETIC TLR-4 AGONIST ADJUVANT FORMULATION INDUCES DURABLE AND FUNCTIONAL IMMUNITY WHEN COMBINED WITH A CLINICAL-STAGE RECOMBINANT WEST NILE VIRUS VACCINE ANTIGEN

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West Nile virus (WNV) is a mosquito-transmitted member of the Flaviviridae family that has emerged in the 21st century to become a public health threat. Given the sporadic nature of WNV epidemics both temporally and geographically, there is an urgent need for a vaccine that can rapidly provide effective immunity. Protection from WNV infection is correlated with antibodies to the viral envelope (E) protein, which encodes receptor binding and fusion functions. Despite many promising E-protein vaccine candidates, there are currently none licensed for use in humans. This study reports the optimization of a WNV vaccine candidate containing a clinical-stage WNV recombinant E-protein antigen (WN-80E) and a TLR-4 agonist adjuvant containing a synthetic Lipid A TLR-4 agonist (SLA) and the saponin QS21 (SLA-LSQ). We have optimized adjuvant components for rapid induction of potent antiviral immunity in murine models, and find that both SLA and QS21 individually stimulate the production of multi-functional Th1 CD4+ T-cells (IFNγ+/TNFα+/IL-2+), as well as an increase in the number of germinal center B-Cells (CD95+/GL7+) in a dose dependent manner. Consistent with induction of Th1 biased cellular immunity, the humoral response following adjuvanted immunization in mice is focused toward production of class-switched IgG2c antibodies, resulting in high levels of virus neutralization activity. Importantly, we observe significantly increased neutralizing titers in mice given formulations which contain both SLA and QS21 compared to either component alone. Using an optimized vaccine formulation, we demonstrate induction of durable immunity (300 days) following a single immunization in mice, and stimulation of functional protective immunity in a Syrian hamster challenge model of WNV disease. Taken together, these studies demonstrate the utility of SLA-LSQ adjuvant formulations for induction of functional and durable West Nile Virus immunity.

607

DIFFERENTIAL MECHANISMS OF WEST NILE VIRUS-INDUCED PATHOGENESIS IN BIRDS

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West Nile virus (WNV) is maintained in North America in an enzootic cycle between highly susceptible passerine birds and mosquitoes. However, the mortality rates and viremia profiles of birds in response to WNV infection vary dramatically between viral strains and avian species. For example, American crows (AMCRs) inoculated with the NY99 strain of WNV manifest high viremias with concomitantly high mortality rates. However, AMCRs inoculated with a strain of WNV containing a pro-to-thr mutation at amino acid 249 of NS3 (NS3-249T) exhibit significantly lower viremias and mortality rates. These viremia profiles are inversely correlated with interferon (IFN)-α expression. In contrast, we found that the viremia and mortality rate of the NS3-249T virus is higher than that of the WT NS3-249P virus in 2-day-old chicks. In order to better understand these viral- and host-specific differences in WNV replication, a chicken fibroblast

cell line was infected with NS3-249T or 249P viruses, with and without chicken IFN-α pre-treatment. While both viruses were sensitive to IFN-α treatment, the NS3-249T virus replicated to a higher titer, suggesting the increase in chick viremia *in vivo* may be due to increased intracellular replication, rather than a differential innate immune response. To identify the cell populations contributing to the replication of WNV in chicks, 2 day-old chicks were inoculated with a WNV mutant restricted for leukocyte replication through the insertion of multiple leukocyte-specific miRNA target sequences into the 3' UTR. Previous studies have shown that a leukocyte-restricted WNV mutant replicates poorly in AMCRs, suggesting leukocytes are important sites of replication. Unexpectedly, the leukocyte-restricted virus exhibited increased viremia and mortality in chicks. This suggests that chick and AMCR leukocytes play very different roles during *in vivo* WNV infection as critical sites of viral replication or effectors of innate immune responses, respectively. Further studies of infected birds, including sequencing of the avian transcriptome, will help to define the avian innate immune response to WNV infection.

608

THE EFFECT OF CO-INFECTION WITH DENGUE, CHIKUNGUNYA AND ZIKA VIRUS ON VECTOR COMPETENCE OF *AEDES* MOSQUITOES

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With the recent emergence of both Chikungunya virus (CHIKV) and Zika virus (ZIKV) in the Americas, there are now three arboviruses cocirculating in large areas of South and Central America that are all transmitted by *Aedes* mosquitoes: dengue virus (DENV), CHIKV and ZIKV. Clinical signs can be similar and while cases of coinfections have been reported to occur, the incidence rate of these is unknown and may be underestimated due to the lack of virus specific diagnostic tools. Coinfected viremic patients could expose feeding mosquitoes to multiple arboviruses. Interestingly, in 2015 an increase in CHIKV infections coincided with a drop in dengue cases in Mexico and Colombia. While this could be due to yearly variation, it could also be related to the introduction of CHIKV which may be outcompeting DENV in mosquitoes. However, the impact of coinfection on the ability of relevant mosquitoes to transmit any of these viruses (i.e. their vector competence) has not been determined. Therefore, we determined the competence of *Ae. aegypti* (Poza Rica, Mexico) and *Aedes albopictus* (Florida) mosquitoes exposed to bloodmeals containing more than one *Aedes*-borne arbovirus. Specifically, mosquitoes were given a blood meal containing American strains of DENV-2, CHIKV or ZIKV in single infections, as well as combinations of the three viruses as double and triple infections. Mosquitoes were kept for 5, 7, 9 and 14 days extrinsic incubation, at which point mosquito bodies, legs and saliva were collected to determine infection, dissemination and transmission rates. Presence of viral RNA was determined by multiplex qRT-PCR for DENV-2, CHIKV and ZIKV in order to determine RNA levels for the individual viruses in mosquitoes exposed to more than one virus. Preliminary results suggest that coinfection may impact vector competence in a virus-specific manner.

609

THE INFLUENCE OF GENETIC BOTTLENECKS, RNA INTERFERENCE-MEDIATED DIVERSIFICATION AND SELECTIVE CONSTRAINT ON THE EVOLUTION OF A TICK-BORNE VIRUS

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Within hosts, flaviviruses exist as a heterogeneous population of closely related viral variants termed a mutant swarm. While considerable attention has been directed toward studying the population dynamics of mosquito-borne flaviviruses, little is known about tick-borne flavivirus populations. This is disconcerting due the increased public health relevance of Powassan virus (POWV), the sole North American member of the tick-borne encephalitis complex. Therefore, we assessed the population complexity of POWV lineage II (deer tick virus; DTV) in both a vertebrate host and invertebrate vector and examined the influence of RNAi targeting on DTV populations. 11-week old mice were intra-peritoneally infected with DTV and subsequently fed upon by Ix. scapularis larvae and nymphs. Infected ticks were collected at each life stage through adulthood and RNA-Seq and sRNA libraries were prepared, sequenced and analyzed. We found that DTV populations were subject to a strong bottleneck once introduced into mice, but then rapidly diversified. Subsequently, the populations experienced another severe bottleneck during horizontal transmission to ticks. During transstadial transmission, however, diversification was constrained by strong purifying selection. Despite the relatively limited diversity observed in ticks, we found that RNAi targeting intensity was significantly positively correlated with the presence of intrahost single nucleotide variants (iSNVs); findings similar to what has previously been described for West Nile virus (WNV) in mosquitoes. While the overall DTV population dynamics greatly differ from those of WNV, these findings provide experimental evidence consistent with DTV ecology and support their slow long term evolutionary trends. Together, these data highlight that selective pressures incurred at a molecular level, such as RNAi, may be similar for most if not all arthropod-borne flaviviruses, but that differences in viral ecology can greatly influence the population dynamics of individual flavivirus members.

610

MOSQUITO MIDGUT FREP1 IS A POTENTIAL UNIVERSAL MALARIA TRANSMISSION-BLOCKING VACCINE

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Malaria remains a devastating disease. Transmission-blocking vaccines (TBVs) have been recently considered as a promising approach for the elimination and eradication of malaria. We recently discovered a mosquito midgut protein FREP1 that facilitates parasite transmission through direct binding to parasites and it is easily accessible to antibodies co-ingested with blood. Here, we demonstrated that anti-FREP1 antibodies blocked transmission of multiple *Plasmodium* species (*Plasmodium berghei*, *Plasmodium vivax,* and *P. falciparum*) to multiple *Anopheles* species (*A. gambiae* and *A. dirus*). Sequence comparison of FREP1 orthologs found that a fibrinogen-like (FBG) domain is highly conserved (>90% identity) among *Anopheles* species from different continents, while the sequence similarity between FBG and human fibrinogens is only about 10%. Immunization of mice with purified recombinant FREP1 shows no significant difference of alanine aminotransferase activity between anti-FREP1 serum and the pre-immune serum. Moreover, the anti-FREP1 serum did not show any cross-reactions with human fibrinogens. Thus, FREP1 is non-toxic or unlikely to cause autoimmune response in mammals. Notably, mice immunized with purified FBG effectively blocked *P. berghei* transmission (82.1% and 70.1% blockade) to *An. gambiae* in two independent bioassays respectively. Anti-FREP1 serum from the immunized mice also blocked over 90% infection of *P. falciparum* in standard membrane-feeding assays (SMFA). In summary, our data support that FREP1 is a promising universal TBV antigen to block the transmission of multiple *Plasmodium* species to multiple *Anopheles* species.

611

SKIN SCARIFICATION WITH *PLASMODIUM FALCIPARUM* CS PEPTIDE VACCINES USING SYNTHETIC TLR AGONIST ADJUVANTS ELICITS CHEMOKINE/CYTOKINE PATTERNS THAT CORRELATE WITH INDUCTION OF SPOROZOITE NEUTRALIZING ANTIBODIES

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Sterile immunity can be elicited by immunization with sporozoites, however, the production, storage and administration of whole parasite vaccines present numerous logistical hurdles. Vaccination by skin scarification (SS), as used for mass immunization during the Smallpox Eradication Programme, may more closely mimic the natural route of sporozoite inoculation by mosquito bite. We investigated SS immunization using synthetic peptides containing minimal T and B cell epitopes of *Plasmodium falciparum* CS protein combined with TLR agonists as adjuvants. In a murine model, SS immunization with CS peptide in the oil emulsion AddaVax containing TLR-7/8 and -9 agonists, but not AddaVax without TLR agonists, elicited high levels of systemic sporozoite neutralizing antibody, Th1- type CD4+ T cells and resistance to challenge by bites of mosquitoes infected with transgenic rodent parasites expressing *P. falciparum* CS repeats. Immunogenicity of SS delivered vaccine was demonstrated with either branched or linear peptide containing minimal T and B cell epitopes, indicating that induction of sporozoite neutralizing antibody was not dependent on antigen form. Standard serological assays for measuring the magnitude, fine specificity or affinity of anti-repeat antibodies were not predictive of the differences in levels of sporozoite neutralizing antibodies detected in functional assays based on transgenic parasites. To determine the immunological mechanisms relevant to the initiation of the enhanced levels of sporozoite neutralizing antibodies, we examined a panel of cytokines and chemokines elicited by SS using various TLR agonists alone and in combination. Cellular sources of these cytokines/chemokines were examined by immunohistology and flow cytometry using fluorescent reporter cells including Langerhan cells and dendritic cells. Patterns of chemokines/cytokines were detected in serum at early time points that correlated with enhanced immunogenicity of SS delivered vaccines.

612

HUMORAL IMMUNE RESPONSES TO AN ADJUVANTED SELF-ASSEMBLING PROTEIN NANOPARTICLE (SAPN) MALARIA VACCINE DISPLAYING THE NANP REPEAT AND αTSR REGIONS OF THE *PLASMODIUM FALCIPARUM* CIRCUMSPOROZOITE PROTEIN

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The Circumsporozoite Protein (CSP) is the predominant protein on the surface of the sporozoite and the most thoroughly studied pre-erythrocytic vaccine candidate. It is composed of an N-terminal domain, a conserved pentapeptide protease cleavage site termed region I, a tetra peptide NANP repeat region, a short conserved sequence termed region III, and a C-terminal region with sequence homology to the thrombospondin type-1 repeat superfamily (TSR). We have previously reported that a self-assembling protein nanoparticle, PfCSP-KMY-SAPN, presenting NANP repeats and three human HLA epitopes of the CSP induced very strong protective immune responses in mice. However, when tested in NHP, immune responses were low even when an adjuvant was used. To improve the vaccine induced immune response we have produced FMP014, a SAPN displaying NANP repeats and the entire αTSR domain of CSP. The αTSR in the SAPN uses two Cys-Cys disulfide bonds to stabilize the hydrophobic pocket and core link found in the native protein. Here we report the analysis of the humoral immune responses of C57Bl/6 mice to FMP014 combined with three different Army Liposomal Formulations containing monophosphoryl lipid A with or without QS-21, and Alhydrogel® (ALFA, ALFQ and ALFQA). Intramuscular injection of C57Bl/6 mice with each FMP014/ALF formulation induced high titer anti-NANP repeat, anti-αTSR domain, and cytophilic IgG2b antibodies with high avidity. However, FMP014 adjuvanted with ALF containing QS-21 (ALFQ) induced significantly higher anti-CSP specific antibodies to the NANP repeats and the αTSR domain compared to those without QS-21. Antibodies to both the NANP repeat region and the αTSR domain conferred protection to mice against challenge from an otherwise lethal dose of a transgenic *P. berghei* parasite expressing the full-length *Plasmodium falciparum* CSP.

613

IMMUNOMECHANISM OF PROTECTION FOR E140, A PRE-ERYTHROCYTIC VACCINE CANDIDATE

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A malaria vaccine to prevent infection is greatly needed and essential for a comprehensive malaria eradication program. Pre-erythrocytic (PE) antigens are capable of inducing an immune response resulting in sterile protection, as shown by the RTS,S vaccine. *Plasmodium falciparum* PE antigens are also targeted by efficacious whole sporozoite vaccines and could be the basis of a subunit vaccine. We have identified a single PE *P. yoelii* antigen (E140) capable of sterilely protecting CD1 mice in the range of 71% to 100%, alone and in combination with other antigens, respectively. Initial examination of E140-specific immune responses showed significant CD4+ and CD8+ T cell responses upon DNA-prime/Ad5-boost immunization. We also observed an antibody response that statistically correlated with sterile protection. We have further characterized the T cell responses to E140 immunization evaluating the function of CD4+ and CD8+ T cells, including IFN-gamma, TNF, IL-2, and MIP1alpha. In vivo T cell depletion experiments in mice are being conducted to ascertain the requirement of these cell types for protection. We are also conducting concomitant antibody transfer studies in mice to establish the role of anti-E140 antibodies to protection. These promising data are evidence of the potential of E140 and support further development of the *P. falciparum* E140 ortholog in a subunit malaria vaccine.

614

DEVELOPMENT OF A SEMI-SYNTHETIC WHOLE PARASITE VACCINE

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We are developing whole parasite vaccines to protect against the blood stages of malaria and showed that chemically attenuated vaccines can protect against rodent malaria parasites. This approach is being translated to the clinic. However, issues relating to production, storage and delivery present obstacles that impede development of this vaccine and other whole parasite approaches. We are therefore developing a synthetic vaccine delivery system in which killed blood stage parasites are encapsulated within liposomes that are targeted to dendritic cells using mannosylated lipid core peptides (MLCPs). MLCP-liposomes were taken-up efficiently by antigen presenting cells which then upregulated expression of MHC-II and co-stimulatory molecules, CD80 and CD86. Immunization

of mice with MLCP-liposome vaccine formulations, without adjuvant, generated enhanced levels of activated T cells in peripheral blood and vaccinated mice were completely protected from challenge infection with different species of rodent malaria. Liposome formulations are highly promising delivery systems for a human malaria vaccine.

615

CORD BLOOD ANTI-PFSEA-1 AND PROTECTION FROM SEVERE MALARIA IN INFANTS

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Plasmodium falciparum malaria is a leading cause of morbidity and mortality in developing countries, infecting hundreds of millions of individuals and killing up to one million children in sub-Saharan Africa each year 1,2. In holoendemic areas, children suffer the most from malaria, particularly after six months of age. Both the relative resistance to infection and severe malarial disease (SM) expressed by neonates and young infants, as well as the hypothesis that this resistance is mediated by maternally derived IgG has been recognized for decades 3-5. Despite these early observations, the targets of protective, maternally derived cord blood antibodies remain elusive. Recently, we demonstrated that antibodies to Pf Schizont Egress Antigen-1 (PfSEA-1) predict decreased risk of SM in 1.5-4 yr olds living in a holoendemic area of Tanzania 6. Here we demonstrate, in the same cohort, that maternally derived anti-PfSEA-1 antibodies cross the placenta, are detectable in cord blood, and cord blood levels of these antibodies predict significantly decreased risk of SM in infants for up to 12 months after birth. Further, in maternal vaccination studies in mice, pups born to dams that were immunized with PbSEA-1 prior to pregnancy had significantly lower parasitemia and longer survival times following lethal *P. berghei* ANKA challenge compared to pups born to dams treated with adjuvant alone. Together, these results identify, for the first time, a parasite specific target of maternal antibodies that transfer to the fetus and are associated with protection from SM and suggest that vaccination of pregnant women with PfSEA-1 may afford a survival advantage to their offspring.

616

IMMUNOGENICITY OF CHAD63/MVA ME-TRAP MALARIA VECTORED VACCINE IS NOT AFFECTED BY CO-ADMINISTRATION WITH ROUTINE EPI VACCINES IN A RANDOMIZED CONTROLLED TRIAL IN GAMBIAN INFANTS AND NEONATES

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Recent global estimates show that *Plasmodium falciparum* malaria remains a major public health concern. An effective vaccine could complement existing control measures. Heterologous prime-boost vaccinations using chimpanzee adenovirus 63 (ChAd63) and modified vaccinia Ankara (MVA) encoding ME-TRAP have consistently shown acceptable safety, excellent immunogenicity and substantial efficacy in African adult and paediatric populations. If licensed, this vaccine will be given to infants, who receive routine childhood immunizations. Here, we evaluate the immunogenicity and possible interference of ChAd63/MVA ME-TRAP when co-administered with routine Expanded Programme Immunization (EPI) vaccines in young infants. We enrolled 65 Gambian infants and neonates into 3 groups aged either 16, 8 or 1 week old at first vaccination and randomized them to receive either ME-TRAP vaccine or control. All participants received EPI vaccines according to the national programme. Safety was assessed by the description of vaccines-related adverse events including clinical assessments, biochemical and haematological tests. Immunogenicity was evaluated using anti-TRAP IgG ELISA, interferon-gamma ELISPOT and flow cytometry. Serology was performed to confirm all infants achieved protective titres to EPI vaccines. The vaccines were well tolerated in all age groups with no vaccine-related serious adverse events. High-level TRAP specific IgG and T cell responses were generated after boosting with MVA. Particularly, $CD8^+$ T cell responses, previously found to correlate with protection, were induced in all groups. Antibody responses to EPI vaccines remained at protective levels. While difficult to induce in neonates with protein or polysaccharide vaccines, potent humoral and cellular immunity were generated by heterologous prime-boost immunization with ChAd63/MVA ME-TRAP in young infants and neonates. Co-administration of routine EPI vaccines did not interfere with these responses. The EPI vaccines also retained protective antibody titres following administration of the malaria vaccines, supporting further evaluation of this regimen in infants.

617

FIVE-YEAR OUTCOMES OF A RANDOMIZED TRIAL OF SCHOOL VS. COMMUNITY-BASED MASS DRUG ADMINISTRATION FOR *SCHISTOSOMA MANSONI* CONTROL IN WESTERN KENYA

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Current WHO mass drug administration (MDA) recommendations for schistosomiasis control are based on prevalence in school aged children. At the time they were developed, the guidelines were contingent upon two hypotheses: 9-12 year old children are a reliable indicator of the overall level of infection in the population, and once annual MDA will be sufficient to reduce infection levels. Further, the guidelines were originally developed for morbidity control and may no longer be adequate for the WHA 54.19 and 65.21 resolution targets which now includes elimination of schistosomiasis. We conducted a large trial involving 150 communities in western Kenya as part of the Schistosomiasis Consortium for Operational Research and Evaluation (SCORE) to compare different MDA distribution approaches and frequency of delivery on *Schistosoma mansoni* infection prevalence and intensity. For 4 years, community wide (CWT) or school based treatment (SBT) was provided annually, every other year or only the first 2 years to 25 randomly assigned communities in each of 6 study arms. In the fifth year, communities in all arms were evaluated. At the end of the 5 years, communities that had received a total of 4 treatments had lower prevalence ($P = 0.0041$) and intensity ($p = 0.0024$) than those that had received 2 treatments. However, calculating impact relative to cost suggests that even though 2 treatments reduced prevalence less than 4 treatments, the 2 treatment approach may be more cost effective. We evaluated first year pupils in years 1, 3 and 5 as a proxy measure of the force of transmission and showed that both CWT and SBT approaches appeared to impact community transmission levels. Despite the general reduction in prevalence and intensities of *S. mansoni* infections, 44% of the 50 communities that received annual treatment,

either by CWT or SBT, had < 20% change in prevalence over the five year period, indicating that MDA alone may not be sufficient to achieve control or elimination.

618

FEMALE GENITAL SCHISTOSOMIASIS IN ABEOKUTA, NIGERIA

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Female Genital Schistosomiasis (FGS) is an emerging public health problem for young girls and women of childbearing age living in urogenital schistosomiasis endemic areas. The disease is associated with vaginal itching and discharge, post-coital bleeding, genitopelvic discomfort, marital discord, infertility, preterm labour, anaemia, menstrual disorders, and painful sexual intercourse. Over 44 million women living in sub-Saharan Africa (SSA) are currently affected by FGS. However, this disease has received little or no attention in Nigeria, which is the most endemic country in sub-Saharan Africa. A study was conducted in four *Schistosoma haematobium* endemic communities of Abule-titun, Imala-Odo, Apojola and Ibaro in Abeokuta to investigate the occurrence of FGS and its associated risk factors in young girls and women of childbearing age (age range 5-49 years). A total of 317 females were examined, of which 150 (47.3%) of them had haematuria (blood in urine), and 149 (47.0%) had pre-patent ova of *Schistosoma haematobium* in their urine respectively. There was significantly ($p < 0.05$) higher prevalence (121, 64.7%) and intensity of infection (1.0659±0.1251) in younger girls (aged 5-15 years) than their older counterparts. Using the standardised virgina discharge colour chart, 4(1.3%) cases of FGS were identified. Full gynaecological examination of 20 participants confirmed 14 (70.0%) cases of FGS. Gynaecological abnormalities included 10 (71.4%) of the females with grainy-sandy patches, 6 (42.9%) with yellow sandy patches, 1 (7.1%) with nabothian cysts and rubbery papules in their vaginal and cervical wall. Bathing (92.7%), fetching (52.4%), fishing (93.4%) and washing clothes (96.5%) at the local dam were reported risk factors associated with *S. haematobium* infection. This study has confirmed that the presence of FGS in Abeokuta, Nigeria. Its impacts and implications on the reproductive health of young girls and women of childbearing age living in urogenital schistosomiasis endemic communities are yet to be documented.

619

PROGRESS TOWARDS SCHISTOSOMIASIS CONTROL AND ELIMINATION FROM 2004 TO 2015 IN 28 HEALTH DISTRICTS IN MALI

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Mali has implemented the national schistosomiasis control program since 2005, supported initially by Schistosomiasis Control Initiative and then by Helen Keller International with funding from the USAID's NTD projects managed by RTI International. The main activities include mass drug administration (MDA) and impact evaluations. During 2014-15, 28 health districts (HDs) in Segou, Sikasso, Kayes, Mopti and Koulikoro regions were evaluated. Two or three sites in each HD with 50-60 school-age children at each site, for a total of 59 sentinel sites and 3575 children assessed. All HDs had received six or seven rounds of MDA, except two untreated. The aim of the studies was to evaluate the current status of schistosomiasis and adjust the MDA strategies in each district. The data from 2014-15 were compared to data from the baseline in 2004-05 to understand how prevalence has decreased over time. At baseline, the median prevalence of infection was 49.7% (range 0.29% to 98.5%) and the median prevalence of heavy infection was 12.6 % (0.3% to 78.9%). During the 2014-15 evaluation, the median prevalence of infection was 13.2% (range 0.6% to 83.6%) and the median prevalence of heavy infection was 3.4% (range 0.03% to 25.8%). Thus the median prevalence decreased greatly following the rounds of MDA. As a result ten out of 28 HDs (35.7%) will change their MDA cycle and targets for 2017. Eight out of 28 (28.6%) have achieved schistosomiasis morbidity control, meaning less than 5% of heavy infections in the sentinel population and another eight (28.6%) HDs had achieved the criteria for schistosomiasis elimination, meaning less than 1% of heavy infections in sentinel populations. To move towards the control and elimination goals, additional activities have been identified: snail control, sanitation improvements of river banks and health education. Despite the success, some questions remain, for example, understanding the reasons for persistence of elevated prevalence in three HDs. The evaluation provided an opportunity for the national program to accelerate progress towards elimination and/or control in districts with differing epidemiological profiles.

620

COMMUNITY DIALOGUES FOR PREVENTION AND CONTROL OF SCHISTOSOMIASIS IN MOZQAMBIQUE

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A prerequisite for successful schistosomiasis prevention and control is that affected communities have an understanding of the disease. Malaria Consortium conducted a study in four districts of Nampula province to explore whether the community dialogues approach is effective in improving schistosomiasis prevention and control at community level. The approach prompts communities to select volunteers who subsequently receive training and conduct regular dialogues. This allows communities to explore how they are affected, identify locally relevant solutions and plan for taking communal action. For this study, 157 volunteers were trained and equipped with a visual toolkit. Two six-month cycles of community dialogues were conducted. The study used a mixed-methods design: i) representative household surveys of knowledge, attitudes and practices (KAP) at baseline (n=791) and endline (n=795), ii) 25 focus group discussions with volunteers and community members and four in-depth interviews with district health officials, iii) analysis of monitoring and evaluation forms (n=1,462) and observation reports (n=11), and iv) analysis of stories of change written up by community dialogue participants (n=51). At baseline, correct knowledge of how schistosomiasis is transmitted or prevented was low. KAP indicators improved over the lifetime of the project. At baseline 18% could name at least one risk behaviour correctly. At endline, this had increased to 30%. Community participation also increased; for example, several communities initiated the construction of latrines. Volunteers appreciated being agents of change within their own communities. Participation levels were high, with an average of 70 participants per dialogue. The approach was particularly well received by women. Efforts to maximise prevention and control of schistosomiasis need to take community perceptions into account. Community dialogues are an effective tool to improve KAP and to increase community ownership of health issues. The approach has potential applicability in other health areas requiring communal behaviour change in resource-poor settings.

621

EXPERIENCES WITH A URINE-BASED RAPID DIAGNOSTIC TEST FÜR *SCHISTOSOMA MANSONI* INFECTION IN MIGRANTS

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Schistosomiasis affects more than 250 million people, mainly in sub-Saharan Africa. Intestinal schistosomiasis is primarily caused by *Schistosoma mansoni*. Morbidity in chronically infected individuals can be subtle, but severe long-term consequences may arise (e.g. hepatic fibrosis). While the diagnosis of intestinal schistosomiasis relies on stool microscopy, it is important to note that low-intensity infections are easily missed. Here, we present experiences with a new point-of-care (POC) test that detects a schistosome-specific circulating cathodic antigen (CCA) in the urine of infected individuals. Since March 2015, individuals presenting to the University Medical Center in Homburg, Germany were tested for schistosomiasis upon specific infectious disease consultation. If the patients had lived in an endemic area and signs and symptoms were compatible with a parasitic infection (e.g. abdominal pain, eosinophilia), a urine sample was subjected to a POC-CCA test and stool and urine microscopy for parasites were performed. Patients with confirmed schistosomiasis were treated with praziquantel. Within 12 months, eight patients with a positive POC-CCA urine test have been identified. Upon further investigation, *S. mansoni* eggs were detected by stool microscopy in six of these patients. Of note, examination of a single stool sample failed to detect the infection in four patients. All individuals with confirmed schistosomiasis were migrants from Eritrea (age range: 16-34 years). Peripheral blood eosinophilia was present in three patients and ranged between 6% and 34%. Follow-up samples were obtained from three patients and gave a negative result on the POC-CCA test within 7-10 days after treatment. Our findings suggest that a POC-CCA urine cassette test is highly sensitive for detection of intestinal schistosomiasis in migrants from endemic areas. The high rates of migration into Europe and elsewhere will likely lead to an increase of imported schistosomiasis cases. Hence, a wider implementation of POC-CCA tests in hospitals will contribute to an improved diagnosis and management of otherwise undetected *S. mansoni* infections.

622

ASSOCIATION OF SCHISTOSOMIASIS WITH IMPAIRED FERTILITY IN EAST AFRICA

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Many case reports and pathology series have suggested associations of Female Genital Schistosomiasis of the Fallopian tubes with infertility and ectopic pregnancy. Geographic distribution of infertility (which in Africa is most commonly due to tubal disease) has been reported but not explained. In this cross-sectional study, interpolated prevalence maps for *Schistosoma haematobium* and *S. mansoni* in East Africa were created using data from two open-access Neglected Tropical Diseases databases. Prevalence was extracted to georeferenced survey sample points for Demographic and Health Surveys for Ethiopia, Kenya, Tanzania and Uganda for 2009-2011 and 1999-2001. Outcomes included primary and secondary infertility (no births) and infecundity (no pregnancies) and history of pregnancy loss. Exploratory spatial analyses of outcomes (Moran's I, univariate and bivariate Local Indices of Spatial Autocorrelation) showed that outcomes were not spatially random and mapped clustering, hotspots, and areas of co-location of outcomes and exposures. Weighted multilevel logistic regression analysis found that women living in high compared to absent *S. haematobium* locations had significantly higher odds of secondary infertility (1999-2001: OR 1.8 [CI95 1.4, 2.3]; 2009-2011: OR 1.23 [1.1, 1.5]) and of primary infertility (1999-2001: OR 1.8 [1.3, 2.7]; 2001-2011: OR 1.58 [1.1, 2.3]). Living in high compared to absent *S. mansoni* locations did not affect the odds of any outcome. Women living in high *S. haematobium* compared to high *S. mansoni* locations had significantly higher odds of secondary infertility (1999-2001: OR 1.7 [1.3, 2.3]; 2009-2011: OR 1.6 [1.1, 2.0]), and of primary infertility (2010: OR 2.7 [1.5, 4.9]). For 1999-2001, history of pregnancy loss was significantly associated with high compared to absent *S. haematobium* (OR 1.3 [1.1, 1.6]) and with high *S. haematobium* compared to high *S. mansoni* (OR 1.4 [1.0, 1.8]). There is increasing evidence of the clinical and public health consequences of schistosomiasis to women's health and the importance of inclusion of girls and women in control strategies.

623

REDUCED EFFICACY OF PRAZIQUANTEL AGAINST *SCHISTOSOMA MANSONI* IS ASSOCIATED WITH MULTIPLE-ROUNDS OF MASS DRUG ADMINISTRATION: EPIDEMIOLOGICAL AND GENOMIC DATA FROM UGANDA

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Mass drug administration (MDA) with praziquantel is the cornerstone of schistosomiasis control in sub-Saharan Africa. The effectiveness of this strategy is dependent on the continued high efficacy of praziquantel, however drug efficacy is rarely monitored using appropriate statistical or genomic approaches that can detect early signs of wane. We conducted a repeated cross-sectional study, examining children infected with *Schistosoma mansoni* from 6 schools in Uganda that had previously received between 1 and 9 rounds of MDA with praziquantel. We collected up to 12 *S. mansoni* egg counts from 414 children aged 6-12 before and 25-27 days after treatment with praziquantel. We estimated individual patient egg reduction rates (ERRs) using a statistical model to explore the influence of covariates, including the number of prior MDA rounds. In addition we sequenced whole-genomes of *S. mansoni* parasites before and after treatment. The average ERR among children within schools that had received 8 or 9 previous rounds of MDA (95% Bayesian credible interval (BCI) 88.23%, 93.64%) was statistically significantly lower than the average in schools that had received 5 (95% BCI 96.13%, 99.08%) or 1 (95% BCI 95.51%, 98.96%) round of MDA. We estimate that 5.11%, 4.55% and 16.42% of children from schools that had received 1, 5, and 8/9 rounds of MDA respectively had ERRs below the 90% threshold of optimal praziquantel efficacy set by the World Health Organization. The genomic population structure of parasites collected before and after treatment were compared to elucidate targets of selection against praziquantel. The reduced efficacy of praziquantel in schools with a higher exposure to MDA may pose a threat to the effectiveness of schistosomiasis control programs. We call for the efficacy of anthelmintic drugs used in MDA to be closely monitored.

624

THE PREMONITION TRAP: LABORATORY TRIALS OF A ROBOTIC SMART TRAP FOR MOSQUITOES WITH SPECIES AND SEX RECOGNITION

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Using traps for surveillance of vector and pathogen populations is an important component of many vector management programs, but this technology has remained largely unchanged over many years. It is inaccessible in developing countries and limited in developed countries, largely due to costs and personnel requirements. Project PREMONITION is developing a robotic "smart" mosquito trap that captures live mosquitoes individually for higher-throughput lower-cost pathogen surveillance and elucidation of host associations. The PREMONITION trap uses infrared sensors and algorithms to identify flying insects by wing beat frequency, capturing only target mosquito species and reducing non-targeted captures. In addition to recording putative species identification, additional parameters including precise time of capture, temperature, humidity and ambient light, are recorded, effectively providing foraging activity data throughout the collection period and association with key abiotic factors. Due to a unique design, each specimen is tagged with the data it produced, enabling new bioinformatic analyses. Greenhouse trials of the PREMONITION trap with *Aedes aegypti* and *Culex quinquefasciatus* and different bait regimes including CO_2, skin odor and UV light evaluated the ability to trap mosquitoes, test behavioral activity throughout the collection period and differentiate between these taxa. Field testing will further evaluate the PREMONITION trap under native environmental conditions and exposure to a myriad of flying arthropods.

625

REFINING ESTIMATES OF DENGUE VIRUS TRANSMISSION POTENTIAL IN WILD TYPE AND WMEL-INFECTED *AEDES AEGYPTI*: FIELD REARING CONDITIONS ALTER VIRUS SUSCEPTIBILITY

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Wolbachia- (wMel) infected *Aedes aegypti* have been released at field sites in Australia, Southeast Asia and now South America, with the intention of curbing dengue virus (DENV) transmission using wMel's virus inhibition phenotype. Under optimal rearing conditions in the laboratory, wMel induces reliable protection against DENV. However, mosquitoes reared in the field are subject to changeable conditions (eg: exposure to pathogens, fluctuating temperatures, variable nutrition/rearing densities), and such factors may alter wMel-induced blocking of DENV infection. We aimed to quantify the effect of field and laboratory rearing conditions on DENV susceptibility of wild type (WT) and wMel-infected *Ae. aegypti*, and to examine how this might influence virus transmission dynamics. Our approach utilised weekly deliveries of both WT and wMel mosquitoes collected directly from our Vietnamese field site. 'Lab-reared' eggs were hatched and reared under standard laboratory conditions. 'Field-reared' 4th instar larvae and pupae completed only the final stages of development (without added nutrition) in the laboratory. All adults were fed in parallel with the blood of 33 NS1-positive dengue patients admitted to the Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam. As expected wMel mosquitoes were less susceptible to DENV than WT females. At the level of abdomen infection, logistic regression subgroup analysis showed that field rearing increased virus susceptibility in WT females, but did not affect wMel females. For virus transmission, WT field females more frequently transmitted virus in their saliva compared to lab-reared counterparts, suggesting lab experiments underestimate WT transmission potential in the field. The opposite held true for wMel females; wMel field females were less susceptible to DENV transmission than those reared in the lab, suggesting previous lab estimates of wMel-induced virus protection are conservative.

626

ESTIMATING HUMAN/MOSQUITO CONTACT AND RISK OF EXOTIC ARBOVIRUS TRANSMISSION FROM EGGS COUNTS IN OVITRAP: A CASE STUDY FOR *AEDES ALBOPICTUS* IN ROME (ITALY)

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Aedes albopictus public health relevance is not only associated to its aggressive daytime biting behaviour, but also to its capacity to transmit arboviruses, such as Chikungunya (CHIKV), Dengue and Zika. The Zika epidemics occurred in 2015-16 in Latin America, in addition to the large number of Dengue cases/year worldwide, increases the possibility of virus importation to non-endemic countries where the species has now become a permanent pest. The likelihood of vector-borne disease transmission and spread is commonly estimated by assessing R0, i.e. the number of secondary infection arising from a primary case. However, R0-models heavily rely on the accuracy of estimates of relevant biological parameters for *Ae. albopictus* which may be very difficult to be determined and universally applied. We here exploited the most easily obtainable field data on *Ae. albopictus* density and dynamics (i.e. egg counts in ovitraps) to estimate one of the key epidemiological parameters for risk model (i.e. human/mosquito contact). Based on results from extensive field activities carried out in summer 2014 in two sites in Rome - one of the most heavily *Ae. albopictus* infested urban areas in Europe - we found a positive relationship between counts of eggs in ovitraps (N=25120) and host-seeking females collected by Human Landing Catches (HLC; N=5578). The estimated linear regression coefficient for the mean number of eggs/site/day was 0.21, meaning that an increase of 10 in the mean number of eggs in ovitraps corresponded to an increase of 2 in the mean number of daily host-seeking *Ae. albopictus*. Computation of R0 for CHIKV using observed HLC data showed values >1 in several periods along the sampling season. The computation of CHIKV outbreak probability highlighted two phases with a >75% probability of successful transmission of imported CHIKV. The approach proposed, if validated in different ecological/geographical settings, would represent a valuable and affordable option for assessing risk of exotic arbovirus transmission in non-endemic countries based on ovitrap data gathered during routine *Ae. albopictus* monitoring activities.

627

IMPACT OF SEASONAL PATTERNS AND PARASITE ASEXUAL STAGE ON *ANOPHELES GAMBIAE* SUSCEPTIBILITY TO *PLASMODIUM FALCIPARUM* INFECTION IN BURKINA FASO

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Transmission reduction is a key component of global efforts to control and eliminate malaria. A wide range of novel transmission-reducing drugs and vaccines are currently under development. Human to mosquito transmission is influenced by many factors. Actually, it is unclear how the densities of parasites stages or season influence infection rate and intensity. Here, we describe the importance of the parasites stages seasonal pattern in infections success. Gametocytes carriers' infectiousness to mosquitoes was determined at the peak/end of transmission season and dry season via membrane feeding assay. Asexual parasites prevalence was higher in the peak of the wet season (69.1%, 329/476) compared to the dry (56.8%, 50/88) and the end of the wet season (60.5%, 161/266). Of gametocytes positive slides (N=189), 86.2% had asexual parasites. Gametocytes prevalence did not significantly vary between seasons. Asexual forms median density was 993 (IQR: 317-3759) with a significant difference between seasons (p=0.0004). However gametocytes median density 40 (IQR: 16-78) did not shown any seasonal variation (p = 0.1). For feeding, around 28062 mosquitoes offered blood meal and 29.6% fed and survived until dissection. The average number of dissected mosquitoes 75 (range 18 - 207) was quite the same according to the assays period. In 71.8% (79/110) of feeding experiments, at least one mosquito was infected. The median percentage of infected mosquitoes per infectious experiment was 15.7% (IQR: 07.3- 89. 2 %) with a median oocyst number of 2 (range 1 - 101). The prevalence of infected blood meal was similar across season (70.0%, 72.7% to 70.1% at the dry, the peak, and the end of the wet season. Mosquitos' infection rate also did not show any significant variation within season. The infection success was higher for asexual parasites carriers (91%) than non carriers (9%). However, mosquitoes' infection rate and oocyst load did not significantly vary according the asexual forms carriage. This highlights the need to carefully interpret evaluations, regarding asexual parasites and transmission season for malaria control program.

628

INVESTIGATING THE INFLUENCE OF *PLASMODIUM* INFECTION ON THE HUMAN VOLATILE ODOUR PROFILE IN AN ENDEMIC SETTING

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There is some evidence to suggest that *Plasmodium* parasites manipulate the attractiveness of human hosts to *Anopheles* vectors. This is in accordance with the theory of parasite manipulation, whereby transmission is favoured by an effect of the parasite on host biology or behaviour. If malaria parasites can alter human attractiveness, the repercussions would be far reaching: this would likely have a profound influence on the way that malaria spreads through populations. Despite evidence that variations in human host attractiveness to biting insects are manifested through differences in the human odour profile, the association between such odour profile and malaria infection has never been studied. In this large-scale, field-based trial we used air entrainment techniques to capture the chemicals released by individuals' skin (the 'volatile odour profile', VOP). We measured the VOP of both uninfected school-age children in Western Kenya, and those with parasitaemia of both sexual and asexual *Plasmodium* stages. The same volunteers were measured following treatment with antimalarials. In addition to field diagnostics, parasite infection profile was characterised by the use of molecular techniques, including qt-NASBA and qPCR, for parasite stage and density respectively. Chemical odour profiles were examined using gas chromatography, then further investigated using coupled GC-electroantennography to determine entomological responses to constituent compounds. We will present results showing associations between human odour profile, parasitological parameters of infection and electrophysiological responses of the *Anopheles* vector. Malaria remains one of the most important diseases worldwide. As the global strategy for malaria control and elimination evolves to combat both parasite and vector resistance to drugs and insecticides, the need for innovative tools intensifies. Greater understanding of the factors that influence transmission will allow more precise epidemiological modelling. Additionally, measurable changes in the VOP of infected individuals could form the basis of a novel diagnostic tool.

629

CHANGE IN MOSQUITO BEHAVIOR AFTER DISTRIBUTION OF BEDNETS RESULTS IN DECREASED PERSONAL PROTECTION FROM INFECTIVE BITES

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Behavioral resilience in mosquitoes poses a significant challenge to the control and elimination of malaria. Generalist host-seeking behaviors enable mosquito populations to evade indoor interventions which target host-seeking or resting mosquitoes. It is unclear whether behavior changes, as seen in anopheline vectors over the last decade, compromise the efficacy of vector control in reducing malaria transmission. In this study, we quantified human exposure to both bites and infective bites of a major malaria vector in Papua New Guinea over the course of four years surrounding a nationwide long-lasting insecticidal bednet distribution. Using age-stratified sleeping patterns and the biting density indoors and outdoors, we estimated the protective efficacy of LLINs against the biting population just prior to the distribution and for the next three years. We observed a shift in bite exposure to earlier hours of the evening, before individuals are protected, following the bednet distribution. Protective efficacy was greatest in children under five years old (65%), but significantly lower in the adult population (35%) who may be an important reservoir for transmission. The personal protection in all age groups decreased significantly over the study period and infective mosquitoes were found host-seeking before 10pm. As a result, exposure to infective bites was higher in net users and non-users alike, following the distribution. The result of the decrease in personal protection over several years appears to be a rebound in the entomological inoculation rate to levels that surpass pre-intervention estimates. This study highlights the necessity of validating and deploying vector control measures targeting outdoor exposure if malaria is to be controlled and eliminated.

630

ACCELERATED ARBOVIRAL TRANSMISSION AND MORE EXPLOSIVE OUTBREAKS IN A WARMER WORLD

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Arboviruses are expanding into new regions, and outbreaks of these diseases are becoming more common. Several studies have quantified basic reproduction number, R0, based on temperature. However, none have quantified epidemic growth rate, r, an important temporal measure in the dynamics of these highly epidemic arboviral diseases. We seek to characterize the sensitivity of the epidemic growth rate to changes in both R0 and the generation interval, which is defined as the average time elapsed between consecutive cases in humans and also depends on temperature. We first developed a description of R0 for dengue based on temperature and, for the first time, a fully characterized generation interval distribution for dengue, also as a function of temperature. We then combined these estimates of R0 and the generation interval as functions of temperature to obtain a description of epidemic growth rate r as a function of temperature. To assess the implications of this temperature relationship for projected future temperature increases, we calculated r as a function of monthly, location-specific temperatures globally to identify areas of the world and associated populations that may be subject to increases or decreases in arboviral epidemic growth rates under future climate change. By 2050, as many as 3.2 billion people are projected to live in areas expected to experience increasingly rapid epidemics of arboviral diseases, indicating the need for surveillance systems to remain vigilant as the future explosiveness of outbreaks worsens in many areas.

631

EXPOSURE OF EPITOPE RESIDUES ON THE OUTER FACE OF THE CHIKUNGUNYA VIRUS ENVELOPE TRIMER DETERMINES ANTIBODY NEUTRALIZING EFFICACY

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Chikungunya virus (CHIKV) is a reemerging alphavirus that causes a debilitating arthritic disease and infects millions of people and for which no specific treatment is available. Like many alphaviruses, the structural targets on CHIKV that elicit a protective humoral immune response in humans are poorly defined. Here we used phage display against virus-like particles (VLPs) to isolate seven human monoclonal antibodies (MAbs) against the CHIKV envelope glycoproteins E2 and E1. One MAb, IM-CKV063, was highly neutralizing (50% inhibitory concentration, 7.4 ng/ml), demonstrated high-affinity binding (320 pM), and was capable of therapeutic and prophylactic protection in multiple animal models up to 24 h post-exposure. Epitope mapping using a comprehensive shotgun mutagenesis library of 910 E2/E1 mutants with alanine mutations demonstrated that IM-CKV063 binds to an intersubunit conformational epitope on domain A, a functionally important region of E2. IM-CKV063 blocks both virus entry and release steps. MAbs against the highly conserved fusion loop have not previously been reported but were also isolated in our studies. The fusion loop MAbs were broadly cross-reactive against diverse alphaviruses but were non-neutralizing. Fusion loop MAb reactivity was affected by temperature and reactivity conditions, suggesting that the fusion loop is hidden in infectious virions. Visualization of the binding sites of 15 different MAbs on the structure of E2/E1 revealed that all epitopes are located at the membrane-distal region of the E2/E1 spike. Interestingly, epitopes on the exposed topmost and outer surfaces of the E2/E1 trimer structure were neutralizing, whereas epitopes facing the interior of the trimer were not, providing a rationale for vaccine design and therapeutic MAb development using the intact CHIKV E2/E1 trimer.

632

BRIDGING THE GAP BETWEEN IMMUNOGENICITY AND SAFETY: AN INSECT-ONLY VIRUS AS A VACCINE PLATFORM

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Traditional approaches in vaccine development have typically involved live-attenuation or inactivation strategies. However, these approaches tend to offer unbalanced safety and immunogenicity profiles with live-attenuated vaccines providing robust yet reactogenic immune responses, while inactivated vaccines present a safer alternative often requiring multiple doses for protective immunogenicity. We propose the use of an insect-specific virus, Eilat virus (EILV), as a novel platform for alphavirus vaccines that provides exceptionally safe yet long-term, robust immunogenicity. EILV was isolated from a pool of mosquitoes collected in the Negev desert of Israel and is completely restricted to replication in insects only due to an inability to enter and replicate its RNA genome in vertebrate cells. To address the growing concern over chikungunya disease as it has now become a global threat, we developed a proof-of-concept chikungunya vaccine using the EILV platform. Here we report that EILV chimeras with chikungunya structural proteins are antigenically identical to their pathogenic counterpart, mimic early stages of the virus replication-cycle from attachment and entry to viral genome delivery, yet remain restricted to replication in mosquito cells only, providing an extremely safe phenotype in vertebrate animals with balanced, long-lived humoral and cellular immune responses following a single-dose vaccination. In non-human primates, EILV/CHIKV elicited rapid and robust neutralizing antibodies against chikungunya virus and provided protection against telemetrically-monitored disease. The EILV platform represents the first application of an insect-only virus in vaccine development and highlights the broader application of such viruses in vaccinology.

633

PROTECTION AGAINST TWO LETHAL HETEROLOGOUS VIRUSES AFTER SIMULTANEOUS DUAL-AEROSOL CHALLENGE USING A NOVEL IMMUNOTHERAPEUTIC

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No specific therapeutic prevents or treats Venezuelan Equine Encephalitis (VEE) or other alphaviruses. VEE causes human epidemics and is a select agent. Influenza can cause severe mono-infection or a nosocomial co-infection in hospitalized patients admitted for other infections. Passive immunotherapeutics with mono-specific immunoglobulin (IgG) effectively treats toxin, venom and pathogen-mediated diseases and are either polyclonal IgGs, derived from human or animal plasma, or monoclonal antibodies. Effective multi-pathogen/toxin hIgGs could have great clinical benefits. Transchromosomic bovines (Tc-bovine) provide an alternative method to economically produce large quantities of multi-target IgG with fully-human antibodies. Tc-bovines have had their repertoire of bovine antibody genes deleted, and instead carry a human artificial chromosome containing the full repertoire of human antibody genes. Tc-

bovines can rapidly produce up to 600 grams per month of hyperimmune, multi-pathogen, fully-human immunoglobulin (hIgG). Tc-bovines were hyperimmunized with psoralen inactivated Trinidad-Tobago (Td-Tb) VEE virus and a trivalent spilt virion seasonal influenza virus (pH1N1, H3N2, type B) and a Tc-bovine hIgG with very high titers to these viruses was produced. Multiple groups of BALB-C or DBA2 mice (n=5) were aerosol challenged with 300 PFU of wild-type pDNA electroporated strain Td-Tb VEE (.5 PFU LD50) or dual challenged with 30 PFU Td-Tb VEE and 600 PFU pH1N1 (30 PFU LD50) on day 0. VEE aerosol challenged mice received 100 ug (5 mg/kg) IP at -12/+48h (prophylactic) or +12/48h (therapeutic). Dual VEE and pH1N1 aerosol challenged mice received 200 ug (10 mg/kg) IP at -12/+48h (prophylactic) or +12/48h (therapeutic). Controls received irrelevant hIgG. Prophylactic and therapeutic groups had 80-100% survival. All controls had 0% survival. To our knowledge, this is the first study demonstrating protection after simultaneous challenge with two lethal heterologous viruses (alphavirus + orthomyxovirus) by any route in any animal model. Multi-pathogen Tc-bovine hIgGs could be produced and tested in human clinical trials.

634

A VIRUS-LIKE PARTICLE VACCINE ELICITS BROAD NEUTRALIZING ANTIBODY RESPONSES IN HUMANS AGAINST DISTINCT CHIKUNGUNYA VIRUS GENOTYPES

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Chikungunya virus (CHIKV) is a mosquito-borne alphavirus that is endemic to many regions of Asia and Africa, and has recently emerged in the Western hemisphere. The rapid emergence of CHIKV is partly attributed to an adaptive mutation that facilitated efficient transmission by a widely distributed mosquito species, *Aedes albopictus*, highlighting the threat of further CHIKV spread and the need to prioritize vaccine development. There are three CHIKV genotypes: Asian, East/Central/South African (ECSA), and West African, which share 95.2 to 99.8% amino acid identity. CHIKV particles are enveloped, and encapsidate a positive-sense, single-stranded genomic RNA that encodes four non-structural and five structural polyproteins. Expression of the structural polyproteins gives rise to virus-like particles (VLPs), which are highly immunogenic. In a recent phase 1 trial, we demonstrated a CHIKV VLP vaccine based on a West African strain to be safe, tolerable, and to elicit robust neutralizing antibody (NAb) responses against an ECSA strain. Here, we investigated the breadth of the VLP vaccine-elicited NAb response against eight additional CHIKV strains representing all three genotypes to further evaluate the potential of this vaccine candidate to reduce CHIKV spread. We generated fully infectious chimeric viruses in which the non-structural genes of Semliki Forest virus (SFV), a related alphavirus, were complemented with CHIKV structural genes for use in neutralization assays against sera obtained from 12 CHIKV VLP vaccine recipients. SFV-CHIKV viruses encoding the structural genes from all nine CHIKV strains were infectious, demonstrated similar *in vitro* growth kinetics, and were potently neutralized by sera from vaccinees (average half-maximum inhibitory dilution per vaccinee: 2209, range: 315 - 6423). These results suggest that the CHIKV VLP vaccine-elicited NAb response could confer cross-protection against diverse CHIKV genotypes, further supporting the potential of this vaccine candidate to combat CHIKV spread. This vaccine is currently being evaluated in a phase 2 clinical trial in the Caribbean.

635

HIGH-RESOLUTION IMAGING OF CENTRAL NERVOUS SYSTEM INVASION BY ALPHAVIRUSES DELIVERED BY SUBCUTANEOUS OR AEROSOL ROUTES OF EXPOSURE

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Encephalitic alphaviruses, including Venezuelan and Eastern equine encephalitis viruses (VEEV, EEEV), have caused sporadic outbreaks in the human population and can result in severe neurologic impairment and death. Although in nature virus is delivered subcutaneously by the bite of a mosquito, these viruses have potential for use as bioweapons, and in this case, they are likely to be delivered by aerosolization. Currently, no specific anti-viral treatments or FDA approved vaccines are available for VEEV or EEEV. Thus, a detailed understanding of the routes of brain entry and the dynamics of spread that differentiate the two will be important for the development of prophylactics or post-exposure interventions that may mitigate disease. In mouse models of disease, VEEV and EEEV demonstrate distinctly different cellular tropisms due to microRNA binding sites in EEEV that limit its growth in myeloid cells and natural heparan sulfate (HS) binding by the virus, which further limits myeloid cell infection but exacerbates brain replication. As a result of these phenotypic differences, the dynamics of neuroinvasion and spread within the brain are also expected to differ between the viruses. Using high speed ribbon scanning confocal microscopy and the prototypic strains VEEV Trinidad Donkey and EEEV FL93-939, we are collecting large-area, high resolution, images of brain tissue in three-dimensions. With these data we can resolve, at the cellular and macro levels, early sites of virus infection. Importantly, we can differentiate patterns of infection following different routes of exposure for both VEEV and EEEV. Initial results indicate that, while the timing of neuroinvasion differs between the two viruses, primarily dependent upon the HS binding phenotype, the olfactory bulb is a principal site of early infection for both viruses when delivered peripherally and via aerosol. We expect these data to provide a guide for the development of therapeutics that can be specifically targeted for the post exposure timing and route of infection.

636

ATTACK RATE OF CHIKUNGUNYA IN NICARAGUAN CHILDREN DURING THE FIRST TWO WAVES OF THE EPIDEMIC, 2014-2016

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Chikungunya was recently introduced into the Americas, causing explosive epidemics throughout the region. Autochthonous transmission of Chikungunya was detected in September 2014 in Nicaragua. To study the introduction and transmission of Chikungunya in Nicaragua, we included testing for chikungunya virus (CHIKV) in an ongoing pediatric dengue cohort study in District II of Managua, the capital city. Children were enrolled prospectively, and data was systematically recorded on all medical visits. Participants are encouraged to come in at first sign of illness, and all medical care is provided free-of-charge through the study. Participants who present to the health center with suspected chikungunya or undifferentiated fever are tested for chikungunya by RT-PCR and serological assays. Between September 2014 and March 2016,

3,788 children participated in the study. From September 2014 to March 2015, the first wave of the epidemic, the clinical attack rate of laboratory-confirmed CHIKV infection was 2.9% (95% CI: 2.3%, 3.4%). From July 2015 to January 2016, the second wave of the epidemic, the clinical attack rate of laboratory-confirmed CHIKV infection was 13.9% (95% CI: 12.7%, 15.1%). Age was significantly associated with symptomatic CHIKV infection, with 2-4 year olds experiencing the lowest attack rate (9.4%) and 11-14 year olds experiencing the highest attack rate (20.2%). In the first year of the study, the proportion of children in the study with inapparent infections was 58.3% (95% CI: 51.5%, 65.1%). Poverty was an independent risk factor for CHIKV infection, with a prevalence ratio (PR) 1.33 (95% CI: 1.11, 1.58), as was having ≥8 hours per day without running water in the home, PR 1.31 (95% CI: 1.05, 1.63). The number of inapparent infections and the ratio of symptomatic:inapparent infections, along with risk factor analysis, is ongoing for the second wave of the epidemic. This study is providing critical data on the epidemiology and transmission of chikungunya in the Americas.

637

TWELVE-MONTH ASSESSMENT OF PERSISTENT ARTHRALGIA ASSOCIATED WITH THE 2014-2015 CHIKUNGUNYA VIRUS OUTBREAK IN THE U.S. VIRGIN ISLANDS

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Chikungunya virus (CHIKV), an alphavirus transmitted by *Aedes* spp. mosquitoes, causes fever and polyarthralgia. Symptoms often resolve within 7-10 days; however, up to 79% of cases in previous outbreaks have reported persistent arthralgia (defined as joint pain at least once per week) for up to 36 months following acute infection. To enhance our understanding of the long-term impact of CHIKV illness, persistent arthralgia and functional outcomes of laboratory-positive cases in the U.S Virgin Islands (USVI) were evaluated at 6 and 12 months after symptom onset. Outcomes of individuals with similar healthcare seeking behaviors were compared to those of cases. A similar healthcare seeker was defined as a USVI resident who did not report experiencing sudden onset of fever and joint pain in June 2014-June 2015, and attended a healthcare facility during the last week of June 2015. Six months after illness onset, 165 cases (65% female, median age: 52) were interviewed and 12 months after illness onset, 128 of the 165 cases were interviewed. During the 12-month follow-up of cases, 167 similar healthcare seekers (64% female, median age: 34 years) were interviewed. The difference in prevalence of persistent arthralgia between cases and the comparison group at 6 months was 32% (95% confidence interval [CI]: 23-40%) after adjusting for age, sex and self-reported history of arthritis; at 12 months after onset, the difference in prevalence was 19% (95% CI: 11-28%). Twelve months after illness onset, cases were 1.81 (95% CI: 1.08-3.02) times more likely to have difficulty walking, 1.96 (95% CI: 1.24-3.12) times more likely to have difficulty climbing stairs and 2.63 (95% CI: 1.31-5.29) times more likely to have difficulty getting in and out of a car compared to similar healthcare seekers. Furthermore, 22% (95% CI: 15-29%) of cases compared to 10% (95% CI: 5-14%) of the comparison group reported that their health was either somewhat or much worse compared to one year prior. These findings highlight the long-term impaired physical functionality of CHIKV cases and the need for therapeutic and vaccine research to manage and prevent acute illness and long-term morbidity.

638

HIGH SEROPREVALENCE OF MIDDLE EAST RESPIRATORY SYNDROME CORONAVIRUS (MERS-COV) IN CAMELS IS NOT ASSOCIATED WITH MERS-COV SERO-POSITIVITY AMONG CAMEL PASTORALISTS IN KENYA

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High seroprevalence of MERS CoV among camels has been reported in Kenya and other countries in Africa. To date, the only report of MERS CoV sero-positivity among humans in Kenya is of two livestock handlers with no known contact with camels. We assessed whether persons exposed to seropositive camels at household level had serological evidence of infection. A total of 798 human and 879 camel sera were collected from 265 households in Marsabit County in 2013. Household data collected included human and animal demographics, animal ownership and type of contact with camels. Human and camel sera were tested for antiMER-CoV IgG using a commercial ELISA test. Human samples were considered positive if positive on both ELISA and a confirmatory plaque reduction neutralization test (PRNT). PRNT was not performed on camel samples. Multivariable logistic regression was used to identify factors associated with MERS CoV sero-positivity and adjusted odds ratios (aORs) reported. The median age of persons sampled was 30 years (range 5-90) and 50% were males. A quarter (197/760) of the participants reported having had contact with camels defined as milking, feeding, watering, slaughtering or herding. Of the 798 human sera tested, 18 (2.2%) were positive on ELISA but negative by PRNT. Of the 879 camels sera tested, 90% (n=791) were positive. On multivariate analysis, older camels > 4 years and those raised under nomadic pastoral versus agro-pastoral production system had significantly increased odds (aOR 21.3; 95% CI 10.3, 43.6 and 18.5; 95% CI 7.4, 46.1, respectively) of being sero-positive, while the number of cattle in the herd and in households that had sold livestock were associated with decreased odds (aOR 0.9; 95% CI 0.96, 0.97 and 0.3; 95% CI 0.1, 0.9) of being seropositive. Despite high sero-prevalence among camels, there was no serological evidence of MERS CoV infection among camel pastoralists in Marsabit County. High seropositivity among camels suggests that MERS CoV or other closely related virus continues to circulate in camel herds particularly those raised in nomadic production systems, and highlights ongoing potential for animal to human transmission.

639

REDRAWING THE BOUNDARIES OF KYASANUR FOREST DISEASE (KFD) IN INDIA- EARLY RESULTS OF GHSA-SUPPORTED ACUTE FEBRILE ILLNESS SURVEILLANCE

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Kyasanur Forest Diseases (KFD) is a tick-borne flavivirus disease first described in 1957 from the Shimoga district of Karnataka, India.KFD detection remained restricted to persons living in the Shimoga forest region until 2012 when it was identified in Chamarajanagar, 400km away.

Characteristically, this disease presents as an acute haemorrhagic febrile illness. In 2014 we initiated hospital-based laboratory-supported acute febrile illness (AFI) surveillance) at sub-district level sentinel hospitals across several states to map the aetiology of AFI in general, and KFD in particular. The project is supported under the Global Health Security Agenda (GHSA). All admitted AFI patients from June 2014 to March2016 with fever <15 days were enrolled. We recorded the demographic and clinical parameters of all cases, and tested for bacterial, viral and parasitic diseases, including leptospirosis, dengue, influenza, scrub typhus, chikungunya, typhoid, brucellosis, and KFD. Serological and molecular diagnostic assays were performed, including real-time PCR to detect viral RNA in serum for KFD confirmation. We enrolled 4693AFI patients from five contiguous Indian states. Of these, 302 (6.4%) were KFD-positive: Karnataka (98/2970), Kerala (45/1290), Tamil Nadu (1/29), Goa (143/391), and Maharashtra (15/15). KFD-positive patients ranged from 5 to 65 years (median age 40 years); 59% were female. Their clinical spectrum included myalgia (89%), generalized weakness (79%), prostration (22%), nausea (60%), vomiting (51%), abdominal pain (34%), diarrhea (24%), hemorrhagic fever (2%), and altered sensorium and or seizures (1%). Of 302 cases, 80% were living near the forest edge and 84% reported visiting the forest in the last 2 weeks. This study documents that KFD is not restricted to the Shimoga forest region, and has a more diverse clinical presentation than previously observed. Further, in our surveillance, we recorded cases without confirmed forest incursion.AFI platforms, as are being built under GHSA, are critical for the comprehensive characterization of known pathogens and may lead to detection of novel pathogens.

640

RISK FACTORS FOR ACUTE LEPTOSPIROSIS IN NORTHERN TANZANIA

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Leptospirosis is increasingly recognized as a major cause of febrile illness in Africa but little is known about risk factors for human infection. Patterns of sero-reactivity in Tanzania have indicated that livestock may be important sources of human leptospirosis. To test this hypothesis we conducted a prospective cohort study of acute leptospirosis in northern Tanzania. We enrolled pediatric and adult patients with fever from two referral hospitals in Moshi, Tanzania and performed *Leptospira* microagglutination antibody testing on acute and convalescent serum. Cases were patients who had either a four-fold rise in *Leptospira* antibody titers or a single reciprocal titer ≥800, seropositivity required a single titer ≥100, and controls had titers <100 in both sera. We administered a standardized questionnaire to assess behaviors over the preceding month. We calculated odds ratios (OR) for individual behaviors, and combined behaviors to form exposure scales to livestock, rodents, and surface water. Of 1,446 patients enrolled from February 2012 through September 2014 the analyzed cohort included 24 (1.7%) cases, 179 (12.4%) seropositive participants and 592 (40.9%) controls. Among cohort members the median (range) age was 26.0 (0.2, 95.3) years and 422 (54.7%) were female. On bivariate analysis, acute leptospirosis was associated with age >12 years (OR 7.7, p<0.01), high level of cattle contact (OR 3.3, p=0.05), feeding cattle (OR 3.9, p=0.02), cleaning cattle waste (OR 4.3, p=0.03), working in fields (OR 2.9, p=0.02) and rice fields (OR 14.4, p<0.01). Seropositivity was associated with age >12 years (OR 2.2, p<0.01), Maasai ethnicity (OR 7.0, p=0.03), high level of cattle contact (OR 1.7, p=0.04), keeping cattle inside the house (OR 4.7, p=0.04), high level of goat contact (OR 1.9, p=0.03), slaughtering livestock (OR 1.7, p=0.02), and working in rice fields (OR 3.9, p=0.01). Behaviors and scales for exposure to rodents and surface water were not associated with either acute leptospirosis or seropositivity. Our findings suggest livestock contact and working in rice fields are important risk factors for leptospirosis in northern Tanzania.

641

PRODUCTION FARMS, CLASS-1 INTEGRONS, AND ANTIBIOTIC RESISTANCE IN *E. COLI* ISOLATES FROM RURAL ECUADOREAN CHICKENS AND HUMANS

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While it is clear that industrial-scale farming operations affect antibiotic resistance (AbR) in livestock and humans, the extent to which smaller community-level farming operations impact AbR in surrounding communities, particularly in developing nations, is poorly described. We hypothesized that small-scale production operations contribute to elevated levels of AbR bacterial isolates in livestock and people living in close proximity to these operations by selecting for genetic mobile elements such as integrons that allow sharing of resistant gene cassettes between isolates. To investigate the relationship between community-level farming, mobile elements, and AbR, we conducted a serial cross-sectional community-based study collecting *E. coli* fecal isolates from over 1,000 chickens and humans in rural villages from northern Ecuador. Isolates were typed for 4 mobile elements using a DNA microarray platform; phenotypic resistance was assessed for 12 antibiotics using standard disc diffusion assays. We compared AbR levels in isolates from production chickens (broiler and laying hens raised for sale and fed commercial feed containing antibiotics) to AbR among isolates from household chickens (raised for domestic use and fed antibiotic-free feed). AbR for all markers was higher in production chickens compared to household chickens. There was a significant downward trend in AbR levels across birds from production operations, household birds in villages with production operations, and household birds in villages with no production operations, suggesting that proximity to a production operation can influence AbR in non-production chickens. In addition, the risk of phenotypic AbR appears to be modified by the presence or absence of class-1 integrons, suggesting a complex relationship between the environment, integrons, and AbR. These analyses (which will be expanded to include humans in houses associated with production or household chickens) suggest that small-scale production poultry farming selects for class-1 integrons in both chicken types, which has important public health implications.

642

IDENTIFYING CHALLENGES AND OPPORTUNITIES FOR ONE HEALTH SYSTEMS STRENGTHENING IN GUINEA

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The recent Ebola virus disease outbreak in West Africa highlighted the importance of emerging zoonotic diseases, and provided an impetus for renewed emphasis on "One Health" capacity building with respect to zoonotic disease control. To guide capacity building efforts in Guinea, we sought to first identify the existing systems and structures in place for zoonotic disease control, specifically highlighting existing multi-sectoral approaches. Adapting our methodology for compatibility with the Global Health Security Agenda targets, we worked with the government ministries responsible for human, animal, and environmental health to identify a list of diseases - rabies, anthrax, brucellosis, viral hemorrhagic fevers (including Ebola fever, Lassa fever, yellow fever, and Rift Valley fever), trypanosomiasis and highly pathogenic avian influenza - as the Government of Guinea's top priorities. We then used each priority disease as a case study to identify existing processes for prevention, surveillance, diagnosis, laboratory confirmation, reporting and response across all sectors, with an emphasis on examining elements of cross-sectoral coordination or communication. Results were used to produce disease-specific systems "maps," which highlighted commonalities across all systems, as well as gaps and opportunities. Overall, we identified five major categories of gaps, each with a corresponding set of recommendations: 1) Coordination; 2) Training; 3) Infrastructure; 4) Public awareness; and 5) Research. These recommendations have been provided to the Government of Guinea to assist with the development of a One Health strategic plan. Overall, the project demonstrates an effective methodology for mapping systems and structures for zoonotic diseases, and the benefit of conducting a baseline review of systemic capabilities prior to embarking on capacity building efforts.

643

PARASITES IN THE PARK: AN EPIDEMIOLOGIC STUDY OF NYC PARKS FOR *TOXOCARA* SPECIES

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Toxocara species are common pet parasites that can be found in the stool of dogs and cats. The infective larvae released in the stool survive in soil for many years and can subsequently be ingested by children who encounter them in sandboxes or on playgrounds. The CDC lists Toxocariasis as one of five neglected parasitic infections in the US and states that 'we urgently need to know more about who is at risk and how they are affected'. It is considered a neglected disease of poverty in the US, posing a significant disease burden in certain groups, remaining largely underdiagnosed. Infection in humans, paratenic hosts, can lead to visceral or ocular larva migrans, blindness, and silent brain infection that can diminish neurological cognition. New York City is pet-friendly, and known for its sprawling parks and play areas. It has been suggested that certain NYC neighborhoods may pose a higher risk for *Toxocara* infection, particularly in lower socioeconomic and predominately immigrant communities, although no study of actual *Toxocara* burden in NYC has been reported. The goals of this study are to:1)determine the burden of *Toxocara* in parks by examining sand/soil in playgrounds, 2) to determine if a disparity exists in neighborhood distribution, and 3) to explore which species of *Toxocara* is more prevalent. To accomplish these objectives multiple samples will be taken from more than 100 parks and playgrounds and analyzed by standard flotation and microscopy methods. Species will be identified by multi-parallel quantitative real-time PCR (qPCR) using specific primers to *T. cati* and *T. cani*. Parasite burden will be calculated. Results will be tabulated geospatially and correlated with US Census maps of income, housing and ethnicity. Preliminary results from 10 of 100 sites in this study identified *Toxocara* eggs in 40% of samples, suggesting that *Toxocara* is common in many play areas. At the conclusion of this project, we hope to provide the first epidemiologic geospatial survey of *Toxocara* species in NYC parks that may be helpful in identifying parks that may pose the highest risk of *Toxocara* transmission.

644

USE OF TRACKING PLATES TO IDENTIFY HOTSPOTS OF RAT ABUNDANCE IN SLUM COMMUNITIES WITH HIGH ENDEMIC TRANSMISSION OF LEPTOSPIROSIS

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At present, there are no effective measures to prevent leptospirosis in slum environments, where the domestic rat is the primary reservoir. A critical barrier to mounting rodent control strategies is the lack of reliable approaches to estimate rat abundance and their distribution in complex urban settings. We developed a tracking plate method that quantifies the abundance of rats by identifying rat-specific markings on lampblack-coated tiles. We used this method to create high-resolution risk maps for rat abundance by placing tracking plates at 440 spatially randomized locations in a Brazilian slum community (0.46 km2) with high leptospirosis infection rates (35.4% CI, 30.7 - 40.6 per 1,000). We performed environmental surveys at sampling points and used satellite imagery to derive spatially relevant covariates. We analyzed the data using an interval-censored mixed model to evaluate the association between tracking board metrics and environmental characteristics. Among the 402 (91.4%) points that were successfully sampled, 173 (43%) had signs of rat-specific markings. Tracking plate-ascertained rat abundance was associated with the number of rat burrows (OR 1.32 CI 1.18 - 1.48), rat trails (OR 2.10 CI 1.8 - 2.62) and sites with rat feces (OR 1.39 CI 1.17 - 1.64). The distribution and intensity of rat abundance was highly heterogeneous. Clustering of rat abundance was identified throughout the study site and generally <20m in diameter. Clusters were associated with domestic areas (OR 1.47 CI 1.21 - 1.79) and areas with access to open sewers (OR 2.05 CI 1.69 - 2.48). Conversely, impervious surfaces (OR 0.55 CI 0.38 - 0.80) and increasing distance from flood-prone areas (OR 0.29 CI 0.17 - 0.50) and public trash dumps (OR 0.74 CI 0.60 - 0.96) were associated with decreased risk of rat abundance. By using tracking boards to create high-resolution risk maps, we identified defined environmental features of slum communities, which predict rat abundance. We also found that while rat abundance is high, there is marked spatial heterogeneity in the microenvironment, which may offer an opportunity for targeted rodent control for leptospirosis.

645

FRACTION OF ALL UNDER FIVE DEATHS CAUSED DIRECTLY OR INDIRECTLY BY MALARIA IN SUB-SAHARAN AFRICA FROM 2000-2015

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Malaria cases have declined in sub-Saharan Africa due to the scale-up of interventions, and a consistent reduction in child mortality since 2000 has been recorded. In order to quantify the effect of malaria interventions on child mortality rates, we estimated the proportion of all-cause deaths attributed to malaria by direct and indirect causes. A detailed dataset of 978253 birth histories was assembled using cross sectional household surveys. The dataset included information on social and health factors. Birth history data were merged with *Plasmodium falciparum* prevalence rates and environmental and socioeconomic covariates obtained by remote sensing. Bias analysis was applied to identify confounders and effect modifiers among social and health covariates. A spatio-temporal hazard model was applied to estimate the relative risk of malaria for the surveyed communities. A structured additive regression (STAR) model was applied to generate a risk map for sub-Saharan Africa. The results of the STAR model were used to estimate the proportion of all-cause under 5 deaths due to malaria as direct and indirect cause at country level from 2000 to 2015. The proportion of all-cause deaths linked with malaria reduced drastically from 2000 to 2015. The reduction was more marked in Western and South Africa than in Central and Eastern Africa. Countries which have increased artemisinin-based combination treatments for children with non-complicated malaria showed a marked reduction of child deaths. The estimates of under 5 deaths indirectly or directly caused by malaria were higher than those calculated for malaria as direct one-cause-one death. The reduction of malaria transmission had significant impact on child mortality rates. The number of lives saved by the scale-up of malaria interventions was higher than previously estimated.

646

THE DISTRIBUTION OF DHPS MUTATIONS IN AFRICA AND THEIR ASSOCIATION WITH DRUG PRESSURE AND TRANSMISSION INTENSITY

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Sulphadoxine-pyrimethamine (SP) was formerly widely used as a first-line treatment for *Plasmodium falciparum*, and continues to be important for prevention of malaria in pregnant women and children. Parasite mutations which confer resistance to SP spread earlier and more rapidly in some areas than others. Understanding why some areas are vulnerable to resistance could help inform surveillance and resistance prevention, and assess whether currently available data are sufficient to identify areas at risk. Here, we updated a systematic review on the K540E and A581G mutations in the dhps gene in Africa to 2016, previously completed in 2011. We geolocated each estimate, and linked each resistance data point with data from the same area and time, where available, on (a) slide prevalence, using Malaria Atlas Project estimates, and (b) SP use amongst febrile under five year olds, and coverage of intermittent preventive treatment in pregnancy (IPTp) from Demographic & Health Surveys and MICS Surveys, as well as SP market share from ACTWatch. Associations between resistance, drug pressure and transmission intensity were analysed using weighted regression. The systematic review resulted in 362 estimates of the prevalence of K540E and 220 A581G. In East Africa, the prevalence of both mutations generally increased or remained high, despite the end of first-line SP treatment policies. Here, the A581G emerged in locations where the prevalence of K540E was >50%. In West Africa, the K540E prevalence still did not reach levels as high as East Africa. In some sites where it emerged, later surveys no longer detected it. However, here the A581G mutation occurred when K540E was absent. We found a strong association between SP drug use in under-fives and K540E prevalence ($p<0.001$), but not A581G prevalence. Neither mutation was associated with IPTp coverage, nor SP market share data, nor transmission intensity. We are currently adding further covariates to the analysis, including age, presence of symptoms, and cotrimoxazole use. Our analysis could help inform surveillance for SP resistance mutations and potentially future emergent strains resistant to other antimalarials.

647

PLASMODIUM FALCIPARUM AND *P. VIVAX* GAMETOCYTE CARRIAGE IN SOUTH AMERICA, ASIA AND THE SOUTH PACIFIC

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Reducing human-to-mosquito transmission is crucial to control and eliminate malaria, yet not everybody infected is infective to mosquitos. To identify those contributing most to transmission, cross-sectional surveys of the general population were conducted in Brazil, Thailand, Papua New Guinea and Solomon Islands, including 18,979 individuals. *Plasmodium falciparum* and *P. vivax* were diagnosed using microscopy and highly sensitive qPCR assays. Over 80% of individuals were asymptomatic, yet they accounted for 84% of *P. falciparum* and 86% of *P. vivax* gametocyte carriers. Blood-stage parasite density was the main predictor for gametocyte positivity in all surveys. Each 10-fold increase in parasite density resulted in a 1.5-fold and 3.9-fold increase in the odds of *P. falciparum* and *P. vivax* gametocyte positivity. For *P. vivax* a close correlation between parasite and gametocyte densities was found. By microscopy asexual stages and/or gametocytes were detected in 37-72% of individuals positive for *P. falciparum* gametocyte by RT-qPCR, and in 42-91% of *P. vivax* gametocyte carriers. Across all surveys, 95-99% of the total gametocytes biomass was found in microscopy positive samples, with no apparent correlation between transmission level and the proportion gametocyte carriers identified by microscopy. Microscopy is thus a valuable tool to identify asymptomatic infections contributing to malaria transmission. While in high transmission settings a large proportion of all gametocyte carriers and 85-99% of all gametocytes were found in children below 6 years, gametocytes were evenly distributed across all ages in low transmission settings. This suggests that interventions to reduce transmission in high transmission areas will have the greatest effect when targeted towards children, but in order to achieve elimination in low transmission settings individuals of all ages must be targeted.

648

DIABETES AND OBESITY AS RISK FACTORS FOR SEVERE MALARIA: AN OBSERVATIONAL STUDY OF COMORBIDITIES IN *PLASMODIUM FALCIPARUM* CASES DIAGNOSED IN SWEDEN OVER 20 YEARS

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Non-communicable diseases and obesity are increasing global health problems, also in malaria endemic countries. The impact of these conditions on the severity of malaria needs to be established. The aim of this study was to assess if comorbidity, in terms of chronic diseases and obesity, is associated with severe malaria in adults with *Plasmodium falciparum*. Patients aged >18 years with *P. falciparum* malaria diagnosed at multiple sites throughout Sweden 1995-2015 were included in the study. Medical records were retrospectively reviewed regarding demographics, travel history, clinical presentation, weight, height, and comorbidity. Severe malaria was defined according to WHO criteria and hyperparasitaemia >5%. Obesity was defined as body mass index (BMI) ≥30, according to WHO classification. Data was analysed using multivariable logistic regression models. 937 patients with *P. falciparum* malaria were included in the study, 547 (58.4%) originated from endemic countries in Sub-Saharan Africa and 388 (41.4%) from non/low endemic countries. In total, 92 patients fulfilled the criteria of severe malaria, of which 22 (23.9%) had at least one comorbidity included in the Charlson Comorbidity Index compared to 84 (9.9%) among non-severe cases ($p < 0.001$). Age, health care delay, origin in non/low endemic country, diabetes, hypertension, cardiovascular disease, HIV and BMI ≥30 were associated with severe malaria in univariable analyses. In multivariable analysis adjusted for age, health care delay, patient origin and HIV, both diabetes and obesity were associated to severe malaria. Patients with obesity together with another metabolic risk factor (hypertension, dyslipidaemia or diabetes) had an even more pronounced risk of severe malaria in adjusted analysis. In conclusion, diabetes and obesity were independently associated with an increased risk of severe malaria in adults diagnosed with *P. falciparum* in Sweden. These metabolic comorbidities need to be considered in the acute management and prevention of malaria in adults. Moreover, their role in human malaria pathology needs to be further investigated.

649

THE DESCRIPTIVE EPIDEMIOLOGY OF PEDIATRIC SEVERE MALARIAL ANEMIA IN MALI AND TANZANIA

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Severe malarial anemia, defined as hemoglobin <5 g/dL in the presence of a *Plasmodium falciparum* infection, is the most common manifestation of severe malaria among young children in regions with very high malaria transmission. However, basic questions persist about the natural history of this profound anemia and the factors that contribute to an individual's risk of developing this syndrome during malaria infections. Drawing on birth cohort data collected from over 75,000 study visits with 880 children in the Mother Offspring Malaria Studies Project (2002-2006) in Muheza, Tanzania, and 1647 children in the Malaria Research and Training Centre-Laboratory of Malaria Immunology and Vaccinology Immuno-Epidemiology Observational Study (2010-2016) in Ouélessébougou, Mali, we aimed to describe in detail the epidemiology and pathogenesis of severe malarial anemia in early life. While severe malarial anemia occurred at older ages in the seasonally endemic Malian study site (median (IQR): 63 (39, 100) weeks) than in the perennially endemic Tanzanian setting (median (IQR): 34 (29, 52) weeks), these data suggest that the pathogenesis of malarial anemia is largely conserved across transmission settings. We will present data describing the associations between severe malarial anemia risk and parasite density, baseline hemoglobin levels, and bioavailable iron in early life. We will also use individual case data to illustrate that, although a subset of cases arise from gradually declining hemoglobin profiles associated with repeated infections, severe malarial anemia more commonly presents as an acute drop in hemoglobin. From a public health perspective, these findings reinforce the value of campaigns designed to interrupt malaria transmission and to reduce high density infections.

650

TRAVEL PATTERNS AND DEMOGRAPHIC CHARACTERISTICS OF MALARIA CASES: THE CASE STUDY OF SWAZILAND, 2010-2014

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As Swaziland gets closer to its 2018 national elimination goal, importation of parasites into receptive areas becomes increasingly important; imported infections have the potential to instigate local transmission and sustain local parasite reservoirs. Travel history (reported travel in the last 8 weeks) from Swaziland's routine surveillance data from 2010 to June 2014 was extracted to describe and compare travel patterns of RDT-confirmed index cases (identified passively) to travel patterns of individuals that tested negative (during re-active case detection, where the contacts in the vicinity of an index case within receptive areas were tested). Reported destinations of travel were geo-located to the smallest administrative boundary possible. Of 1,517 confirmed index cases 67% reported travel history, whilst 105 (1%) of the 9,859 contacts screened during the reactive case detection were RDT positive. 876 index cases travelled internationally. The proportion of index cases reporting travel history increased by year with a 48% increase per annum for international travel and 27% increase for travel within Swaziland. 25% of screened contacts reported international travel, of which 22% tested positive for malaria. Mozambique was the most likely travel destination of positive individuals, with Maputo City, Inhambane and Gaza being the most likely destinations in Mozambique. 97% percent of RDT positive international travellers were either Swazi (52%) or Mozambican (45%), however Swazis were more likely to test negative. All international travellers were unlikely to have a bed net at home or use protection while travelling. 84% of the 755 males and 60% of the 440 females that travelled abroad tested positive. Overall, 24 to 45 year olds were most likely to report travel. Additionally, paths of transmission, important border crossings and means of transport were identified. Results from this analysis can be used to direct national and well as cross-border targeting of interventions, over space, time and by sub-population. Collaboration between neighbouring countries is needed to tackle the importation of malaria at the regional level.

651

PLASMODIUM KNOWLESI MALARIA IN CHILDREN IN MALAYSIA: NO SEVERE DISEASE DESPITE AN INCREASED RISK OF ANAEMIA COMPARED TO ADULTS

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Plasmodium knowlesi is now the most common cause of malaria in Malaysia, but prospective studies describing the clinical spectrum have only detailed adult disease. In our prospective study (2012-ongoing) at 3 district hospitals in Sabah, Malaysia, previously untreated, non-pregnant patients of any age hospitalised with PCR-confirmed malaria includes: 500 *P. knowlesi* (49 [9.8%] children ≤12 yrs), 204 *P. vivax* (83 [40.7%] children), 103 *P. falciparum* (33 [32%] children), 26 *P. malariae*, 1 mixed *Pk/Pf* and 1 mixed *Pv/Pf*. Preliminary data include a lower baseline parasite count for *P. knowlesi* malaria patients (median 2480/µL, IQR 538-8481) than *P. falciparum* (9600/µL [IQR 1446-25440]; $p<0.001$), and *P. vivax* (3882/µL, [1454-9608]; $p=0.016$). Parasite counts were higher in adults with *P. knowlesi* compared to children (median 2800 vs. 1535/µL; $p=0.019$). Anaemia (WHO criteria) was present in 32% (95%CI 27-36) of adults vs. 81% (95%CI 70-93) of children with knowlesi malaria ($p<0.001$); comparable to that seen in children with *P. vivax* (82%; $p=0.948$) or *P. falciparum* (76%; $p=0.551$). Acute kidney injury (AKIN criteria and/or creatinine >132mmol/L) was found in 26% (95% CI 22-30) of those with *P. knowlesi* malaria including 111/437 (25%) of adults and 13/46 (26%) of children ($p=0.673$). There was a higher prevalence of acute kidney injury in children with *P. knowlesi* malaria (26%) compared to *P. vivax* (12.5%; $p=0.027$) but not *P. falciparum* (30.3%; $p=0.844$). Severe malaria (modified WHO 2010 criteria) was present in 6.4% (95%CI 2.4-9.6%) of those with *P. knowlesi* overall: 32/451 (7.1%) in adults but none in children. In comparison, severe disease was seen in 6/204 (2.9%; $p=0.065$) with *P. vivax* and 4/103 (3.9%; $p=0.326$) with *P. falciparum*. There were no treatment failures in knowlesi malaria patients seen at 28 days. There was one *P. knowlesi* malaria death, an adult (age 62) with delayed parenteral artesunate due to misreported hyperparasitaemia. Overall *P. knowlesi* malaria predominantly affected adults, and while anaemia was more common in children, parasitemia was lower and severe disease was not seen.

652

ASSESSMENTS OF WILDLIFE RESERVOIRS OF *TRYPANOSOMA CRUZI* AND THEIR INTERACTIONS WITH TRIATOMINE VECTORS ACROSS TEXAS

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Despite the importance of various wildlife species for perpetuating *Trypanosoma cruzi,* agent of Chagas disease*,* in nature, little is known about the relationship between infection and cardiac disease in wildlife, or the relative importance of wildlife species for feeding bugs. Using a cross-sectional study design, we collected cardiac tissue and blood from potential *T. cruzi* reservoirs across Texas, a state with widespread endemic, infected triatomines. Target species included migratory bats, nuisance rats (*Rattus rattus*), feral swine (*Sus scrofa*), and hunter-harvested predators including raccoon (*Procyon lotor*), coyote (*Canis latrans*), gray fox (*Urocyon cinereoargenteus*), and bobcat (*Lynx rufus*). Concurrently, we collected triatomines from regions of wildlife trapping and through a statewide citizen science campaign. Wildlife samples were tested for *T. cruzi* and parasite lineage was ascertained from the TcSD5D gene sequence. Vector-host interactions were determined using a bloodmeal analysis to amplify the vertebrate cytB gene in bug hindguts. No rats (n=152), a single bat (n=593), 5.6% of feral swine (n=54), 13.8-14.3% of bobcats, coyotes, and foxes (n=156), and 70% of raccoons (n=70) were infected with *T. cruzi*. The bat harbored lineage TcI, whereas TcIV was found in all raccoons that were typed. Although a histologic survey of right ventricles showed 21.1% of PCR-positive raccoons had mild lymphoplasmocytic infiltration, no other lesions were observed in raccoons, and none of the positive pigs showed cardiac pathology, suggesting infection is associated with minimal cardiac disease in these species. Diverse wildlife species were identified as bloodmeal hosts in an analysis of 76 bugs, including rats, raccoons, rabbits, feral swine, squirrel, fox, skunk, opossum, deer, and toads, but the majority of hosts were dogs (50%) and humans (22.4%), likely reflecting the nature of bugs encountered in the citizen collection program. Characterization of the robust sylvatic transmission cycles of *T. cruzi* combined with analyses of local triatomine feeding patterns will lead to ecological interventions to reduce Chagas disease risk.

653

LEISHMANIA INFANTUM PARASITEMIA IN ASYMPTOMATIC BLOOD DONORS IN AN ENDEMIC REGION OF NORTHEAST BRAZIL

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Leishmania infantum causes fatal visceral leishmaniasis (VL), but a majority of infections are asymptomatic. Transmission by blood transfusion has been documented in Europe, but the potential impact of *L. infantum* in blood donations in Latin America has not been assessed. We hypothesized that blood donations contaminated with *L. infantum* would be found at the blood bank serving Natal, northeastern Brazil, a city with a high prevalence of periurban *L. infantum* infection. Blood donations were examined for occult *Leishmania* infection in the Natal blood center. Three-hundred blood units initially rejected on the basis of positive screening for pathogens including *T. cruzi* or insufficient volume, and 254 samples from routine blood donors were assessed for *Leishmania* by culture, qPCR, and serology (ELISA). Remarkably, 18 of 300 (4%) rejected blood units were culture positive for *L. infantum*. qPCR was positive for *Leishmania* in 8.7%, and 22% were seropositive for Leishmania. Anti-*Leishmania* antibody levels correlated with *Leishmania* load by qPCR. Of the 254 blood samples that tested negative for other pathogens, 28.4% were seropositive for *Leishmania*, 7.7% were qPCR positive and one was culture positive. *T. cruzi* ELISA detected only 14/18 culture positive, 38/107 seropositive, and 22/37 qPCR positive donations. In conclusion, asymptomatic *Leishmania infantum* infections are associated with infected blood donations in northeastern Brazil. DNA testing by qPCR seemed the most sensitive and specific donor screening method. Serological assays for *T. cruzi* detected many but not all of the Leishmania-infected donors, likely due to cross-reacting antibodies and/or dual infections. The data suggest a need to screen blood donations for *L. infantum* in residents of, and immigrants from endemic regions.

654

CUTANEOUS AND MUCOCUTANEOUS LEISHMANIASIS IN INTERNATIONAL TRAVELERS: RESULTS FROM THE GEOSENTINEL SURVEILLANCE NETWORK

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Due to increasingly exotic travel, displacement of migrant populations, and expanding vector ranges, cutaneous leishmaniasis (CL) is emerging among international travelers and migrants, and limited data exist on mucocutaneous leishmaniasis (MCL) in travelers. We describe the epidemiology of travel-associated CL and MCL among international travelers and immigrants over an 8-year period through descriptive analysis of GeoSentinel data. Demographic and travel-related data on returned international travelers diagnosed with CL or MCL at a GeoSentinel Surveillance Network site between March 15, 2007 and August 31, 2015, were analyzed. Eight-hundred, twenty-eight returned travelers or new immigrants were diagnosed with CL or MCL during the study period, 824 (99.5%) of which were travel-acquired. Of travel-acquired cases, immigrants accounted for 8% (n=66). For all non-immigration travelers, the most common source countries were Bolivia (n=150, 19.8%) and Costa Rica (n=89, 11.7%), while for new immigrants, they were Afghanistan (n=18, 27.3%) and Syria (n=15, 22.7%). Eighty-one travelers (9.8%) acquired their disease on trips of ≤2 weeks. Species identification was available for 218 cases (26.5%). *Leishmania Viannia braziliensis* was the most well represented strain (n=93, 45.6%), followed by L. major (n=31, 15.2%), and *L. V. panamensis* (n=27, 13.2%). Thirty-five cases of MCL occurred, most of which were in tourists (n=26, 74.3%) and acquired in Bolivia (n=16, 45.7%). CL is predominantly a disease of tourist travelers to areas such as Bolivia where risk of acquiring L. V. braziliensis and subsequent MCL is high. That many travelers acquired their illness on trips lasting ≤2 weeks challenges the common notion that CL is a disease of prolonged travel. New immigrants from areas of conflict and political instability, such as Afghanistan and Syria, were well represented, suggesting that as mass migration of refugees continues, CL will be increasingly encountered in intake countries. Initiatives to enhance awareness and assure adequate resources for diagnosis and management of leishmaniasis are needed.

655

ASSOCIATIONS BETWEEN PARASITOLOGICAL AND SEROLOGICAL INDICATORS OF INFECTION AND THE DEVELOPMENT OF CLINICAL VISCERAL LEISHMANIASIS IN ETHIOPIA

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Visceral leishmaniasis (VL) is a vector-borne parasitic disease that results in 40,000 deaths annually. In East Africa, human infection by *Leishmania donovani* occurs via anthroponotic transmission, while the role of zoonotic routes remains unclear. Increased risk for VL is associated with malnutrition, male sex, and animal ownership. A recent upsurge in case counts and spatial spread of the disease in Ethiopia have demonstrated the need for more effectively implemented control policies. Data from a prospective cohort study conducted between 2011-2013 in northern Ethiopia provided longitudinal distributions of infection intensities (assessed by qPCR) and rates of seropositivity (assessed by direct agglutination test, DAT). Multivariate logistic regression with model selection allowed for evaluating independent risk factors for *L. donovani* exposure, seropositivity, and development of clinical VL. At baseline, 14.1% of 4,722 individuals had >0 parasites per ml of blood and 3.3% were seropositive at DAT titers >1:800. Despite similar distributions of infection intensity to females, males were significantly more likely to be seropositive during the study (6.8% vs 3.6%, P<0.001). After adjustment for age and body mass index, seropositivity was associated with infection intensities >100 parasites per ml blood. Seventy-five incident VL cases were recorded during the study period. Progression to clinical disease was significantly related to male sex (OR: 1.68, 95% CI: 1.01, 2.80), DAT positivity (OR: 4.60, 95% CI: 2.25, 9.41) and high infection intensity, specifically 101-1000 parasites per ml blood (OR: 4.67, 95% CI: 1.80, 12.13). Significantly increased odds of infection, seropositivity, and clinical disease were associated with having a seropositive household member. Simultaneous seropositivity and high infection intensity were significantly associated with progression to clinical VL. Males tended to exhibit these risk factors more than females. Identifying people with DAT titers >1:800 and then performing routine qPCR to determine the subset with high infection intensity could be a strategy for targeted intervention.

656

PROGRESSION AND MORTALITY RATES FOR MODELLING THE BURDEN OF CHAGAS DISEASE

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Accurate estimates of morbidity and mortality due to Chagas disease are needed to improve burden of disease evaluations. For parasitic infections, disease burden models link infection frameworks with associated morbidity (sequelae) and mortality, a much needed area of research for a better understanding of the impact and cost-effectiveness of control interventions. Such modelling efforts require elucidating the relationship between infection and disease as well as its rigorous and robust parameterisation. A systematic review was conducted to identify observational (longitudinal) studies comparing disease progression and mortality rates in populations with and without Chagas disease. Literature databases were searched without restrictions on publication language or date; 8,935 potentially relevant references were screened. Information on

selected papers was extracted and analysed using a random-effects model for both disease progression and mortality rates. The results of a previously developed force-of-infection model (for Colombia), yielding incidence and prevalence trends were linked to the progression and mortality rates thus quantified. This disease model was used to calculate the Disability Adjusted Life Years (DALYs) attributed to Chagas disease in Colombia. For assessment of progression rates, 19 studies were selected which provided 49,792 patient-years of follow-up. The general progression rate was 2% per year (95%CI: 1.7-3.4). For mortality rates, 25 studies were selected, providing data on 53,346 patient-years of follow-up, and 2,739 events. Pooled estimates revealed that Chagas disease patients have significantly higher annual mortality rates (AMR) compared with non-Chagas disease patients (0.18 vs. 0.10; RR = 1.74, 95 % CI 1.49-2.03). The application of these progression and mortality rates to burden of disease models allows us to estimate figures and burden metrics by clinical stage. A more refined analysis would allow us to estimate progression to megaesophagous, stroke and other outcomes.

657

DEFINITIONS AND FEASIBILITY OF ELIMINATION OF VISCERAL LEISHMANIASIS

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Visceral leishmaniasis (VL) is the second deadliest parasitic disease globally and has been targeted by the WHO for elimination and control by 2020. Two separate modeling approaches have been used to look into the feasibility of reaching the targets with current intervention strategies. 'Elimination of VL as a Public Health problem', the target for the Indian subcontinent, is predicted feasible in low and medium endemic regions with optimal implementation of interventions. However, highly endemic areas and regions with suboptimal interventions are likely not to achieve the target on time and will require additional efforts. 'Elimination of Disease', the target for the rest of the world, can be achieved by more regular serology testing, to identify which individuals are likely to develop clinical symptoms, so that they can be treated promptly. This should reduce the intensity of transmission and could lead to 'Elimination of Transmission'. The remaining knowledge gaps in the disease dynamics of VL, such as the contribution of asymptomatic individuals to transmission, present a challenge to reaching and sustaining the elimination targets.

658

VISCERAL LEISHMANIASIS IN THE INDIAN SUBCONTINENT: HOW MUCH DO ASYMPTOMATICS CONTRIBUTE TO TRANSMISSION AND HOW DOES TRANSMISSION DECREASE WITH DISTANCE FROM A CASE?

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A key unknown in the transmission of visceral leishmaniasis is how much asymptomatically infected individuals contribute to transmission. Since they significantly outnumber individuals who develop clinical symptoms (by 4-10 times in the Indian subcontinent) and are often infected for a long period, they may contribute substantially to transmission even if their relative infectivity to sandflies is low. This has important implications for attempts to control and eliminate the disease, as there is currently no safe treatment for asymptomatic infection, and interventions are focussed on reducing transmission through rapid diagnosis and treatment of symptomatic cases combined with indoor residual spraying of insecticide in endemic areas. Efforts to determine the infectivity of asymptomatic and symptomatic individuals via xenodiagnostic trials are ongoing, but these critical parameters are not known. Another unknown factor that has major implications for control is how the risk of transmission varies with distance from infectious individuals. This is key to determining whether responsive insecticide spraying strategies, in which houses within a certain radius of an index case are sprayed, will reduce transmission. To start to address these questions we have developed an individual-based spatiotemporal model of visceral leishmaniasis transmission and fitted it to detailed epidemiological and serological data from three highly endemic villages in Bangladesh to estimate the infectivity of asymptomatic individuals and the spatial kernel of transmission. Using Bayesian MCMC methods we have been able to account for the unknown times of infection of asymptomatic and symptomatic individuals, variation in infected individuals' infectivities over time, and potential false positives from serological tests. Our results suggest that the contribution of asymptomatic individuals to transmission in this highly endemic setting was small compared to that of individuals with clinical VL, and that the risk of infection was greatest for individuals within 40m of a VL case within 6 months of their onset of symptoms.

659

DOES ARTIMISININ BASED COMBINATION THERAPY INFLUENCE MOSQUITO FITNESS AND HOST-SEEKING BEHAVIOR?

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Artemisinin-based combination therapy (ACT) is recommended against malaria in many endemic areas, and thus widely used. Surprisingly little is known about the effect of ACTs on mosquitoes that transmit malaria parasites. Our objectives were to (1) determine whether ACTs have a direct impact on mosquito fitness when added to the bloodmeal, and (2) evaluate whether ACTs influence mosquito host-seeking behavior by changing the intrinsic attractiveness of human skin odor. Our study was done with *Anopheles gambiae* s.l., which is the main vector of malaria in sub-Saharan Africa. Mosquitoes that were fed on blood with ACT survived equally long as mosquitoes fed on control blood. ACT-fed and control mosquitoes also laid equal numbers of eggs, thus fitness was not affected by the treatment. To investigate host-seeking behavior of mosquitoes, adult malaria-free men were given a treatment dose of an ACT, and skin odor was collected on nylon socks before, during and three weeks after treatment. A screenhouse choice test showed no preference of *An. gambiae* females between socks worn by the same person before, during or after ACT-treatment. Relative attractiveness of nylon socks to An. coluzzii in a dual-choice olfactometer was also not influenced by ACT-treatment although mosquitoes appeared to be more responsive to skin odor collected three weeks after ACT-treatment. We conclude that ACT-treatment does not affect fitness and host-seeking behavior of malaria mosquitoes. Our results are important in light of possible transmission of gametocytes from ACT-treated people to malaria vectors.

660

UNEXPECTEDLY LOW HUMAN BLOOD INDEX ASSOCIATED TO HIGH *PLASMODIUM* SPOROZOITE RATES IN *ANOPHELES GAMBIAE* COMPLEX SPECIES FROM A LLIN-PROTECTED VILLAGE IN BURKINA FASO

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The global effectiveness of long-lasting insecticidal nets (LLINs) in reducing malaria transmission is indisputable. However, in areas where malaria transmission levels are extremely high, substantial reductions in transmission intensity only led to a modest reduction in human parasitaemia. A paradigmatic case is represented by Burkina Faso where, after a few years of mass distribution of LLINs, the burden of malaria has not significantly changed as highlighted by WHO Country statistics and statistical bureau of Burkina Faso. We here report the results of a longitudinal survey on host choice and *Plasmodium* sporozoite rate (SR) in malaria vectors belonging to *Anopheles gambiae* complex in a rural village of Burkina Faso where LLINs were broadly distributed the year before the sampling (August - November 2011). The human blood index (HBI) was 18.8% (N=112) and 8% (N=75), in An. coluzzii and An. arabiensis, the two most abundant malaria vectors in the area. These values are much lower than usually reported particularly for An. coluzzii, which is known as a highly anthropophilic species, but consistent with the hypothesis that LLINs reduced the availability of human hosts to mosquitoes. Unexpectedly, *Plasmodium* sporozoite rates (*An. coluzzii*: 7.6%, N=449; *An. arabiensis*: 5.2%, N=229) were found to be in the range of those reported in the region before LLIN implementation when much higher HBIs were observed. This suggests that, despite LLINs have significantly reduced human/vector contact, this has not apparently yielded to a substantial reduction of mosquito infection rates. Further investigations are needed to confirm these results; however, they are fully consistent with the lack of effectiveness of LLINs in stemming malaria transmission in the study area.

661

THE WMEL STRAIN OF WOLBACHIA REDUCES TRANSMISSION OF ZIKA VIRUS IN *AEDES AEGYPTI*

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Zika virus (ZIKV) is an arbovirus that belongs to the family Flaviridae. It currently is causing an explosive outbreak of febrile disease in the Americas. During the current outbreak, a causal relationship has been established between prenatal ZIKV infection and microcephaly and other serious brain anomalies. Most human cases around the globe result from ZIKV emergence into a human-mosquito cycle involving *Aedes aegypti* and/or other urban or peri-urban *Aedes* species. Despite the continued spread of the virus, there remain no effective antiviral therapies or licensed vaccines. Thus, with its continued invasion of the new world, the only tools presently available to combat Zika target mosquito populations, mostly with insecticides and larval source reduction. But, these strategies have not prevented invasion of this virus into new locales and have not been adequate to control the virus upon arrival. A promising candidate for arbovirus control and prevention relies on the introduction of the intracellular bacterium Wolbachia into *Ae. aegypti* mosquitoes. This primarily has been proposed as a tool to control dengue virus transmission; however, evidence suggests Wolbachia infections confer protection for *Ae. aegypti* against chikungunya virus as well. Although this approach holds much promise for limiting virus transmission, at present our understanding of the ability of ZIKV to infect, disseminate, and be transmitted by wMel-infected *Ae. aegypti* currently being used at Wolbachia release sites is unknown. Using *Ae. aegypti* infected with the wMel strain of Wolbachia that are being released in Medellin, Colombia, we report that these mosquitoes have reduced vector competence for ZIKV. In fact, we were not able to detect infectious ZIKV in the saliva of mosquitoes at any timepoint assayed. These data argue for the expansion of this technology to ZIKV in South and Central America and are useful and germane in the broader context of ZIKV-mosquito interactions. Finally, we also describe a biologically relevant model for studying ZIKV transmission dynamics (feeding on a viremic host) that does not rely on animal blood spiked with cultured virus.

662

COMBINING CONTACT TRACING WITH TARGETED INDOOR RESIDUAL SPRAYING SIGNIFICANTLY IMPACTS DENGUE TRANSMISSION

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The current paradigm for dengue virus (DENV) control relies on reactive measures aimed at containing virus transmission around the home residence of confirmed symptomatic cases. Unfortunately, the impact of such strategy on preventing virus transmission is very limited. Furthermore, the recent emergence of Chikungunya and Zika infections in the Americas elevates the need for more efficacious tools for virus surveillance, *Aedes aegypti* control and disease mitigation. We quantitatively investigated the epidemiological value of performing location-based contact tracing (incorporating potential out-of-home exposure locations by phone interviews associated with ongoing DENV passive surveillance) in improving identification of local dengue transmission foci across a metropolitan area (Cairns, Australia). Using space-time interaction tests applied to 2,064 potential exposure locations reported by contact tracing from 902 DENV-confirmed cases we statistically identified DENV transmission chains across the metropolitan area. We used such estimates of transmission to empirically evaluate the epidemiological impact of targeted indoor residual spraying (TIRS) with insecticides (application of residual insecticides at *Ae. aegypti* indoor resting sites). The city of Cairns was identified as a central hub for DENV transmission (95.2% of transmission events of Cairns residents and 60.4% of transmission events of residents of satellite towns were tracked to locations found within Cairns). Out-of-home exposure accounted for 57.2% of all putative transmission sites. Performing IRS in contact locations lead to a significant protective efficacy (0.86-0.96) in preventing DENV transmission. We provide quantitative evidence of the positive value of enhancing surveillance of urban DENV by performing location-based contact tracing and targeting indoor residual spraying operations at putative transmission sites identified from such data. While this approach was applied in a developed urban center, the potential for its implementation in DENV endemic areas will need to be evaluated.

663

ZIKA VIRUS IN THE AMERICAS: A MODEL-BASED ASSESSMENT OF FACTORS AFFECTING EMERGENCE

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Zika virus is a mosquito-borne pathogen that has emerged as a major threat to human health as the virus continues to spread throughout Latin America. Zika virus has now been linked to severe birth defects, as well

as an increase in Guillain-Barré syndrome. The parameters that affect transmission of the virus between mosquitoes and humans are still poorly understood beyond extrapolations from similar viruses. It is known that the virus is transmitted by mosquitoes in the genus *Aedes* (*Ae. aegypti* and *Ae. albopictus*), two invasive species that are both well-established within the United States. These species are closely associated with human habitation and breed in a diverse array of peridomestic containers that hold standing water. We have developed a deterministic model accounting for spatio-temperal heterogeneities in temperature throughout the United States to predict seasonal limits and peaks of Zika virus infection in order to focus vector control efforts and anticipate potential diagnostic testing demands. The model is informed by emerging field and laboratory data, including vector studies from our laboratory, and we contrast our Zika estimates for the U.S. to those for a dengue-endemic area in Iquitos, Peru to understand whether housing characteristics such as the availability of window screening and air conditioning in the U.S., are adequate to prevent Zika outbreaks.

664

WIND-ASSISTED LONG-DISTANCE MIGRATION OF MALARIA MOSQUITOES IN THE SAHEL

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Over the past decade, malaria control efforts have greatly reduced its burden even in its home base - Africa, raising hopes for malaria eradication in some reader's lifetime. Malaria transmission in Africa extends from the Equatorial Zone to the Sahel where it is confined to the short rainy season. Persistence of anopheline vectors in areas without surface waters for 3-8 months a year attests for the sophisticated strategies employed by the vectors. Recent evidence suggests that *Anopheles coluzzii* persists locally throughout the dry season by a form of diapause (aestivation), whereas *A. gambiae* and *A. arabiensis* rely on long-distance migration from areas where reproduction continues year round. This contradicts the widely accepted view that these vectors do not disperse beyond a few kilometers in a lifetime. Here, we summarize aerial sampling of insects 100-300 m above ground conducted between May 2012 and November 2015 in four Sahelian villages. A total of 30 *A. gambiae* s.l. and 117 *A. pharoensis* were captured among >3,000 mosquitoes and over half a million other insects during 747 aerial night collections. No mosquitoes were captured in 502 control captures raised briefly to 120 m during launches and retrievals, corroborating that these species were intercepted at high altitudes rather than near the ground. A high proportion of the mosquitoes were gravid, indicating that they might carry human pathogens. Because such movements of mosquito vectors are regular and involve many thousands of mosquitoes per night, they have important implications for disease emergence and reemergence as well as for disease elimination programs. A comprehensive analysis of these data will be presented.

665

GENERIC AND STANDARDIZED DATA COLLECTION FORMS AND DATABASE APPLICABLE TO DIVERSE ENTOMOLOGICAL STUDIES OF MOSQUITOES

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Studies of malaria vectors and other vector-bone diseases encompass a remarkably diverse array of designs and rapidly generate large data volumes. Such data is normally collected or recorded using experiment-specific forms that require frequent, error-prone redesign leading to badly or inconsistently structured data, difficulty in sharing or linking. Standardised data schema, databases and even public data repositories exist for genetic data for malaria parasites and for their human and mosquito hosts and similar controlled and standardised systems are available for epidemiological studies of malaria-infected human beings. However, equivalent systems for studies of the mosquitoes which mediate transmission do not exist. We have developed 1) a generic schema, 2) paper-and electronic-based customizable data collection forms, and 3) a database web-based application - to provide structure to data at the point of mosquito data collection and streamline use of databases that consistently link field and laboratory data. The database is built in such a way that it can be linked to other system such demographic surveillance systems and epidemiological based-databases. As a result, data from diverse mosquito studies conformed to a developed generic schema, with data collection forms recording the experimental design, sorting of collections, details of sample pooling or subdivision, and additional observations using standardized formats. The database stores and links data, generates summarized reports, enhances data sharing and dissemination from multiple experiments, projects, and studies. Currently, the user uptake includes 20 experiments, 10 projects, and 20 users at 3 research and control institutes in 3 African countries, resulting in 13 peer-reviewed publications. This vector database is expected to advance vector control research especially for resource-limited tropical settings lacking specialized software or informatics support.

666

IMPLEMENTATION OF A MULTI-COUNTRY RESPONSE TO THE EBOLA VIRUS DISEASE EPIDEMIC IN WEST AFRICA: LESSONS FROM THE FIELD

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The recent West African outbreak of Ebola virus disease (EVD) is the largest ever recorded. Starting from September 2014, International Medical Corps (IMC) opened five Ebola Treatment Units (ETUs) in Liberia and Sierra Leone, which cumulatively collected nearly 25,000 pages of epidemiologic, clinical, and laboratory data. To extract this data, each chart was either manually copied across the fence between the high-risk zone and low-risk zone or was imaged in the high-risk zone using a GoPro camera. Data were then entered into separate electronic databases, which were combined into a single relational database. Data quality assurance identified an overall final error rate of 1.2%. The full IMC database includes 2768 patient presentations, of which 2485 were admitted to an ETU, and 2329 had outcome data available. Of these, 1506 (65%) patients were from Sierra Leone while 823 (35%) were from Liberia. 54% of patients were male, and the median age was 30 (IQR 18-43). 10% of admitted patients were < age 5 and 11% were > 55. Among all admitted patients, 461 (20%) tested positive for EVD. 192 recovered to discharge, 5 were transferred to other facilities, and 264 died, for a case fatality ratio (CFR) of 58%. Among EVD negative patients, 154 of 1868 died, for a CFR of 8%. Average length of stay was 14.6 days for EVD positive patients who recovered and 5.6 days for those who died. Although more males were admitted as suspect patients than females, a larger proportion of females were diagnosed as EVD positive (26% vs 15%). EVD positive patients aged 15-24 had the lowest CFR (37%), while patients < 5 and > 55 had the highest CFRs (93% and 70%, respectively). CFRs were also higher in Sierra Leone (61%) than Liberia (53%). While several prior reports have documented the experiences of individual ETUs, this study is the first to present data from multiple ETUs across two countries run by the same organization with similar clinical protocols. Our experience

demonstrates that even in austere settings under difficult conditions, it is possible for humanitarian organizations to collect high-quality clinical and epidemiologic data during a major infectious disease outbreak.

667

THE NATURAL HISTORY OF EBOLA VIRUS DISEASE: A RETROSPECTIVE STUDY OF THE WEST AFRICA EPIDEMIC

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This retrospective study documented the natural history of EVD among confirmed patients utilizing data from five Ebola Treatment Units operated by International Medical Corps in Sierra Leone and Liberia in 2014-2105. All patients were treated as per standard treatment protocols based on guidelines developed by the World Health Organization and Medecins Sans Frontieres. Self-reported and observed data on presence of symptoms were collected at admission and during daily rounds by clinical staff. Progression of EVD symptoms were explored using descriptive statistics, differences in mean number of days symptomatic among subgroups were analyzed using t-tests and ANOVA, and survival analyses were conducted utilizing Kaplan-Meier estimators and Cox proportional hazards models. Of 470 confirmed EVD cases treated, 297 met the inclusion criteria of positive EVD diagnosis, known outcome, and reported symptom onset date. To assess progression of symptoms by week, a subset of patients for whom rounding dates were also present were analyzed (n=253). The mean number of symptomatic days at admission was 4.3; this varied significantly by sex (females: 3.9, males: 4.8, $p = 0.036$) and by age category (0-4: 2.4, 5-24: 3.7, 25-44: 4.6, 45+: 5.0, $p = 0.009$). The three most common symptoms by week symptomatic were: Week 1 (n=253) - weakness (67%), anorexia (63%), fever (55%); Week 2 (n=134) - weakness (70%), fever (69%), diarrhea (63%); Week 3 (n=52) - fever (44%), bone/muscle/joint pain (35%), headache (33%). The overall survival rate was 36.9% with no significant difference by sex. All age groups had a lower risk of death when using patients 0-4 years as the reference: 5-24 years, hazard ratio (HR)=0.34 (95% confidence intervals (CI): 0.19-0.59, $p<0.001$); 25-44 years, HR=0.45 (95% CI: 0.27-0.77; $p=0.003$); 45+ years, HR=0.54 (95% CI: 0.31-0.93; $p<0.025$). Among patients who died, mortality occurred in 86% by day 13 of experiencing symptoms. The natural history of EVD among patients in Liberia and Sierra Leone demonstrated consistent progression of nonspecific symptoms and high overall mortality with significantly higher mortality among patients 0-4 years.

668

BIAS ADJUSTMENT OF CASE FATALITY RATE ESTIMATES IN THE EBOLA OUTBREAK IN WEST AFRICA

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The recent Ebola outbreak in West Africa caused an officially reported 28,646 cases and 11,323 deaths by 30 March 2016, however, the true burden was likely considerably higher. The case fatality rate (CFR), defined as the proportion of cases that die, is the most important indicator of severity. It is however surprisingly difficult to estimate accurately from data collected routinely during an outbreak. The most reliable estimates in this outbreak were obtained by only considering cases for which the outcome (death or survival) was recorded, however, with only half of the reported cases having an outcome recorded there is substantial scope for biases in this simple estimate if the probability of reporting the outcome depends on the outcome itself. By considering the strong age-dependence of CFR and comparing the proportion of cases with recorded outcome between age groups we assess the differential outcome reporting probability between survivors and fatalities, and adjust CFR estimates accordingly. While in Guinea outcome reporting was near complete for cases entered into the VHF database, we estimated that fatal cases were more likely than survivors by 31% in Liberia and 131% in Sierra Leone to have the outcome reported, leading to an upwards bias in raw CFR estimates. When adjusting for this bias, CFR estimates were corrected from 65.6% (95% CI 63.8 - 67.3%) to 58.8% (51.4 - 65.4%) in Liberia and from 71.2% (69.7% - 72.7%) to 51.7% (45.2 - 61.4%) in Sierra Leone, while estimates in Guinea remained ad 59.2% (57.1 - 61.4%). CFR estimates adjusted for differential reporting were therefore more consistent between countries. Accurate estimates of disease severity are crucially important for public health planning, but are challenging to obtain from data collected typically during an outbreak. The bias of differential outcome reporting is likely a problem in many settings, and the method developed here will therefore be useful in future outbreaks of novel pathogens.

669

QUANTIFICATION OF THE IMPACT OF SAFE AND DIGNIFIED BURIALS DURING THE 2013-2016 WEST AFRICAN EBOLA VIRUS DISEASE EPIDEMIC

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Over 28,600 people were infected with Ebola virus disease (EVD) and over 11,000 died in the 2013-2015 West African epidemic. The EVD status of individuals who die in the community, outside of Ebola Treatment Centers, is unknown at the time of death however burial of EVD infected individuals poses a serious risk for continued transmission. Consequently an important component in EVD response is safely burying infected individuals. This pillar of the response was led by the International Federation of the Red Cross and Red Crescent Societies, who buried 47,505 individuals, 2,205 of which were EVD positive. Here we quantify the impact of the Red Cross Safe and Dignified Burial Program (SDB) on the EVD epidemic. Epidemiological and anthropological investigations were completed in communities in Sierra Leone, Guinea and Liberia that had carried out unsafe burials. Forty-five unsafe burials were investigated and 310 contacts identified. Approximately 7 individuals per unsafe burial were reported to have had contact with the index case (IC) and 1.8 infected secondary cases were generated, although this varied by district (range: 0.6-5.5). Contact with the IC during their acute illness and post-mortem was reported for 46% of contacts. Contact with fluids of the IC was the most strongly predictive of transmission followed by physical contact with the IC during their acute illness. Those having contact with the IC before death were 2.5 - 6 times more likely to be infected with EVD, relative to those with post-mortem contact only. By averting 1,477 to 10,452 secondary EVD cases, SDB reduced the size of the epidemic by 5.2 to 36.5%. Through this study it is impossible to ascertain, for those individuals who had contact with the index case before and after death, the exposure that caused their infection. Nevertheless, these results underline the importance of isolating individuals infected with EVD early in order to further limit community transmission. We also quantify for the first time, the importance of SDB as a fundamental EVD control measure and provide an estimate the number of additional infections averted by SDB.

670

MORTALITY OUTCOMES AMONG PATIENTS WITH VARIABLE INFECTION STATES WITH EBOLA VIRUS DISEASE AND MALARIA IN SIERRA LEONE: A RETROSPECTIVE COHORT STUDY

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This retrospective cohort study investigated the relationship between infection with Ebola Virus Disease (EVD) and/or malaria and case fatality ratio (CFR) among patients admitted to three Ebola Treatment Units (ETU) in Sierra Leone between December 2014 and September 2015. Standardized care with artemisinin-based combination therapy for empiric malaria treatment was provided to all patients based on International Medical Corps protocols. The cohort was stratified by infection status as: malaria negative/EVD negative (m-/e-), malaria positive/EVD negative (m+/e-), malaria negative/EVD positive (m-/e+) and malaria positive/EVD positive (m+/e+). Mortality outcomes were explored using descriptive statistics and Cox proportional models. Survival regression analyses adjusted for age, and the m-/e- group was set as the baseline comparator. Mortality time of event was derived from chart documentation and all survivors were censored at 28 days from admission. Among 1548 cases treated, 753 met inclusion criteria. The cohort cases included 431 m-/e-, 180 m+/e-, 108 m-/e+ and 34 m+/e+. In the m-/e- group, the CFR was 11%. For m+/e- cases the CFR was 5%, with an adjusted hazard ratios (aHR) of 0.5 (95% CI: 0.3-1.0, p=0.07). Cases found to be m-/e+ had 53% mortality and a greater than five-fold increased risk of death (aHR=5.7, 95% CI: 3.9-8.5, p<0.001). The m+/e+ group had the highest CFR, at 59%, with an aHR of 7.7 (95% CI: 4.5-13.1, p<0.001). Patients admitted to ETUs with confirmed EVD had the highest mortality, and concomitant infection with malaria increased the risk of death in the studied population. Among patients without EVD who were admitted to ETUs, risk of death was nearly twice as high in those without malaria infection as opposed to those with malaria infection, likely due to empiric treatment of all patients with antimalarial medications.

671

IMPLEMENTING SEASONAL MALARIA CHEMOPREVENTION (SMC) IN THE CONTEXT OF EBOLA VIRUS DISEASE (EVD) IN GUINEA

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An overlap of symptoms of the EVD and malaria in Guinea created a context for integrated case management of the two diseases, as untreated malaria cases were linked to increased malaria mortality and fever cases (a shared symptom for both diseases) which was deemed to impede the EVD response. SMC began in Guinea during the onset of EVD and in the context of severely diminished demand for health services; EVD was the estimated cause of approximately 74,000 fewer malaria cases seen at health facilities in 2014. The NMCP used EVD case management structures and implemented SMC through a house-to-house strategy in six SMC-eligible districts targeting 210,000 children 3-59 months. A Health Service Delivery Coverage exercise designed to examine perceptions of service delivery acceptability (including SMC) was conducted through focus group discussions and key informant interviews in each of the SMC Districts. It engaged caregivers, local leaders and CHWs who observed a flattening in demand for health services throughout Guinea in reaction to EVD - contributing to the 46,968 confirmed cases of malaria and 86 deaths within the under-five target population in 2014. The total cases of confirmed malaria in the areas that received SMC in 2015 decreased by 29% and by 26% for malaria deaths among the under-five population. Whereas in the two SMC-eligible Districts of Siguiri and Madiana where SMC was not implemented in 2015 there were increases in malaria cases in the under-five population from 2014 to 2015. In Siguiri there was an increase in confirmed malaria cases by 92% (3,352 cases in 2014 and 6900 cases in 2015) and an increase in malaria deaths 7,350% (4 deaths in 2014 and 298 deaths in 2015); while in Madiana there was an increase in confirmed malaria cases by 2,450% (740 confirmed cases in 2014 and 19,633 confirmed cases in 2015) and no increase in malaria deaths. SMC in Guinea, reached 100% of eligible children: demonstrating that even in the context of EVD or potentially other outbreaks, SMC can be delivered at scale and save lives. In 2016, SMC will be scaled up to cover Siguiri and Madiana and the NMCP will continue SMC implementation in all eligible areas in 2017 and beyond.

672

EFFECTS OF FUNCTIONAL LATRINE DENSITY ON HOUSEHOLD DRINKING WATER CONTAMINATION, SOIL-TRANSMITTED HELMINTH INFECTION AND DIARRHEA: A SPATIAL ANALYSIS

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India accounts for 60% of the 2.4 billion people practicing open defecation worldwide. A large cluster-randomized trial (CRT) in Orissa found no village-level health impacts of the Government of India's Total Sanitation Campaign. Village-level coverage varied greatly; latrines went unused, often due to poor construction/functionality. Using geospatial data from households in 50 intervention villages of the CRT in Orissa, we assessed environmental health impacts of *functional* latrine density on a fine spatial scale. Coverage of all latrines and functional latrines within 25m, 50m, 100m, and 200m of households was calculated via a multiple ring buffer analysis in ArcGIS. Outcomes were household drinking water contamination (N=1009), soil-transmitted helminth (STH) infection (N=822), diarrhea among all ages (N=1275) and children <5 (N=1017). Multivariate regressions adjusted for village-level clustering with Generalized Estimating Equations. Covariates included location of water source, household population, and whether or not a household, itself, owned a functional latrine. Increased latrine coverage in 200m was associated with decreased levels of thermotolerant coliform (TTC) in household drinking water (β=-5.05, 95% CI -9.81, -0.29). Each additional functional latrine in 25m was associated with a decrease of 28.9 cfu TTC per 100 mL (CI -57.5, 9.8). Odds of STH infection decreased by 10% for each additional latrine, regardless of functionality, in 25m (β=0.903, CI 0.819, 0.994). For every 10 additional latrines in 25m, regardless of functionality, household longitudinal diarrhea prevalence (all ages) *increased* by 2.13 days per 1,000 person days (CI 0.06, 4.2). A 10% increase in functional latrines in 25m was associated with 1.4 fewer days of diarrhea per 1,000 person days (CI -5.5, -0.1). Households owning functional latrines, themselves, had 8 fewer days of childhood diarrhea per 1,000 person days (p<0.05). Ensuring 100% sanitation coverage and functionality within the immediate surroundings of the home is critical for reducing exposure to pathogenic feces that cause diarrheal diseases.

673

FECAL CONTAMINATION ALONG MULTIPLE ENVIRONMENTAL PATHWAYS IS ASSOCIATED WITH SUBSEQUENT DIARRHEA AMONG CHILDREN IN RURAL BANGLADESH

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Pathogens associated with diarrhea are transmitted from feces to new hosts through multiple environmental pathways: drinking water, ambient water, hands, soil, food, and flies. Understanding the relative risks of exposure to fecal contamination along each pathway could be valuable in the design of interventions to interrupt transmission and reduce diarrhea. We conducted a prospective study among 1843 households in rural Bangladesh to quantify levels of fecal contamination along multiple environmental pathways and assess their association with subsequent risk of under-five child diarrhea. Measurements were collected during two sequential visits to each study household. During the first visit, sampling at each household included: child hand rinse, stored drinking water, source drinking water (tubewells), pond water, soil from the child's play area, food served to young children and flies captured from the food preparation area. All samples were analyzed for fecal indicator bacteria (most probable number [MPN] of *E. coli* and fecal coliforms) using the IDEXX Colilert-18 Quanti-Tray system. During the second visit (conducted 4-10 days after the initial visit to encompass typical incubation periods for gastrointestinal pathogens), interviewers collected caregiver-reported child gastrointestinal symptoms. We used generalized linear models with robust standard errors to estimate the relationship between the presence and concentration of fecal indicator bacteria and subsequent diarrhea prevalence. Child diarrhea prevalence following the field team's previous visit to the household increased by 31% with *E. coli* presence on child hands (PR=1.31, 1.06-1.63) and 17% for each log10 increase in fecal coliform counts in soil (PR=1.17, 1.04-1.32). Our findings suggest that child hands and household soil are important transmission pathways for diarrheal illness among children under five in rural Bangladesh.

674

QUANTIFYING FECAL CONTAMINATION LEVELS OF DRINKING AND AMBIENT WATERS, HANDS, FOOD, SOIL AND FLIES IN THE DOMESTIC ENVIRONMENT IN RURAL BANGLADESH

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Fecal-oral pathogens are transmitted from feces to hosts through a variety of environmentally mediated pathways. Characterizing levels of fecal contamination along these pathways can inform the development of targeted interventions to reduce fecal exposures. We quantified fecal indicator bacteria along different transmission routes in the domestic environment in 1843 households in rural Bangladesh. We collected samples of source (tubewell) and stored drinking water, pond water, child hand rinse, weaning food, flies caught in the food preparation area and soil collected from children's outdoor play area. We analyzed samples for *E. coli* with the IDEXX most probable number (MPN) method and calculated the geometric mean of *E. coli* concentration for each type of sample. 24% of tubewells and 58% of stored water samples were contaminated with *E. coli*. A typical pond water sample contained almost 4-log MPN *E. coli* per 100 mL. 40% of children had *E. coli* on their hands; children ≥ six months old had 0.3-log MPN per two hands more *E. coli* than children < six months of age (p<0.001). A typical soil sample had approximately 5-log MPN *E. coli* per dry gram. Soil in the vicinity of human or animal feces had 0.2-log MPN higher *E. coli* than soil collected from areas with no feces (p<0.001). Soil from sunlit areas had 0.2-log MPN fewer *E. coli* than soil from shaded areas (p<0.001), and levels of *E. coli* were positively correlated with the moisture content of samples (p<0.001). 59% of stored food samples contained *E. coli*; food stored in a covered container had 0.2-log MPN fewer *E. coli* than food from uncovered or partially covered containers (p=0.02). 54% of captured flies had *E. coli* contamination; a typical fly had approximately 3-log MPN *E. coli.* Fecal indicator bacteria levels were higher among all seven pathways in the rainy season (p≤0.01). Our findings demonstrate ubiquitous fecal contamination along multiple pathways in rural Bangladeshi households and highlight the occurrence of high levels of fecal indicator bacteria in ponds and especially courtyard soil in this setting, drawing attention to these understudied pathways for diarrheal disease transmission.

675

UNSAFE CHILD FECES DISPOSAL IS ASSOCIATED WITH ENVIRONMENTAL ENTEROPATHY AND IMPAIRED GROWTH

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This study was undertaken to investigate the relationship between unsafe child feces disposal, environmental enteropathy (EE), and impaired growth, we conducted a prospective cohort study of 216 young children in rural Bangladesh. Unsafe child feces disposal, using the WHO/ UNICEF Joint Monitoring Program definition, was assessed using 5 hour structured observation and caregiver reports. Anthropometric measurements were collected at baseline and at a nine month follow-up. Stool was analyzed for fecal markers of EE: alpha-1-antitrypsin, myeloperoxidase, neopterin (combined to form an EE disease activity score), and calprotectin. Eighty four percent of households had an unsafe child feces disposal event during structured observation and 75% had caregiver reported events. There was no significant difference in observed unsafe child feces disposal events for households with or without an improved sanitation option (82% vs. 85%, p=0.72) or by child age (p=0.96). Children in households where caregivers reported unsafe child feces disposal had significantly higher EE scores (0.82 point difference, 95% confidence interval (CI): 0.11, 1.53), and significantly higher odds of being wasted (Weight for Height z-score (WHZ) <-2 SDs) (9% vs. 0%, p= 0.024). In addition, children in households with observed unsafe feces disposal during structured observation had a significantly reduced change in Weight for Age z-score (-0.34 (95% CI: -0.68, -0.01) and WHZ (-0.52 (95% CI: -0.98, -0.06)). In conclusion, unsafe child feces disposal was significantly associated with EE and impaired growth in a pediatric population in rural Bangladesh. Interventions are needed to reduce this high risk behavior to protect the health of susceptible pediatric populations.

676

THE MAPUTO SANITATION (MAPSAN) TRIAL: ASSESSING A SANITATION INTERVENTION'S IMPACT ON HELMINTHIASIS IN CHILDREN <5 YEARS OLD IN MAPUTO, MOZAMBIQUE

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The MapSan study, in Maputo, Mozambique, is a first of its kind, controlled before and after trial of the impact of a shared sanitation intervention (pour-flush latrines) on soil-transmitted helminth (STH) and enteric infections in children <5 years. Despite progress in urban sanitation coverage, residents of urbanizing, unplanned communities in large cities of Sub-Saharan Africa experience elevated disease risks associated with poor sanitation. Dense urban environments are critical settings for targeted sanitation improvements since the risks of unsafe excreta disposal can be much greater within a dense urban population compared with a low-density rural population. For the study, stools were collected from children in intervention and matched-control arms to assess baseline STH prevalence prior to intervention exposure. Stools were analyzed by Kato Katz (N=671) to quantify several STHs (including *Trichiura trichuris*, *Ascaris lumbricoides*, and hookworm) and will be shipped to the US for multiplex-STH qPCR analysis. Overall baseline prevalence for any STH infection by Kato-Katz is 46% and is similar between study arms. As expected, prevalence increases with age [in years (OR 1.96, CI: 1.67, 2.31)]. T. *trichuris* and A. *lumbricoides* are the most commonly observed STHs with prevalences of 23% and 38%, respectively. Coinfection with multiple STH was observed in 16% of samples. Post-exposure sample collection is ongoing and data will be available starting in Summer 2016. The most recent nationwide survey of STH in school-aged Mozambican children (2005-2007) found a combined prevalence of 53.5%. Our baseline results provide a more complete picture of the STH burden in Mozambique by reporting on younger children who are often missed in school-based prevalence surveys. Our pending endline results will add evidence to the conversation of how to best serve the approximately 2.5 billion people who currently lack access to safe sanitation.

677

WATER, SANITATION AND HYGIENE (WASH) AND ENVIRONMENTAL RISK FACTORS FOR SOIL-TRANSMITTED HELMINTH INTENSITY OF INFECTION IN TIMOR-LESTE, USING REAL TIME PCR

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No investigations have been undertaken of risk factors for intensity of soil-transmitted helminth (STH) infection in Timor-Leste. We present the first analysis of combined water sanitation and hygiene (WASH), environmental, and socioeconomic risk factors undertaken using intensity of infection classes developed from qPCR diagnosis of STH. Questionnaires were used to collect WASH and demographic data from 24 villages in Manufahi District, Timor-Leste. An algorithm was developed to correlate PCR cycle threshold values to eggs per gram of faeces equivalents, using seeding experiments. Open-access environmental variables were obtained. A socioeconomic quintile was developed using principal component analysis. Multinomial mixed-effects regression was used to assess risk factors for intensity of Necator americanus and Ascaris infection in 2152 participants. In adjusted models incorporating WASH, socioeconomic and environmental variables, environmental variables were generally associated with infection intensity for both N. americanus and Ascaris spp. Precipitation (in centimetres) was associated with increased risk of moderate-intensity (adjusted relative risk (ARR) 6.1; 95% confidence interval (CI) 1.9-19.3) and heavy-intensity (ARR 6.6; 95%CI 3.1-14.1) N. americanus infection, as was sandy-loam soil around household (moderate-intensity ARR 2.1; 95%CI 1.0-4.3; heavy-intensity ARR 2.7; 95%CI 1.6-4.5; compared to no infection). For Ascaris, alkaline soil around the household was associated with reduced risk of moderate-intensity infection (ARR 0.21; 95%CI 0.09-0.51), and heavy-intensity infection (ARR 0.04; 95%CI 0.01-0.25). Few WASH risk factors were significant. Our novel approach of assigning infection intensity classes to PCR-diagnosed STH infection requires further research. In this high-prevalence setting, significant risk associations with environmental factors suggest that anthelmintic treatment should be integrated with other interventions, as conditions are favourable for ongoing environmental transmission. Integrated STH control strategies should be explored as a priority.

678

WASH FOR WORMS: END-POINT RESULTS FROM A CLUSTER RANDOMIZED CONTROLLED TRIAL OF THE IMPACT OF A COMMUNITY-BASED INTEGRATED WASH AND DEWORMING PROGRAM ON SOIL-TRANSMITTED HELMINTH INFECTIONS

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Soil-transmitted helminth (STH) infect an estimated 1.45 billion people worldwide, therefore remaining a global health problem. Deworming programmes are effective in reducing STH prevalence but rapid reinfection occurs in the absence of decreased environmental contamination. Therefore WASH interventions are obvious candidates for sustainable control. WASH for WORMS is a cluster-randomised controlled trial to quantify the impact of a community-based WASH intervention integrated with periodic mass distribution of albendazole on infections with STH and protozoa, compared to mass deworming alone. In this trial, initiated in 2012, all participating communities in Timor-Leste received mass deworming every 6 months (for 2 years) and half of them also received the WASH intervention. The WASH intervention was implemented by WaterAid, Australia and included promotion of household latrines (based on "Community Led Total sanitation"), improved access to water and hygiene promotion. Infection prevalence and intensity were measured by qPCR. At baseline, the prevalence of STH in the 24 villages was high, with 62.3% of the participants infected with *Necator americanus*. In the intervention arm, *N. americanus* decreased from 62.8% to 32.2% at the 1st follow-up (FU1), with a further decrease to 21.7% at the 2nd follow-up (FU2), one year after completion of the WASH intervention. In the control group, *N. americanus* decreased from 61.8% to 36.9% at FU1, reaching 20.5% at FU2. At this time point, 77.7% of households in the intervention arm had a latrine whereas in the control arm 20.5% of the houses had one. Participating villages were followed for an additional year, with data collection ending in April 2016. Results will be presented for the study end-points and discussed in the context of the uptake of the WASH intervention. This trial is the first reported RCT evaluating the impact of integrated WASH and deworming interventions on STH infection; and will provide essential evidence for optimizing integrated STH control programmes.

679

THE NEW WORLD HEALTH ORGANIZATION APPROACH TO SURVEILLANCE FOR TRACHOMA, EXPERIENCE IN NEPAL AND ADDED BENEFIT OF ANTIBODY PREVALENCE TO *CHLAMYDIA TRACHOMATIS* PGP3 PROTEIN: NESTS STUDY

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As part of the global effort to eliminate trachoma, the leading cause of preventable infectious blindness in the world, the World Health Organization (WHO) requires a second survey for trachoma at least two years after districts have stopped mass drug administration, to determine if re-emergence has occurred. Useful markers, like antibodies to chlamydial antigens, and chlamydial DNA testing for elimination of trachoma, are being investigated for utility in these surveillance situations. Using the new WHO guidelines, this study was undertaken to conduct surveillance surveys and to evaluate the possible role of tools in surveillance programs in 4 districts in Nepal. 15 randomly selected clusters within four districts were chosen. In each cluster, 50 randomly selected children ages 1-9 year olds and 100 adults ≥15 years old were examined for TF±TI and TT respectively. Eye swabs were taken from all children to test for *C. trachomatis* (CT) infection using the Cepheid GeneXpert platform. Dried blood spots were collected from children to determine antibody positivity to the *C. trachomatis* antigen pgp3. Blood spots were processed on the Luminex-100 platform following standard procedures. Data were analyzed as simple frequencies, and age stratified proportions. Results: Districts were 2, 4, 8, and 10 years from last program activities. In the sampled 4,042 children, only 11 TF cases and 3 CT infection were found. Overall antibody positivity was found in 1.9% of samples with no increase in frequency by age. There was no evidence for clustering of antibody positivity by community. Once adjusting for TT already known to the health system, rate was <1/1,000 population in all districts. In conclusion, no evidence of re-emergence of trachoma was found in four districts in Nepal during surveillance surveys as late as 10 years after cessation of all program activities. The absence of an increase in age seroprevalence suggests this tool may measure interruption of transmission of *C. trachomatis*. This study provides an even stronger empirical basis for the new WHO guidelines for surveillance of trachoma, and adds new knowledge on surveillance for trichiasis.

680

SERO-SURVEILLANCE IS AN INFORMATIVE INDICATOR OF TRACHOMA TRANSMISSION INTENSITY

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Trachoma remains the world's leading infectious cause of blindness. The World Health Organization (WHO) has a target of elimination of trachoma as a public health problem by 2020. Validation of elimination of trachoma requires that the clinical sign trachomatous inflammation—follicular (TF) be less than 5% in children aged 1-9 years old. However, in settings where transmission is low, grading TF can be challenging, as other bacterial infections and dust may aggravate the eyelid, producing TF-like inflammation that may not be associated with ocular chlamydia infection. Serology has been successfully used as an indicator of transmission intensity for a number of pathogens, and recent work has evaluated the potential for antibody-based testing in children for trachoma surveillance. We used serological data collected from a range of trachoma transmission settings—including high (TF greater than 30%), medium (TF 10-30%), and low (TF less than 10%) prevalence settings with ongoing transmission in Tanzania as well as post-elimination validation surveys in Nepal—to estimate the sero-conversion and reversion rates with a sero-catalytic model for all sites for which appropriate data was available. We then developed a mixed effects regression model to assess which key epidemiological variables (age, gender, number of known past rounds of antibiotic treatment) across multiple surveillance sites were significant predictors of antibody titre, allowing them to vary by trial site. We found that TF prevalence and age are good predictors of antibody titre within the community. Our findings demonstrate that prevalence of antibodies against chlamydial antigens may be informative indicators of transmission intensity and that serological surveillance could be a valuable approach for-post-elimination validation surveys.

681

INFLUENCE OF INDIVIDUAL AND ENVIRONMENTAL FACTORS ON THE PREVALENCE OF TRACHOMA IN THE HEALTH DISTRICT OF MOKOLO AFTERTHREE YEARS OF MASS TREATMENT WITH ZITHROMAX AND TETRACYCLINE

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It is commonly believed that personal hygiene and the state of cleanliness of the environment are significant factors in the spread of trachoma. The strategy for control is through SAFE (Surgery, Antibiotic treatment, Facial cleanliness and Environmental improvement). The health district of Mokolo in the Far-North Region of Cameroon, recognized as endemic with trachoma in 2010, benefited from the azithromycin and tetracycline mass distribution during three consecutive years, supported by HKI with funding from USAID's ENVISION Project, managed by RTI International. Rarely actions in F and E components were registered. We aimed at evaluating the influence of facial cleanliness and the environmental factors on the residual infection of trachoma after the treatment. We carried out a descriptive cross-sectional study based on a stratified random sampling in Mokolo in 2015. Selected were 20 communities representing clusters; with 25 households in each. After an interview with the households and ophthalmic assessments by trained trachoma graders, data were collected using the numerical tablets transferred to a central base, before being analyzed using Software SPSS. Among the 827 children aged 1 to 9 years examined, the prevalence of active trachoma (TF&TI) was 1.7% in 2015 against 18.1% in 2010. The proportion of children having dirty faces was 14.85%. A strong association was found between the facial uncleanliness and active trachoma infections (OR = 14.79; p<0.01). Out of 91.6% of houses with cattle, 94.7% cohabited with the animals inside the homes. However 60.8% of the households were located within less than 30 min from a source of water. Despite drastic reduction of the disease prevalence to the threshold of the stopping MDA, the individual and environmental factors remain a strong influence. This could compromise the sustainable elimination of trachoma in this health district. More efforts on the F and E components of the SAFE strategy is needed.

682

A SPATIAL ANALYSIS OF ENVIRONMENTAL FACTORS AND TRACHOMATOUS-INFLAMMATION FOLLICULAR AMONG CHILDREN 1-9 YEARS IN SOUTH GONDAR, ETHIOPIA

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South Gondar zone in the Amhara region of Ethiopia has a high prevalence of trachomatous-inflammation follicular (TF), a clinical sign of trachoma, among children ages 1-9 (25.9%). This may not be entirely explained by individual behaviors such as facial cleanliness or household factors like latrine access. Environmental covariates like rainfall, altitude, vegetation, and temperature may impact TF prevalence but there are few published studies assessing the spatial relationships of environmental factors and TF clustering in a hyperendemic setting. Using a multi-stage, cluster randomized design, 12,108 households were surveyed in 313 villages in 12 districts in South Gondar in 2011. We assessed TF prevalence in all children 1-9 years in each household. TF prevalence was aggregated by village for those with valid GPS coordinates. A point pattern analysis of 313 villages was performed using weighted K functions for global clustering and a Getis G* for local clustering (|z|>3.71). Altitude, global precipitation measurement (GPM), naturalized difference vegetation index (NDVI), and land surface day temperature raster images were overlaid with TF clusters. The weighted K function did not identify aggregation up to 100km. Local Getis G* tests identified clustering starting at 10km. Hot spots, areas where high prevalence villages tend to occur near each other, were identified in northern districts of Ebinat and Libo Kemkem. Cold spots, areas where low prevalence villages occur near each another, were identified in central districts of Debre Bahir Town, Lay Gayent, and Estie. Preliminary descriptive analyses suggest that cold spots may be associated with higher elevations and NDVI. Hot spots may be associated with lower elevations and NDVI. Subsequent analyses will include descriptive mapping of select environmental factors and multivariate logistic regressions to identify environmental factors associated with TF clustering. Risk maps will be generated with the associated environmental factors to identify areas of heightened risk which may help inform trachoma control activities in these areas.

683

USE OF MULTIPLE STRATEGIES TO MOBILIZE TRACHOMATOUS TRICHIASIS CASES FOR SURGERY IN 4 DISTRICTS OF KATSINA STATE, NIGERIA

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Trachomatous Trichiasis (TT) is blinding stage of trachoma and could cause blindness if not treated. To prevent the blindness, surgical intervention is required. In Katsina State, Nigeria, the ultimate intervention goal (UIG) for TT surgery in 8 Local Government Areas (LGAs) is 15,760. With the support of the Queen Elizabeth Diamond Jubilee Trust, 19 ophthalmic nurses were trained to provide surgeries using the bilateral tarsal rotation (BTR) method. A single TT case mobilization strategy was recommended and initially implemented, i.e. by trained TT case finders to identify and refer cases for surgery. There was a low turn-out of TT cases. To maximize the productivity of surgery, different methods were then tested in order to mobilize patients to access the free service. Barriers to access, such as fear of surgery, distance, poverty level of the affected people and gender barrier, informed the decision to adopt context specific case mobilization method in 4 selected LGAs. TT surgery camps were organized in Daura emirate comprising of Zango, Daura, Mai'adua and Baure LGAs between October and December 2015. A combined strategy of mobilization of TT patients was used in the weeks preceding the outreaches. These included radio announcements, town announcers, announcements in public places such as mosques and market places, in addition to trained TT case finders. With combined mobilization strategy, there was a resultant spike in the productivity as well as the frequency of outreaches being conducted. Two surgery camps were conducted in October and December which resulted in high turn-out of TT cases for surgery. Thus 7 outreaches were conducted within December alone, yielding 245 persons operated in 293 eyes. This caused an increase in productivity as compared to previous months when only the case finder method was used. The number of persons presenting themselves for surgery more than doubled during the same period. Regular outreaches are very important for TT surgery. However, multiple mobilization strategies employed for outreach activities are required in order to reach the UIG and achieve the year 2020 trachoma elimination goal in Nigeria.

684

CHLAMYDIA TRACHOMATIS INFECTION IN AMHARA, ETHIOPIA 2011-2015

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Trachoma is caused by the bacteria *Chlamydia trachomatis* (Ct). Infection data, collected in the programmatic setting, would help in understanding the impact of mass drug administration (MDA) interventions as well as the relationship between the clinical signs of trachoma and infection. This study aimed to understand the effect of multiple years of MDA on Ct infection in the Amhara region, Ethiopia by describing the prevalence of Ct infection in a representative sample of children ages 1-5 years. Population-based trachoma impact surveys were conducted in all districts of Amhara, from 2011 to 2015, following 5 years of SAFE interventions. Ocular specimens were collected from randomly selected children ages 1-5 years whose households were included in the surveys to estimate the zonal prevalence of Ct infection. Samples from each district were pooled and the Abbott Realtime PCR assay was used to detect Ct DNA using the m2000 system. District prevalence was determined from the district pooled prevalence using maximum likelihood techniques. Zonal prevalence and confidence intervals (CI) were estimated using survey procedures in Stata. A total of 15,636 samples were collected across 10 zones of Amhara. The prevalence of trachomatous-inflammation follicular (TF) in children ages 1-9 years in Amhara region was 26.1%, (95%CI: 25.1, 27.1), zonal range: 13.6% to 54.6%, and the regional prevalence of trachomatous-inflammation intense (TI) was 5.6% (95%CI: 5.2, 6.0), zonal range: 3.4% to 13.6%. The prevalence of Ct infection in children ages 1-5 years was 5.5% (95%CI: 4.2, 6.7), with zonal estimates ranging from 1.0% in Awi zone to 15.3% in Waghemra. Ct infection and TI were very highly correlated at the zonal level (Spearman correlation(r)= .92; P=0.0002), while Ct infection and TF were moderately correlated (r= .57; P=0.084). To our knowledge, this is the first report of Ct infection data at a regional level within a programmatic setting. Despite over 5 years of MDA, a considerable amount of Ct infection remains in Amhara. TI was highly correlated with Ct infection and may represent a potential marker of infection that programs could use in measuring impact.

685

TRACHOMA IMPACT SURVEYS IN MAINLAND TANZANIA: LESSONS LEARNED FROM IMPLEMENTATION OF THE SAFE STRATEGY

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Tanzania started implementation of the surgery, antibiotics, facial cleanliness and environmental change (SAFE) strategy for trachoma control in 1999. Tanzania has made substantial progress towards meeting Global Elimination of blinding Trachoma by the year 2020 (GET2020) objectives. By the end of 2015, 37 out of 55 districts under SAFE implementation had achieved the goal for stopping mass drug administration (MDA) of trachomatous inflammation-follicular (TF) prevalence <5% in children aged 1-9 years. We investigated the trends of TF prevalence and annual MDA coverage. Data from 13 districts were analyzed: 2 had TF<5% (Handeni and Kwimba); 2 had TF>10% after 1st impact survey (Kilindi and Kongwa); 2 had TF=5-9.9% after 1st impact survey (Manyoni and Mpwapwa); 3 had stopped MDA due to programmatic challenges (Monduli, Longido and Ngorongoro); and 4 had baseline TF=5-9.9% and no MDA (Morogoro Rural, Mvomero, Singida Urban and Rombo). Time trends of TF prevalence and MDA coverage were plotted. In 2 districts with TF<5% at 1st impact survey, Handeni had 3 consecutive years of MDAs while Kwimba had 1 year of MDA. In 2 districts with TF>10% at 1st impact survey, Kilindi had no change in TF prevalence despite 4 MDA rounds of varying coverage while in Kongwa, TF was >10% despite 14 MDA rounds of varying coverage. Among districts with TF=5-9.9% (at the 1st impact survey), Manyoni had no change in prevalence (at the 2nd impact survey) despite a high coverage MDA; while Mpwapwa had TF<5% after a high coverage MDA. MDA implementation was delayed in Monduli, Longido and Ngorongoro, then implemented with low coverage in Monduli and Longido, and high coverage in Ngorongoro but stopped in all 3 districts due programmatic challenges. In 4 districts where baseline TF=5-9.9%, follow-up surveys after 10 years showed that TF was <5% in absence of interventions. The reduction of TF varied by district. Results suggest that low MDA coverage may contribute to the reduced impact on TF decline. To achieve GET 2020 objectives: MDA needs to be started promptly and sustained to achieve maximum impact; high MDA coverage needs to be achieved; and prompt impact surveys and action are needed.

686

COMPARISON OF EPIDEMIOLOGY, TRANSMISSION DYNAMICS AND CLINICAL PRESENTATIONS OF GENITAL AND SKIN ULCERATIONS CAUSED BY *HAEMOPHILUS DUCREYI*: EXPERIENCE FROM HYPER-ENDEMIC AREAS IN AFRICA AND PACIFIC ISLANDS

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Haemophilus ducreyi (HD), a fastidious gram-negative bacterium, has been known to cause the sexually transmitted genital ulcerations (i.e. Chancroid) in tropical countries and hyper-endemic situations were reported in Southern Africa during the 1990s. Recently, HD has been found to be associated with cutaneous ulcerations among children under 15 years old in yaws endemic areas. A retrospective case review was conducted on 117 laboratory-confirmed HD genital ulcer cases (genital HD) that presented to the Sexually Transmitted Disease (STD) clinic in Johannesburg, South Africa and 60 laboratory-confirmed cutaneous ulcerations (non-genital HD) in children seen during the community-based surveillance activities in Vanuatu. The demographic, clinical presentations (including photographs of lesions) and laboratory findings of two patient population were compared. The laboratory investigations include syphilis serology using RPR and TPPA; multiplex real-time PCRs for HD, herpes simplex virus (HSV), Treponema pallidum subspecies (TP), and Mycobacterium ulcerans (buruli ulcer). In addition, culture for HSV and HD, and HIV serology were performed on samples from the genital-HD group. The mean ages of patients with genital and non-genital HD lesions were 33 years (± 9) and 8 years (± 3), respectively. The majority of patients in both groups presented with large, multiple, punched-out ulcerations with soft edges and purulent bases which are clinically indistinguishable between genital and non-genital HD lesions. Purulent inguinal lymphadenopathy (i.e. Bubo) was observed in 38 (32.5%) genital HD cases compared to none in non-genital HD patients. RPR seropositivity was 15.5% in genital HD group and 35.5% in non-genital HD group. In conclusion, clinicians should be aware of the presence of non-sexual transmission of HD and TP in some geographical areas and carefully consider the potential etiology of cutaneous genital and non-genital ulcerations in residents from those areas. Laboratory confirmation and differentiation of ulcer etiology at a reference laboratory would be required to support the clinical diagnosis and management.

687

DIFFERENCES IN THE CLINICAL AND LABORATORY FEATURES OF ONCHOCERCIASIS IN ENDEMIC AND NONENDEMIC POPULATIONS REFLECT IMMUNE-MEDIATED PROCESSES

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Some filarial infections including *Loa loa* and *Wuchereria bancrofti* may have different presenting features in endemic populations compared with immunologically naïve expatriate hosts. However, few studies have examined this relationship in *Onchocerca volvulus* infection. To study this question more directly, we identified all patients diagnosed with active *Onchocerca volvulus* infection at the National Institutes of Health between 1976 and 2016. Study subjects received a detailed baseline assessment including history, physical examination, ophthalmologic evaluation, and extensive laboratory investigations. Forty returned travellers (TR) and 36 endemic (END) subjects had onchocerciasis acquired almost entirely in West and Central Africa. The TR more frequently reported a pruritic rash and 28% had acute papular dermatitis on examination compared with only 2.8% of END ($p = 0.004$). Skin pigmentation changes, excoriation or lichenification occurred in 47% of the END but in only 18% of TR ($p = 0.007$). Although similar numbers in both groups reported ocular symptoms, documented onchocercal eye involvement occurred in 16.7% of the END and in none of the TR ($p = 0.009$). There were no differences in any other clinical parameters including pruritus, arthralgia, edema, lymphadenopathy, or of the presence of onchocercomata (2 in the TR, 6 in the END; $p = 0.14$). Fourteen (35%) TR and 7 (19%) END patients had microfiladermia ($p = 0.43$). Geometric mean (GM) absolute eosinophil counts were significantly ($p<0.05$) higher in the TR (850/mm3) compared to the END (438/mm3). By contrast, we observed higher serum polyclonal IgE levels in endemic patients (GM = 826.1 IU/mL) compared to the TR (206.3 IU/mL; $p < 0.001$). Parasite-specific IgG4 levels were also significantly higher in END than in TR patients ($p = 0.026$). Although there is substantial overlap in the presentation of *O. volvulus* infection in TR and END populations, the TR had more frequent acute (and possibly eosinophil-mediated) findings that may reflect differences in chronicity and immune tolerance felt to underlie the relative hyporesponsiveness seen in lifelong onchocerciasis.

688

DIAGNOSIS OF LOW-INTENSITY *SCHISTOSOMA* INFECTION IN A NON-ENDEMIC SETTING USING THE ULTRASENSITIVE LATERAL FLOW TEST FOR DETECTION OF SCHISTOSOME CIRCULATING ANODIC ANTIGEN (CAA)

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Schistosomiasis in travelers and migrants is mostly diagnosed by detecting specific antibodies in serum, as microscopy has a pronounced low sensitivity in this particular population. Although serology is sensitive and specific, it cannot distinguish active from past infection and it may take up 6 to 10 weeks for seroconversion to occur. An alternative diagnostic tool is detection of adult worm-derived circulating antigen in serum or urine. Here we explored the diagnostic value of an ultrasensitive robust lateral flow based test for the quantification of the *Schistosoma* Circulating Anodic Antigen (CAA) utilizing fluorescent up-converting phosphor reporter particles (UCP-LF CAA assay) within a non-endemic routine diagnostic laboratory setting. Serum samples from 81 serology positive cases were tested, including 36 travelers of which 14 had proven seroconversion. CAA (>0.1 pg/ml) was detected in 68% of all schistosomiasis cases, including 56% of the travelers and 64% of those who seroconverted. All 19 controls were CAA negative, while all 11 subjects who were positive for microscopy and/or PCR in stool or urine were CAA positive. In travelers CAA was seen as early as four weeks after exposure and the antigen could be demonstrated in four out of five samples collected days to weeks before antibodies were observed. On the other hand, most of the CAA positive travelers (18/20) had marginally to low (<10 pg/ml) serum CAA concentrations, while higher levels were seen in the subjects with chronic schistosomiasis. Consecutive samples were tested in 16 subjects and all showed a rapid decline in CAA concentration, reflecting decreasing worm loads due to anti-schistosomal therapy. This explorative retrospective study indicates the UCP-LF CAA assay to be a highly accurate test for diagnosing schistosomiasis in a non-endemic setting.

689

FIELD-TESTING OF A COST-EFFECTIVE MOBILE-PHONE BASED MICROSCOPE FOR SCREENING OF *SCHISTOSOMA HAEMATOBIUM*

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Schistosomiasis is a parasitic and neglected tropical disease, and affects >200-million people across the world, with school-aged children disproportionately affected. Here we present field-testing results of a handheld and cost effective smartphone-based microscope in rural Ghana, Africa, for point-of-care diagnosis of *Schistosoma haematobium* infection. In this mobile-phone microscope, a custom-designed 3D printed opto-mechanical attachment (~150g) is placed in contact with the smartphone camera-lens, creating an imaging-system with a half-pitch resolution of ~0.87μm. This unit includes an external lens (also taken from a mobile-phone camera), a sample tray, a z-stage to adjust the focus, two light-emitting-diodes (LEDs) and two diffusers for uniform illumination of the sample. In our field-testing, 60 urine samples, collected from children, were used, where the prevalence of the infection was 72.9%. After concentration of the sample with centrifugation, the sediment was placed on a glass-slide and *S. haematobium* eggs were first identified/quantified using conventional benchtop microscopy by an expert diagnostician, and then a second expert, blinded to these results, determined the presence/absence of eggs using our mobile-phone microscope. Compared to conventional microscopy, our mobile-phone microscope had a diagnostic sensitivity of 72.1%, specificity of 100%, positive-predictive-value of 100%, and a negative-predictive-value of 57.1%. Furthermore, our mobile-phone platform demonstrated a sensitivity of 65.7% and 100% for low-intensity infections (≤50 eggs/10 mL urine) and high-intensity infections (>50 eggs/10 mL urine), respectively. We believe that this cost-effective and field-portable mobile-phone microscope may play an important role in the diagnosis of schistosomiasis and various other global health challenges. We discuss the use of these instruments in clinical and public health settings.

690

THE EMERGENCE AND EPIDEMIOLOGY OF ENDEMIC (FLEA-BORNE) TYPHUS IN TEXAS, 2003-2013

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Endemic or murine typhus, a disease spread by fleas and caused by the organism Rickettsia typhi, is a neglected tropical disease in Texas. The purpose of this study was to characterize the epidemiology of murine typhus in the state, identify seasonality of disease transmission, and identify high-risk geographic and demographic populations. We analyzed data on all confirmed and probable cases reported to the Texas Department of State Health Services' Zoonosis Control Branch between 2003 and 2013. Over this time period, 1762 cases were reported (770 confirmed and 992 probable cases). Evidence of emergence was seen both in increased numbers of cases over time (27 cases in 2003 versus 222 cases reported in 2013) and geographical location (9 counties in the southern-most part of the state in 2003 to 40 counties reporting cases by 2013). With regards to demographics, females had a slightly higher attack rate compared to males (7.3 vs. 6.7 per 100,000 population, respectively). When examining risk of disease by age, the highest attack rate (10.4/100,000 population) was found among 5-19 year olds. Of these cases, 1047 (59.6%) were hospitalized. Most commonly reported signs and symptoms included fever (99.7%), headache (77.2%), chills (70.1%), malaise (64.1%), anorexia (52.8%), nausea/vomiting (51.4%), and myalgias (50.8%). Rash was reported by 42.5% of cases, with pediatric cases being statistically more likely to present with a rash when compared to adults (odds ratio = 2.2). Fatality was rare, with 4 deaths being reported (0.2% case fatality rate). Median age of fatal cases was 51.5 years (range 36-55 years). With the increase in reported cases, high percentage of hospitalizations, and geographic expansion of transmission, we want to highlight the importance of public education and raising physician awareness to identify and treat suspected cases. Additionally, further research is needed to better understand the dynamics of transmission and risk of infection in these newly identified geographic regions.

691

ZIKA VIRUS DISEASE AMONG TRAVELERS RETURNING FROM THE AMERICAS BETWEEN JANUARY 2013 AND FEBRUARY 2016: A GEOSENTINEL ANALYSIS

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Since first identified in Brazil in 2015, Zika virus (ZIKV) has rapidly spread to >30 countries and territories in the Americas. Travelers, important sentinels of new disease outbreaks, can facilitate global disease spread. ZIKV disease acquired in the Americas and evaluated at GeoSentinel Surveillance Network sites from January 1, 2013 to February 29, 2016 were analyzed. Interim Council of State and Territorial Epidemiologists classification criteria were used to classify patients as clinically suspect, probable, or confirmed cases of ZIKV disease. There were 62 confirmed, 13 probable, and 16 clinically suspect ZIKV cases of 100 cases submitted. Of the 91 cases, 64% were female, and median age was 41 y (range 3-77 y). Major reasons for travel were tourism (48%) and visiting friends and relatives (40%). Infections were acquired in South America (59%), the Caribbean (24%), and Central America/Mexico (16%); the top three countries were Suriname (22%), Colombia (16%), and Brazil (11%). Diagnoses were made in Western Europe (73%), North America (15%), Middle East (9%), and South America (3%). The first two cases were reported in May 2015 and November 2015, after which there was a rapid rise in cases. The most common sign/symptoms were rash (88%), fever (76%), arthralgia (72%), headache (60%), myalgia (60%), fatigue (47%), conjunctivitis (41%), and pruritus (23%). Less common were nausea, diarrhea, paresthesia, dysgeusia, and arthritis. Of 4 pregnant patients, 2 had normal ultrasounds, one underwent elective termination due to major fetal neurological abnormalities, and one had no documented outcome. Two patients were diagnosed with Guillain-Barré syndrome. This large case series demonstrates the range of symptoms associated with ZIKV infection. While sentinel surveillance does not reflect true incidence and is biased towards identifying more severe disease and influenced by testing availability, GeoSentinel data are important for tracking the geographic spread of emerging infections. We show that travelers infected in the Americas return to locations where they can potentially transmit the virus to sexual partners or competent vectors.

692

OUTCOMES OF PREGNANT PATIENTS PRESENTING TO EBOLA TREATMENT UNITS IN SIERRA LEONE AND LIBERIA: A RETROSPECTIVE COHORT STUDY

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Pregnant women are thought to have increased risk for severe illness and death when infected with Ebola virus disease (EVD). This retrospective cohort study investigated the presenting symptoms and outcomes of pregnant women compared with non-pregnant women of reproductive age admitted to five Ebola Treatment Units (ETUs) in Sierra Leone and Liberia between September 2014 - 2015. Data analysis was completed for women of reproductive age with results for EVD testing, with a focus on those with the report of pregnancy. Standardized care, including empiric malaria treatment and oral antibiotics, was provided to all patients based on International Medical Corps protocols. Among 2,323 patients admitted with outcome data, 723 women of reproductive age were included for analysis. Forty-four women were documented as pregnant; the median gestational age reported was 26 weeks (range of 4 to 40 weeks). There was no significant difference in overall mortality between pregnant and non-pregnant women (13.9% vs 18.9%, p=0.42). Pregnant patients were no more likely to have EVD than non-pregnant women (29.6% vs 23.9%, p=0.39), and were less likely to have fever, bone/muscle pain, nausea, vomiting, diarrhea, anorexia and asthenia as presenting symptoms when compared to non-pregnant women. Thirteen pregnant patients were EVD positive. Six died, six survived to discharge, and one was transferred to a nearby ETU with specialized care for pregnant women (outcome unknown). There was no difference in mortality between pregnant and non-pregnant women with EVD (50% vs 53.7%, p=0.80). There were two live births in the ETU; both infants died before hospital day 9. Training guidelines for providers working in Ebola treatment units focus on maternal deliveries without assistance to minimize risk to healthcare workers considering historical data suggested poor maternal or fetal survival. Our data points to comparable maternal outcomes, though fetal survival in the context of an EVD gestation remained poor. Based on this data, re-evaluating the approach to management of pregnant patients in the ETU setting may be warranted.

693

DECIPHERING THE BIOLOGY OF THE DORMANT MALARIA PARASITE, *PLASMODIUM VIVAX*, VIA AN *IN VITRO* PLATFORM

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Liver stage malaria is an attractive target for all *Plasmodium* species since it provides the opportunity to attack the parasite at an early, obligate, yet clinically silent stage. Of the 4 strains of malaria that infects humans, *P. vivax* is the most frequent and widespread cause of human malaria and poses unique challenges to treatment and eradication because liver stage parasites can develop into dormant small forms, hypnozoites, that remain in this state for prolonged periods. This strain of malaria causes chronic, relapsing infection months to years after the initial infection via reactivation of hypnozoites. Drug discovery could be informed by targeting the hypnozoite stage, however the biology behind hypnozoite formation and reactivation remains to be elucidated. *In vitro* platforms

for studying *P. vivax* are lacking because of challenges associated with keeping primary human hepatocytes phenotypically stable over the long periods of time required for studying dormant parasites. Benefiting from the longevity of our micropatterned primary human hepatocyte cultures, we were, for the first time, able to culture *P. vivax* hypnozoites *in vitro*. To validate the system as an *in vitro* surrogate for *P. vivax* biology applications, we have shown (1) complete *P. vivax* liver stage development, including release of merozoites and subsequent infection of overlaid reticulocytes and (2) formation, persistence and reactivation of hypnozoites. Using the system as a potential discovery tool, we presented evidence of differential drug sensitivity of schizonts and hypnozoites towards both clinically available and yet-in-development liver-acting drugs. Furthermore, leveraging the power of an *in vitro* culture in facilitating rapid testing of biological hypotheses that are otherwise clinically difficult to test, we created a simple deterministic model that recapitulates the behavior of *P. vivax* parasites over time. Fitting experimental data into this model, we addressed two hallmarks of *P. vivax* liver stage biology: lifetime in the liver and hypnozoite reactivation frequency.

694

CELL TRAVERSAL BY MALARIA PARASITES: *PLASMODIUM* CELTOS BINDS AND DISRUPTS PLASMA MEMBRANES FROM THE CYTOPLASMIC FACE TO ENABLE THE EXIT OF PARASITES FROM CELLS DURING HOST AND VECTOR CELL TRAVERSAL

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Traversal of host and vector cells by *Plasmodium* parasites is required for malaria infection and transmission. *Plasmodium* CelTOS has been identified by genetic studies to be critical for the traversal of parasites through the mammalian host and mosquito vector. Additionally, CelTOS is a leading malaria vaccine candidate in clinical trials. Even though CelTOS has critical roles in *Plasmodium* biology and is a malaria vaccine target, the molecular function and mechanism of CelTOS remains unknown primarily due to its lack of sequence similarity to proteins of known function. We determined the structure of *Plasmodium* CelTOS to obtain insight into its molecular function. Unexpectedly we discovered CelTOS is structurally similar to viral fusion proteins and a bacterial pore-forming toxin that bind membranes. Unlike other membrane binding proteins, CelTOS specifically lipids predominantly present in the inner leaflet of plasma membranes. We also observed that CelTOS disrupts liposomes composed of these lipids, and further *in vivo* studies demonstrate that CelTOS disrupts cell plasma membranes. Taken together, these studies demonstrate that CelTOS is the only known malaria parasite protein that enables the exit of parasites from host and vector cells during traversal by having nearly universal activity in binding and disrupting plasma membranes from the cytoplasmic face. By providing insight into the function and mechanism of CelTOS, this study facilitates the design of therapeutics which target CelTOS to protect against malaria.

695

MECHANISM OF FETAL GROWTH RESTRICTION IN PLACENTA MALARIA

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Placental malaria can trigger intervillositis; a local inflammatory response more strongly associated with fetal growth restriction (FGR) than placental malaria infection alone. The underlying mechanisms are unknown but we have shown that placenta malaria-associated intervillositis impairs placental amino acid transport. The mechanistic target of rapamycin (mTOR) signaling pathway regulates fetal growth by modulating transplacental amino acid transport. mTOR activity has also been observed to be decreased in non-malarial cases of FGR. We hypothesized that placenta malaria-associated intervillositis inhibits mTOR signaling pathway, resulting in reduced placental amino acid uptake. Using placental tissue biopsies from Malawian women, we demonstrated that mTOR signalling activity is i) decreased specifically in placenta malaria-associated intervillositis compared to uninfected controls ($p \leq 0.03$); ii) negatively correlated with the degree of inflammation ($p \leq 0.03$; $R < -0.42$) and iii) positively correlated with amino acid uptake ($p \leq 0.03$; $R > 0.36$). Using our established *in vitro* model of placental response to intervillositis, we demonstrated that primary human trophoblast (PHT) cells exposed to placental malaria-associated intervillositis have decreased mTOR signalling activity ($p \leq 0.02$) and reduced amino acid uptake (-63%, $p \leq 0.02$), recapitulating our *ex vivo* findings. Furthermore, constitutive mTOR activation (by silencing the endogenous inhibitor of mTOR) partially restores amino acid uptake (+30%, $p \leq 0.0001$) in PHT cells exposed to placental malaria-associated intervillositis. In summary, we determined that inhibition of placental mTOR activity mechanistically links placental malaria-associated intervillositis and reduced amino acid transport, which may contribute to the pathogenesis of FGR. We propose that restoring mTOR signaling in placental malaria may increase fetal growth and complement malaria control strategies to improve pregnancy outcomes in pregnant women exposed to malaria.

696

THROMBOSPONDIN RELATED SPOROZOITE PROTEIN IS IMPORTANT FOR THE ESTABLISHMENT OF *PLASMODIUM FALCIPARUM* LIVER STAGE INFECTION

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From oocyst to vertebrate host cell, malaria sporozoites must traverse and recognize multiple cell types if they are to successfully continue the parasite's lifecycle. Proteins previously recognized to be important for sporozoite-host-cell interactions; circumsporozoite protein (CSP) and thrombospondin related anonymous protein (TRAP), contain thrombospondin type 1 repeats (TSR). Another less well characterized TSR protein, highly expressed in sporozoites, is thrombospondin related sporozoite protein, which contains a TSR, a transmembrane domain and a putative PEXEL motif, suggesting it may localize to the surface of the sporozoite and/or be exported. TRSP has previously been shown to be important for the successful invasion and establishment of liver stage infection in *P. berghei*. In order to investigate the role and localization of this protein in *P. falciparum*, the species responsible for the vast majority of human malarial morbidity and mortality worldwide, we generated

P. falciparum TRSP knockout parasites, as well as lines expressing GFP and HA tagged TRSP. TRSP deficient parasites show no defect until the sporozoite stage, where they display a mild hepatocyte traversal defect *in vitro*. This does not however result in an *in vitro* invasion defect as seen in *P. berghei*. To further investigate the importance of this traversal defect *in vivo*, ΔTRSP sporozoites were injected into uPA/SCID mice with humanised livers, where a 95% reduction in parasite load was observed six days post infection by qPCR. Preliminary data indicate that ΔTRSP liver schizonts can develop with a morphology and size similar to wildtype and morphological characteristics will be discussed. These data show that TRSP, in common with other TSR containing sporozoite proteins, is important for the establishment of liver stage infection, most likely during the invasion process, however work is ongoing to further define the exact function of TRSP in this important human pathogen.

697

A SYSTEMATIC APPROACH TO THE OPTIMIZATION OF *PLASMODIUM VIVAX IN VITRO* CULTURE

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The WHO estimates that 35% of the global population was at risk for and approximately 16 million people suffered from clinical *Plasmodium vivax* infection in 2013, making *P. vivax* the most globally widespread malaria parasite and an immense health and economic burden. Unfortunately, largely due to its unique biology, a continuous, *in vitro* culture system for this parasite has remained elusive, hindering our ability to make scientific advancements and combat *P. vivax*. A major obstacle to continuous, *in vitro* culture is our lack of understanding the nutrient requirements of *P. vivax* for successful asexual maturation and survival. To enhance *P. vivax ex vivo* growth and to understand the nutrient requirements of the parasite, we designed and executed a screen to identify an optimal culture media. Twenty different media were commercially acquired that represent a range of defined and proprietary formulations, with an emphasis on media developed for cultivation of erythrocytes and erythrocyte precursors. From this screen, we identified a formulation that significantly enhances the survival and asexual maturation of human *ex vivo P. vivax* isolates from ring stages to schizogony and egress. Furthermore, the increased survival and maturation has enhanced our ability to perform invasion assays. While it has been previously demonstrated that *P. vivax* preferentially invades very young red blood cells, reticulocytes, our assays indicate that a currently uncharacterized, more specific subset of reticulocytes is required for efficient invasion and subsequent asexual maturation. To enable the identification of a host cell that fully enables *P. vivax* invasion and maturation, we have developed methods to fractionate very specific subpopulations of human reticulocytes from multiple sources representing the full range of erythropoiesis. In all, this systematic approach to the optimization of *in vitro* culture of *P. vivax* has enabled us to reliably perform short-term growth assays, establish more robust invasion assays, and it has brought us closer to continuous *in vitro* culture.

698

MOLECULAR TECHNIQUES IDENTIFY *ANCYLOSTOMA CEYLANICUM* AND *NECATOR AMERICANUS* AS THE MAJOR HOOKWORM PATHOGENS AMONG MYANMAR REFUGEES PRE-RESETTLEMENT AND DEFINE THEIR DIFFERENTIAL RESPONSE TO ANTHELMINTIC THERAPY

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Human hookworm infection, typically caused by *Necator americanus* (Na) and *Ancylsotoma duodenale* (Ad), remains an important source of childhood growth restriction, iron deficiency, and anemia. Using multi-parallel real-time PCR (qPCR) on stool samples from 233 subjects that were part of a larger study (n=2000) assessing the prevalence of intestinal parasites in refugees from Myanmar (domiciled in refugee camps in Thailand) and the efficacy of pre-resettlement anthelmintic therapy, we assessed Na and Ad prevalence in all subjects from 3 time points (1-following arrival in refugee camps in Thailand, 2-after treatment with albendzaole/ivermectin (A/I) prior to departure for the United States (US), and 3-following arrival in the US after a second treatment with A/I). At entry, 51 of 233 (22%) were infected with Na. QPCR targeting the ITS2 region of *Ancylostoma* spp. revealed 17 of 233 (7%) stool samples were positive upon entry to the refugee camp. However, qPCR targeting a repeated sequence specific for *A, duodenale* was negative for all 17 samples. Sequencing and follow up qPCR revealed all 17 of these samples to be positive for the zoonotic hookworm *A. ceylanicum* (Ac). Following A/I therapy, all subjects with Ac cleared their infection. Of the 51 subjects infected with Na at baseline, 20 (39%) remained infected with Na following the first A/I dose and 14 (27%) remained Na-infected despite 2 courses of A/I. Six previously hookworm-uninfected subjects acquired Na infection while in the refugee camp (positive at timepoint 3), and 2 acquired Ac (positive timepoint 2). Studies are ongoing to sequence the beta-tubulin genes of those Na parasites that responded and failed to respond to A/I. These data identify the zoonotic Ac as an emerging and important human pathogen in Myanmar. Thus, the availability of increasingly sophisticated molecular diagnostic techniques to assess the differential responses of the various hookworm species to anthelmintics and to provide evidence of ongoing acquisition of hookworm infection within refugee camps should guide future planning of pre-departure medical treatment for refugees in Southeast Asia.

699

MULTI-PARALLEL QUANTITATIVE REAL-TIME PCR BASED DIAGNOSTICS AS THE NEW GOLD STANDARD FOR SOIL TRANSMITTED HELMINTHS

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Due to its simplicity and cost-effectiveness, microscopy has seen extensive field-use as the diagnostic standard for the detection of soil-transmitted helminths (STH) in stool samples. However, the sensitivity of microscopy-

based detection is inadequate in reduced-transmission settings where worm burden is oftentimes low. Equally problematic, eggs of closely related species oftentimes have indistinguishable morphologies, leading to species misidentification. In light of these shortcomings, the purpose of this study was to demonstrate multi-parallel quantitative real-time PCR (qPCR) as the new "gold standard" for STH detection. Accordingly, stool samples from non-endemic participants were spiked with limited numbers of eggs or larvae (1 to 40) of five different species of STH. DNA extracts were tested using two unique multi-parallel real-time PCR-based diagnostic methods. These methods employed different target sequences (ribosomal internal transcribed spacer, or highly repetitive non-coding regions), to evaluate the detection of DNA from as little as one egg per sample. There was a statistically significant spearman correlation between egg/larvae counts and qPCR from both methods for each one of the multi-parallel assays; for Ascaris lumbricoides (0.806), *Ancylostoma duodenale* (0.961), *Necator americanus* (1.00), *Strongyloides stercoralis* (0.835), and *Trichuris trichiura* (0.956) ($p < 0.05$ for all STH). Both methods had similar detection rates for 104 stool samples from a rural population in northern Argentina with less than a 25% variance between them for most STH. As parasitic targeting of two independent genomic regions provided reproducible results, we believe that, low cost multi-parallel quantitative real-time PCR-based diagnostics should supplant microscopy as the new gold standard for stool-based detection of soil transmitted helminths in public-health and community settings.

700

DIAGNOSIS OF *STRONGYLOIDES STERCORALIS* FROM FILTERED URINE RESIDUE BY DETECTING CELL-FREE DNA

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One of the most neglected of the neglected tropical diseases is the infection caused by nematodes in the genus *Strongyloides*. Of the two species of *Strongyloides*, *S. stercoralis* is the most prevalent that infect humans and found in tropical and subtropical regions. Detection of *S. stercoralis* infection is arduous and has low sensitivity. This is a major problem because chronic infections may disseminate in the host and lead to a life threatening condition. Even now, the true prevalence of this parasite is practically unknown because the standard practice for stool examinations usually misses evidence of this infection. We present here the evidence for the first time that the infection can be detected by amplifying *S. stercoralis* specific cell-free repeat DNA from urine residue on filter paper that has been collected and processed in the field. We collected 125 specimens from people living in two types of endemic regions in Northern Argentina (rural and peri-urban). Stool specimens were processed fresh using three different coprological methods and 40-50 ml of urine was filtered through a 12.5cm Whatman No. 3 filter paper in the field. The filters were dried and packed individually in sealable plastic bags with desiccant and shipped to Johns Hopkins University where DNA was isolated and amplified with species-specific primers. The estimate of prevalence of infection was almost doubled when detecting the *S. stercoralis* specific repeat compared with coprological diagnosis (from 28% to 44.8%). Only 21.6% positive cases were congruent, as were 27.3% of negative cases. There were 6.4% of cases where parasite larvae were seen but DNA was not amplified. The species-specific DNA detection from urine residue reveals significantly more cases of infection than combined stool examinations and the method is simple and easy to carry out. This is crucial not only to determine the overall public health impact of the pathogen, but also to understand the extent of the infection in communities so that attempts to control or eliminate these pathogens are efficient and cost-effective.

701

MATERNAL POSTPARTUM DEWORMING AS A MEANS OF IMPROVING INFANT GROWTH AND MORBIDITY IN AREAS ENDEMIC FOR SOIL-TRANSMITTED HELMINTHIASIS

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Health and nutrition interventions targeting the critical growth and development period before the age of two years can have the greatest impact on health trajectories over the life course. Compelling evidence suggests that interventions in the postpartum period can be beneficial for both mothers and their children. One such potential intervention is deworming. In this seminal randomized controlled trial, we sought to evaluate the effectiveness of maternal postpartum deworming on infant growth. A total of 1010 mother-infant pairs were recruited in Iquitos, Peru. Prior to delivery, mothers provided stool specimens to determine the presence of soil-transmitted helminth (STH) infection. Following delivery, mothers were randomly assigned to receive either single-dose 400 mg albendazole, or matching placebo. Mother-infants pairs were followed-up at 1 and 6 months postpartum. At 6 months postpartum, there was no statistically significant difference in mean weight gain between infants in the albendazole and placebo groups (4.3 kg ± 0.04 *vs.* 4.4 kg ±0.04). However, *ad hoc* subgroup analyses restricted to mothers who tested positive for STH infections at baseline suggest that infants whose mothers received albendazole had greater growth in terms of mean length gain in cm (mean difference: 0.8; 95% CI: 0.1, 1.4) and length-for-age in Z-score (mean difference: 0.5; 95% CI: 0.1, 0.8). In a study population composed of both infected and uninfected mothers, maternal postpartum deworming with single-dose albendazole was insufficient to impact infant growth indicators at 1 or 6 months of age. Among STH-infected mothers, however, important infant growth benefits were observed. The benefits of postpartum deworming should be further investigated in study populations having different prevalences and intensities of STH infections and, in particular, where the prevalences and intensities of whipworm and hookworm infections are of public health concern.

702

SOIL-TRANSMITTED HELMINTHS (STH) CONTROL, ELIMINATION, AND DEVELOPMENT OF DRUG RESISTANCE: REPERCUSSIONS OF SYSTEMATIC NON-PARTICIPATION TO PREVENTIVE CHEMOTHERAPY

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Preventive chemotherapy (PCT) is a widely applied strategy to control soil-transmitted helminths (STH). However, mathematical models predict that effective control and/or elimination are easily impeded by suboptimal PCT coverage and/or systematic non-participation to PCT by a subset of individuals. Further, suboptimal coverage and systematic

non-participation may on the one hand facilitate the development of drug resistance through prolonged programme duration until elimination, but on the other hand may also delay it by allowing for parasite refugia in suboptimally treated populations. We present a novel individual-based model for evolution of polygenic drug resistance in helminth populations that accounts for aggregation of helminths in human hosts, human demography, patterns in PCT uptake (including systematic non-participation), sexual mating of parasites, genetic drift, and variation and heritability of parasite traits for drug resistance. The model has been quantified for transmission and control of the three major STH species (ascariasis, trichuriasis, hookworm), and has been used to simulate and explore the impact of various levels of drug efficacy, PCT coverage, and systematic non-participation to PCT on the speed at which polygenic drug resistance evolves in STH populations under varying assumptions about variation and heritability of parasite traits for drug resistance. Based on these explorative simulations, we provide a first-time estimate of the time horizon within which we can expect polygenic drug resistance to develop in STH populations as a result of PCT.

703

DIFFERENTIAL IMPACT OF MASS AND TARGETED DEWORMING CAMPAIGNS FOR SOIL-TRANSMITTED HELMINTH CONTROL IN CHILDREN: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Soil-transmitted helminth (STH) infections are an important global health issue, causing significant morbidity among the world's poorest populations. Regular delivery of anthelminthic chemotherapy is the principal strategy for STH control. As children harbour the largest burden of STH-associated morbidity, deworming medications are often targeted to school-aged children. However, recent modelling studies suggest that deworming campaigns should be expanded community-wide to have an impact on STH transmission.This systematic review and meta-analysis aimed to compare the impact of community-wide and child-targeted anthelminthic delivery strategies on STH prevalence in school-aged children.Studies reporting STH prevalence before and after child-targeted or community-wide treatment were identified searching MEDLINE, EMBASE, and Web of Science. Data extracted included drug administration strategy, drug dose, number of deworming rounds, treatment coverage, diagnostic method, follow-up interval, and STH prevalence before and after treatment. Inverse variance weighted generalised linear models were used to examine the impact of community-wide vs child-targeted drug administration on prevalence reduction in school-aged children.56 studies were included. Results of the regression models show a significantly greater prevalence reduction in children following community-wide deworming, compared to child-targeted deworming, for both Ascaris lumbricoides (OR 16.39; 95%CI 2.14-125.85) and hookworm (OR 4.62; 95%CI 1.85-11.57).The results of this meta-analysis suggest that expanding periodic chemotherapy for STH from child-targeted to community-wide is likely to result in reduced prevalence of STH among the high-risk group of school-aged children, which may lead to decreased morbidity. Findings are in support of recent calls for a re-evaluation of global STH control guidelines.

704

RESULTS OF A COMBINED PUBLIC HEALTH INTERVENTION AGAINST *STRONGYLOIDES STERCORALIS* IN AN ARGENTINIAN ENDEMIC REGION MONITORED THROUGH NIE-ELISA

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Northwestern Argentina is a hyperendemic area for soil transmitted helminth infection, where annual deworming programs are carried out in prioritized areas for its control. In the particular case of *Strongyloides stercoralis*, high prevalence was reported in this area (30-50%); therefore, ivermectin has been included in the chemotherapy. In this context, assessing the *S. stercoralis* response to therapy in the treated population has become a major concern. The NIE ELISA was used for this purpose since it proved to be more sensitive than conventional parasitological techniques for the diagnosis of *S. stercoralis*. This community trial was conducted in two groups of patients, classified according to differences in housing and living conditions. After the first sampling and deworming (Massive Drug Administration, MDA), Group 1 (G1) was moved to new households with drinking water access and improved sanitation facilities (MDA+WAS intervention); while Group 2 (G2) remained living in less developed conditions with unimproved drinking water and sanitation (MDA intervention). The mean interval times between baseline (Base) and the follow up (F/U) were 359 days for G1 and 478 for G2. Anti-NIE antibody titers (Optical Density, OD) were measured for each individual before and after interventions (paired sera). A follow up OD ratio ($OD_{F/U}$/OD_{Base}) was calculated to quantify the variation in antibody titers. A significant decrease of the anti-NIE titers ($p<0.0001$) between Base and F/U was observed in both groups. Nonetheless the number of patients who achieved the cure criteria (ODratio<0.6) was different between groups: G1: 75% (24/32); G2: 45% (17/38) ($p=0.038$). We found that NIE ELISA is a useful test for assessing the response to treatment and to evaluate the outcome of control interventions in the population. Following the anthelminthic treatment, we could observe a marked decrease of anti-NIE antibodies over the time. Furthermore, our results support that a combined intervention including deworming and improvements in life conditions is more effective, in terms of number of subjects cured, than deworming only.

705

COMPARISON OF ALPHAVIRUS AND FLAVIVIRUS PREVALENCE IN WESTERN KENYA

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Chikungunya virus (CHIKV) and dengue virus (DENV) are emerging mosquito-borne viruses that are endemic in tropical environments, such as Africa, Asia, South America, and the Caribbean. In rural areas of Africa, DENV and CHIKV infections often go undiagnosed and unreported, as fever presentation is commonly assumed to be a sign of malaria. The goal of this study was to measure and compare the seroprevalence of CHIKV and DENV (serotypes 1-4) among children (ages 5 to 14, n=250) and adults (ages 15 to 85, n=250) in a rural village community centered around Busia, Kenya. Samples were screened for anti-CHIKV and anti-DENV IgG by indirect ELISA. As expected, children were less likely to be

exposed to CHIKV ($p < 0.001$) than adults. For children, 141 samples (56.4%, CI95 0.500 to 0.626) were positive for anti-CHIKV IgG, and 2 samples (0.8%, CI95 0.001 to 0.029) were positive for anti-DENV IgG. Comparatively, 195 samples (78.0%, CI95 0.724 to 0.83), and 6 samples (2.4%, CI95 0.009 to 0.052) of the 250 samples from adult participants were positive for anti-CHIKV IgG and anti-DENV IgG, respectively. Overall, 67.0% of participants showed seropositivity for CHIKV (CI95 0.627 to 0.711), and 1.6% of participants were seropositive for DENV (CI95 0.007 to 0.031). These results confirm the presence of alphavirus and flavivirus exposure in western Kenya, and illustrate a significantly higher severity of transmission compared to previous studies. Given the expansive spread of the endemic in recent years, understanding the true severity, prevalence and burden of infection of DENV and CHIKV is critical for predicting the future impacts of each virus.

706

SEROPREVALENCE OF FLAVIVIRUSES AND ALPHAVIRUSES IN CHILDREN IN COASTAL KENYA: A 2015 SNAPSHOT

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Some of the most emergent and destructive diseases are mosquito-borne viruses. The non-specific symptoms of such viral infections lead to misdiagnosis and minimal case reporting, making the true impact of such infections difficult to determine. This cross-sectional study aims to describe the true prevalence of flaviviruses, such as dengue virus (DENV) and West Nile virus (WNV), and alphaviruses, such as chikungunya virus (CHIKV) and o'nyong n'yong virus (ONNV), in an urban community on the coast of Kenya in 2015. A subset of 700 afebrile children, aged 1-17 years, was selected from an ongoing community cohort study. Questionnaire data, including health history, socioeconomic status, home environment, and mosquito exposure, was used to determine potential risk factors associated with exposure to alpha- and/or flaviviruses. InBios CHIKjj Detect™ and DENV Detect™ IgG ELISA kits were used to identify IgG antibodies against DENV1-4 and CHIKV in follow-up samples. IgG seroprevalence was 2% for CHIKV (CI95 0.8-2.9%) and 1.4% for DENV (CI95 0.6-2.6%). Genus-specific cross-reactivity is anticipated with IgG ELISAs. Seropositivity for anti-CHIKV IgG, indicating previous alphavirus exposure, was associated with frequent outdoor activity ($p=0.003$) and lack of utilization of mosquito avoidance measures ($p=0.025$). Seropositivity for CHIKV ($p=0.025$) and DENV ($p=0.046$) was associated with mosquito bites at night. Gender was not significantly associated with prior alpha- or flavivirus exposure. Children as young as 5 were seropositive for either anti-CHIKV or anti-DENV IgG, indicating active alpha- and flavivirus transmission within the last 5 years. Children aged between 7 and 12 years were more likely to be seropositive for anti-CHIKV and anti-DENV IgG ($p<0.001$) when compared to younger participants. Prevalence data may not accurately represent the severity of exposure, infection, and disease throughout Kenya, as the varied reports of prevalence in other regions indicates differences that may be dependent on geographic region. These results confirm the continued presence of alphavirus and flavivirus exposure in children in coastal Kenya.

707

CHIKUNGUNYA VIRUS INFECTION IS CAUSING ACUTE FEBRILE ILLNESS AMONG CHILDREN IN KENYA

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Several chikungunya virus (CHIKV) outbreaks have occurred in Africa, Asia, and the Americas in the last decades. However, due to limited surveillance, few serologic data are available in Africa and as a result, there is a gap in our understanding of ongoing endemic transmission of CHIKV. Thus the risk for CHIKV infection in African peoples remains unknown. In order to prevent disease emergence, the dynamics of CHIKV infection must be understood and the risk factors for transmission need to be evaluated. For our study, we enrolled children with acute febrile illness who presented to one of four Kenyan health centers (in Chulaimbo and Obama Children's Hospital in western Kenya, and Msambweni and Ukunda on the Kenyan coast). In each region, one of the sites is localized in an urban area and the other in a rural area, in order to determine whether the differences between the two environments have an effect on the transmission rate of CHIKV infection in humans. Serum samples were collected at an initial visit and at a one-month follow-up visit for CHIKV ELISA testing. Questionnaire data were collected to describe demography, education, and household environment, along with clinical data. In our preliminary screening of 125 paired acute and convalescent serum samples by ELISA for anti-CHIKV IgG, we identified 5 cases (4%, 95% CI 1.3% to 9.1%) of seroconversion. These cases demonstrate recent active transmission of CHIKV in Kenya, both on the coast and in the west. Because of the small number of seroconversions in our preliminary analyses, we did not identify any differences in risk of infection associated with either urban or rural locale. We also did not detect any link between exposure to different water sources and seroconversion, however we did find that people who use a river or a pond as water source were more likely to report mosquito bites than people who have access to a public well (88.7% vs 70.2%, respectively, $p<0.0001$ by Fisher's test). Further testing may reveal important risk factors for seroconversion and will help identify potential interventions to reduce risk of developing CHIKV infection in Kenya.

708

IDENTIFICATION OF FACTORS ASSOCIATED WITH CHRONIC CHIKUNGUNYA DISEASE IN PATIENTS IN GRENADA, WEST INDIES

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Chikungunya virus (CHIKV) is a rapidly re-emerging arboviral pathogen worldwide. In July 2014, an explosive CHIKV outbreak occurred in Grenada, West Indies, infecting around 90% of the population, with a wide spectrum of disease reported. In an estimated 50% of cases, CHIKV infection transitions to a non-communicable painful arthritis that can persist for years. Our understanding of the risk factors and mechanisms underlying chronic disease are limited. Here, we conducted one-year follow up with 240 people who were tested for CHIKV during the Grenada outbreak and performed analyses on demographic, behavioral, exposure, and co-morbid health factors to identify associations with chronic disease. Physical examinations were performed and current arthritis/arthralgia symptoms, as well as prior medical history was recorded. Participants

also completed extensive questionnaires so that physical, psychological, social and environmental factors could also be assessed. "Chronic" CHIKV disease cases were defined as individuals who continue to experience arthralgia and/or arthritis >6 months after onset of their acute CHIKV disease that impacts activities of daily living. Demographic factors including age (p=0.56), gender (0.058), ethnicity (0.58) and socioeconomic status did not have an effect on the likelihood of suffering from chronic persistent CHIKV disease. Increased mosquito avoidance behavior also did not reduce the risk of chronic sequelae. Patients who suffered joint pains (0.005), muscle pains (0.042), generalized body ache (0.013) and weakness in the extremities (0.013) during acute CHIKV disease were more likely to have chronic arthritis and arthralgia symptoms, and an increased duration of acute disease (0.001) also increased risk. None of the co-morbidities measured were associated with increased disease risk. These data demonstrate that chronic CHIKV disease affects people across the age, gender, ethnic and socioeconomic spectrum, and is not reduced by vector avoidance activity. Management of acute symptoms and minimization of acute disease duration could reduce chronic sequelae.

709

SIMULATING CHIKUNGUNYA OUTBREAKS IN COLOMBIA USING AN AGENT-BASED MODEL

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The first chikugunya epidemic in the Americas was reported between 2013 and 2016. In this period, around 1.7 million clinical cases were reported. Due to the lack of a vaccine, vector control was the only resource to halt the epidemic. The response of governments to the emergency faced challenges because of uncertainty in the possible magnitude of the epidemic and in the cost-benefit ratio of vector control programs. We developed an agent-based model as a tool to predict transmission dynamics of vector-borne diseases and to assess the value of vector control. We applied the model to chikungunya in Colombia, where half a million cases were reported. By representing large-scale populations with an agent-based model, we are able to reproduce population-level patterns of the epidemic while accounting for heterogeneity of transmission using high-resolution demographic, geographic, and climate characteristics of the population. Also, our model allows for the evaluation of the impact on the epidemic of individual actions, such as vector control. In the model, transmission of the virus occurs upon human contacts with mosquitoes in specific locations. We used population density and climate grids to reproduce the mosquito abundance in space. Moreover, we created and calibrated a synthetic population to represent human demographics, contact patterns, and activities of 45 million inhabitants of Colombia. We calibrated the model with and without vector control to incidence reports of the first 24 weeks of the outbreak. To predict the possible spread of the epidemic, we evaluated various schemes of geographical distribution of vector control. Also, we validated the model predictions using the incidence reports in Colombia available from 2014 to early 2016. These simulation results show that giving priority to dengue-endemic areas, notably reduces the impact of the epidemic. Furthermore, the model predicted the shape and magnitude of the incidence curves reported in five out of six regions of Colombia. Finally, we believe that this platform can be used to evaluate the impact of similar transmitted viruses such as zika or dengue.

710

VACCINES AGAINST EMERGING ALPHAVIRUSES

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Emerging and re-emerging alphaviruses are responsible for several important infectious diseases. Although previously confined to mainly tropical areas, the geographic occurrence of alphaviruses now overlaps temperate regions including urban areas due to environmental and ecological changes, vector-host interactions and more competent viral strains. While some alphavirus infections can be asymptomatic, others such as those caused by Chikungunya, Ross River or O'nyong'nyong virus can cause high fevers, debilitating myalgia, rashes and arthritic disease. In addition, these infections are also accompanied by more severe symptoms such as life-threatening encephalitis, myocarditis or hemorrhagic fevers. At present, there is no specific FDA-approved medical treatment for infection with these viruses although some concerted efforts are directed at vaccine development. AC Immune has developed a number of various vaccines that can generate robust and long lasting antibody responses, independently of T cells. T cell responses raised during natural infection or alphavirus vaccination have been linked to more severe pathology. Moreover, T cell mediated responses are likely associated with brain encephalitis and arthritic disease. Therefore, a highly desirable feature in alphavirus vaccines is to induce a robust antibody response that can neutralize the virus infection and confer lifelong protection against recurrent re-infections, while avoiding reactive T cells. We have designed new vaccines with different linear and conformational relevant CHIKV peptide sequences. Results in murine models suggest that robust and long-lasting antibody responses are raised upon vaccination and are accompanied by low or no T cell responses. Therefore, our vaccines show promising results against alphaviruses causing arthritis-like symptoms, as well as other infectious diseases where T cell mediated responses are preferably avoided.

711

IMMUNE PROFILING AND NETWORK MODELING OF CHIKUNGUNYA INFECTION IN A HOSPITAL-BASED STUDY IN NICARAGUA

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Chikungunya virus (CHIKV) is an important emerging, mosquito-borne arithrogenic alphavirus that causes both explosive epidemics of debilitating rheumatic disease with fever, asthenia, skin rash and occasionally more severe complications that can be fatal. In December 2013, an outbreak of chikungunya in the Caribbean was reported; since then, over one million CHIKV cases are estimated to have occurred, and most countries of the Americas are reporting autochthonous transmission of CHIKV. It is of global concern that the chikungunya epidemic in the Americas is still

growing and the number of CHIKV-infected people is rising every year, yet much remains to be defined regarding the human immune response to CHIKV infection, especially in children. In our study, we collected acute (day 2 or 3) and convalescent (day 15 or 16) samples from symptomatic CHIKV-infected pediatric cases (n=42) presenting to the national pediatric reference hospital in Managua, Nicaragua. Comprehensive innate and adaptive immune responses were investigated by CyTOF, Luminex cytokine assays, and RNA-seq. Initial analyses revealed that the frequencies and phenotype of several major circulating leukocytes, particularly monocytes and dendritic cells, as well as the cytokine and chemokine profile, are significantly different between acute and convalescent samples. Additionally, we have performed RNA-seq analysis at both phases of CHIKV infection to identify differences in gene expression at the transcriptomic level. These data are being analyzed to identify immune signatures and potential biomarkers of CHIKV infection. All data are being integrated for network modeling, consisting of weighted gene co-expression network analysis (WGCENA), dynamic Bayesian networks and key driver analysis to generate global, unbiased maps of regulatory relationships and to uncover novel host-virus pathways and driver genes. Our study will provide the most comprehensive immune profiling and network analysis of the human response to CHIKV infection to date and will help inform future diagnostics and drug therapies.

712

ATYPICAL CHIKUNGUNYA PRESENTATION DURING THE 2014 EPIDEMIC IN VENEZUELA

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In 2014, Venezuela witnessed one of the worst epidemics ever seen caused by a mosquito-borne virus: Chikungunya (CHIK). The virus rapidly spread through the country affecting ~60% of the population. Although CHIK was not considered a severe disease, during the epidemic in Carabobo State, one of the first Venezuelan regions to be affected, patients developing atypical CHIK presentation were soon observed. We aimed to characterize the atypical clinical presentation of these patients and their possible association with CHIK virus infection. Data on socio-epidemiological factors, clinical presentation, co-morbidities, laboratory parameters and other paraclinical investigations were collected after informed consent from patients admitted to the main tertiary hospital of Valencia, the capital of Carabobo State, between September-December 2014. We present data on 16 patients with a mean age of 59 years (range: 34-81) and 62.5% males. All patients reported symptoms compatible with a previous or current CHIK infection. All presented fever and arthralgia while 12 (75%) had also arthritis and/or rash. Incapacitating arthralgias were reported by 10 (62.5%) patients. Thirteen patients (81.3%) presented cardiovascular complications, mainly myocardial infarction (MI, 77%), as well as angina pectoris, heart arrhythmias, acute pulmonary edema and hypertension. The remaining patients presented renal and neurological complications (Guillain-Barré Syndrome and viral encephalitis). Of those initially diagnosed with a MI, one patient developed a cardiogenic shock, resulting in death. Patients were hospitalized on average for 12 days (range: 4-35). Thirteen (81.3%) patients had underlying diseases of which hypertension was the most common (56.3%) followed by diabetes (31%) and obesity (25%). The combination of hypertension and diabetes type II (n= 4; 25%) was the most common comorbidity combination. Detailed clinical data and further analysis on a bigger sample size will be presented. Knowledge on atypical presentations will improve early diagnosis and management of these patients avoiding possible life threatening conditions.

713

PREDICTORS OF PLASMA LEAKAGE IN ADULT DENGUE PATIENTS

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It is estimated to have 390 million Dengue infections with 96 million cases worldwide. Mortality rate can be high ranging from 0.1% to 5%. Plasma leakage is the commonest complication in Dengue resulting Dengue Haemorrhagic Fever (DHF). The severity of leaking varies from individual to individual. Severe leaking can have a high morbidity and mortality if not detected and treated early. Therefore, it is important to detect plasma leakage early as such patients can be monitored carefully and fluid management can be manipulated according to the degree of leakage. Identifying predictors of plasma leakage would enable clinicians to closely monitor patients with such predictors to detect leaking early. Objective of this study was to determine predictors of plasma leaking in adult Dengue patients. All serologically confirmed Dengue patients admitted to Dengue Management Unit, National Institute of Infectious Diseases (formerly IDH), Angoda, Sri Lanka for four months from 1st of July 2014 were included in a prospective Case Control Study. Patients with plasma leakage were identified with serial ultra sound examination and were compared with others on predetermined parameters. There were 1000 patients with confirmed Dengue infection with 546 males and 454 females. Age ranged from 12 to 86 years. (mean 31 yrs.). 43.8% (n= 438) patients had plasma leakage (DHF) while 56.2% (n=562) did not have. There was no sex difference in patients with and without fluid leakage. Out of the warning signs in the WHO 2009 classification severe vomiting, abdominal pain and tenderness were significantly associated with plasma leakage ($p<0.05$) but not mucosal bleed or restlessness. In addition postural dizziness and platelet counts <50,000/microliter had higher risk ($p<0.05$) of developing plasma leakage as well as both overweight (BMI 23-27) and obesity (BMI >27). This study identifies several easily identifiable and observable parameters as predictors of plasma leakage in Dengue. These can be used identify patients likely to develop plasma leakage and to monitor them carefully to detect and to treat plasma leaking promptly thereby reducing morbidity and mortality.

714

EARLY SEASON INCIDENCE, SUSCEPTIBILITY, AND WEATHER PREDICTS ANNUAL DENGUE HEMORRHAGIC FEVER INCIDENCE IN THAILAND

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Dengue is a mosquito-borne infectious disease that places an immense public health and economic burden upon Thailand. Annual outbreaks of varying sizes pose a particular challenge to the public health system because treatment of severe cases requires significant resources.

Accurately forecasting these outbreaks could help public health decision-makers implement and evaluate the efficacy of interventions. Here, we present a statistical model to predict annual dengue hemorrhagic fever (DHF) incidence for each Thai province in April using weather data and observed case counts through March. We used cross validation on data from 2000-2009 to select covariates a negative binomial generalized additive model. Two models - the model that performed best in cross validation and a model including only preseason incidence - were applied to data from 2010-2014 as if conducted in real time. We compared the results of these models to those of a baseline model that predicts the median incidence over the past ten years. The performance of each model varied across the administrative health regions of Thailand. The preseason incidence model performed best overall with better predictions than the baseline model in 63% of observed province years with a 22% reduction in absolute error, averaging across all province years. The best cross-validated model, including covariates for preseason incidence, estimated relative susceptibility, rainfall, temperature, and humidity made better predictions in Northern Thailand where weather and DHF incidence fluctuate from year-to-year. The baseline model had the best performance in Central Thailand, where annual DHF incidence is relatively stable. These results demonstrate that a combination of location-specific prediction models for dengue can aid public health decision-makers in assessing the potential risk of an epidemic in different geographic locations.

715

SOCIAL CONNECTIONS AND CONTEXT IN DENGUE TRANSMISSION

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Dengue Virus (DENV) is a common arboviral disease in tropical and subtropical countries. Given the restricted range of mosquito movement (i.e., <100 m) and long-term immunity against the four DENV serotypes, human activities and herd immunity play critical roles in the spread of dengue. Specifically, individual activity spaces are socially constructed, and are shaped by activities (e.g., daily commuting, household tasks, social activities). Herd immunity is a limit on the number of potentially infectious hosts in a region and provides indirect protection for susceptible community members. In spite of a consensus on the importance of human activities and herd immunity, it is still difficult to accurately estimate their influence on dengue transmission. Epidemiologically significant measures of vector population density are also difficult to obtain. This uncertainty may be responsible for apparent conflicts in the findings of community-scale studies of DENV transmission. In this study agent-based models (ABM) have been used to jointly assess the effects of social networks, herd immunity, and vector density. Sensitivity analysis was used to understand the importance of social network attributes in terms of network type and number of social ties. The local context, in terms of herd immunity and mosquito density, significantly modulated sensitivity to social network specification. The results highlight the importance of characterizing host and vector population heterogeneity in studies of DENV transmission. Variation in model outcomes also helps us to understand factors that regulate the character of dengue transmission in terms of the overall intensity and focality of infections. The findings indicate that ABMs designed to test the effects of vaccination programs, where herd immunity is artificially increased, could be sensitive to the structure of social connections.

716

ZIKA VIRUS INFECTION IN A COHORT STUDY TO ASSESS THE INCIDENCE OF DENGUE, STATE OF SÃO PAULO, BRAZIL, 2015, 2016

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Zika virus (ZIKV) was first identified in Brazil in 2015 by reverse-transcriptase polymerase-chain-reaction (RT-PCR) assays of serum specimens from patients in Northeastern Brazil, who presented with a dengue like illness that was characterized by rash, fever, myalgia, arthralgia, and conjunctivitis. Up to the 9th 2016 epidemiologic week, 22 Brazilian states notified ZIKV infection confirmed by PCR. In August 2014, in Araraquara, a city in the State of São Paulo, Southeastern Brazil, a prospective cohort for dengue surveillance was initiated through weekly phone calls to access the incidence of dengue among children and adolescents aged 2-16 years. 3,514 children and adolescents and their parents agreed to participate in the study and signed an Informed Consent Form. The follow-up showed 1,140 fever episodes and 314 confirmed dengue cases by laboratory diagnosis (NS1, qPCR IgM positive for dengue) in December 2015. PCR was performed for 492 children and adolescent negative for dengue, but with fewer and any sign or symptom, to investigate Zika virus circulations. The RNA isolated was subjected to a Real-Time PCR of a single step ("one-step") using TaqMan Fast Virus 1-Step Master Mix (ThermoFisher, Brazil) and the set of primers and probe as following: Primer Name Sequence SEQ 5' 3' 1086F CCGCTGCCCAACACAAG 1162R CCACTAACGTTCTTTTGCAGACAT 1107-FAM AGCCTACCTTGACAAGCAGTCAGACACTCAA In Araraquara, in 2015 occurred 1,804 dengue cases, being the largest epidemic of recent years. In 2016, 376 autochthones cases had already occurred up to now. In our cohort dengue cases appeared between November 2014 and August 2015 with a peak in April (117 cases). No case was positive for ZIKV in 2014 and 2015. In 2016, until the 16th epidemiologic week occurred 190 fever episodes with 9 dengue laboratory confirmed and 7 ZIKV confirmed by PCR in plasma with 2 also by urine. The most common symptoms were sore though, conjunctival hyperemia, headache, rash and pruritus. The two viruses are circulating in Araraquara, but it seems that there has been a susceptible depletion in relation to dengue with the concurrence of the emergence of the movement Zika virus.

717

INAPPARENT DENGUE VIRUS INFECTION INCIDENCE, SAO PAULO, BRAZIL, 2014-2015

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Inapparent dengue virus infections have implications on the agent's transmission. They should be considered when evaluating control interventions such as vector control and vaccines. We have attempted to quantify the incidence of inapparent infections that occurred during the 2014 - 2015 dengue transmission season. A cohort study to assess the incidence of dengue among children and adolescents, from 2 to 16 years of age, in a previously recognized as a low endemic setting has started in August, 2014. A random sample of children and adolescents was selected from the population of Araraquara, a city in Central São Paulo State. Home visits were made to the families of selected children, to present the study and invite them to participate. The one who agreed to participate signed an Informed Consent Form. An interview on socio-

demographic characteristics was carried out and blood samples from the selected children were drawn for dengue baseline serology. Dengue IgG antibodies were tested by enzyme-linked immunosorbant assay (ELISA). Families are being contacted weekly for fever surveillance. Fever cases are submitted to dengue diagnosis tests. One year after recruitment a new blood sample was collected for IgG serology, in order to assess inapparent infections. The baseline seroprevalence of dengue IgG antibodies among the cohort participants was 15.3%. In the 2014/2015 dengue epidemic season the cumulative incidence of symptomatic laboratory confirmed dengue was 8.9%. The analysis of the one-year follow up samples is still ongoing. So far, results from 3,019 (85.9%) participants are available. Considering those who were seronegative for dengue at baseline and did not present symptomatic dengue infection, the seroprevalence after one year was 14.0% (320/2285), which may be interpreted as the incidence of inapparent infections. The rate inapparent/symptomatic dengue infections was estimated in 1: 1.6. It is lower than reported elsewhere. The sensitive suspect case definition (fever >37.5°C) may be responsible for this result. Unlike the incidence of symptomatic cases, the incidence of inapparent infections was not associated to age nor sex.

718

DENGUE VIRAL INFECTION INDUCED CD95 EXPRESSIONS IN DIFFERENT B CELL SUBSETS

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Excessive expansion of antibody secreting cell (ASC) was observed during acute dengue viral infection. While ASC is directed against dengue viral antigen, a marked increase in plasma cytokine levels and soluble immune function molecules may drive these cells to become more susceptible to undergo apoptosis. Moreover, as previous studies showed that alterations of B cell subsets were observed by a remarkable decrease in naïve and memory B cells. It might be possible that these cells undergo apoptosis induction through interactions between Fas or CD95 expressed on these cells and Fas-L expressed by cross talking immature pDC or activated NK cells. In our study, peripheral blood samples from acute dengue viral infected patients were stained with fluorochrome conjugated monoclonal antibodies against CD3, CD14, CD19, CD20, CD21, CD27, CD38, CD45, CD95 and CD138. The frequency and density of CD95 expression in ASC and B cell subsets based on the expression of CD19, CD20, CD21, CD27, CD38 and CD138 were determined by flow cytometry. Results showed that CD95 expression was observed in all B cell subsets. However, the high levels of surface expression density were observed in plasmablast and memory B cell subsets. Interestingly, the frequency of naïve B cells that express CD95 was increased when compared to healthy subjects. Our study, therefore, provides important information on apoptosis induction of B cell subsets during acute dengue viral infection via the interaction between Fas and Fas-L. The results demonstrated that all B cell subpopulations, especially plasmablast, have a potential susceptibility to apoptosis through Fas signaling pathway. The study also suggested that a decrease in naïve B cells might be due to apoptosis induction in this subset.

719

DENGUE DIVERSITY ACROSS SPATIAL AND TEMPORAL SCALES: LOCAL STRUCTURE AND THE IMPACT OF HOST POPULATION SIZE

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The micro-scale transmission dynamics of dengue ultimately determine who gets sick and the impact of interventions. Phylogeographic methods have been used to characterize pathogen dispersal at global and regional scales, but have yielded few insights into the local spatio-temporal structure of endemic transmission. Dengue virus infects >300 million people annually. Key mechanisms of introduction and maintenance remain unknown, including the number of distinct transmission chains that co-circulate within populations across different spatial scales. We geolocated 17,291 serotyped cases from Thailand from a 17 year period (1994-2010) and sequenced 800 viruses. We developed methods that compare the genetic similarity between viruses with the spatial distance between their locations to estimate the number of discrete transmission chains circulating within any area. We found that on average, 64% (95% CI: 41%-75%) of cases <200m apart in Bangkok were from the same chain (defined as having a common ancestor from the same dengue season) compared to 3% for cases <5km apart (95% CI: 1%-4%). Within 200m there were on average 1.7 transmission chains during a season, and with every 10-fold increase in population the number of chains increased 7-fold. However, there were significant heterogeneities across the city. Further, we found saturation in the number of chains circulating in equal sized areas at population densities >7,000/km2. We replicated these patterns using simulations where incidence is driven by local, density-dependent transmission; suggesting that ecological interactions in high-density environments limit the number of independent chains. We also found evidence for separation between national epidemics across Southeast Asia, suggesting minimal viral flow across borders. These results reveal hyper-local epidemics within a season and self-supporting dynamics within Thailand over the long-term. This study provides a framework for characterizing the number of independent transmission chains circulating within any community, with key implications for understanding local trends, including the impact of control efforts.

720

AEDES ALBOPICTUS MIDGUT CELL LINE: A PRISTINE CELL LINE FOR *IN VITRO* STUDY OF ARBOVIRAL PATHOGENESIS

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The successful vaccine production for many arboviral diseases is hindered due to a very common problem i.e. non-availability of appropriate cell line, which mimics the microenvironment of the viral pathogenesis inside a host, providing an artificial cellular milieu for the virus to grow and multiply *in vitro*. Till date, many cell lines have been prepared from embryo, eggs and larvae of mosquitoes, which are extensively used for culturing arboviruses, but most of the arboviruses like Dengue virus, West Nile virus, Chikungunya virus, Japanese encephalitis virus, etc mainly infect the midgut of the mosquitoes; rendering available mosquito cell

lines inappropriate models for study of host-viral interactions. In this study, wild *Aedes albopictus* mosquitoes collected from different parts of Kolkata, India, were identified by an entomologist and were dissected to separate the midgut. Light microscopic and electron microscopic study of the cellular pattern on the mosquito midgut surface revealed the presence of stem cells, columnar cells, goblet cells and regenerative cells. Stained histological sections of midgut showed mucous coat enclosing the basal lamina on which the cells are embedded. Depending on the number of microvilli and microvilli-associated network, the posterior part of the midgut was targeted for preparation of the cell culture as it is the primary entry site for most of the arboviruses. The prepared cell culture was found stable without any lot to lot variation. The midgut cell culture was then challenged with dengue virus and then one alternative medicine "Rhus toxicodendron 6c" which is believed to be effective in dengue fever was tested for its efficacy as entry barrier and/or escape barrier in the primary cell line along with controls. Morphological changes were also analyzed. The medicine appeared to provide effective protection of the cell lines from dengue virus infection. Thus, the results of this study open up a new platform to study how arboviruses are normally able to surmount midgut infectivity barrier and midgut escape barrier, which will provide a better understanding of the innate host-viral interaction mechanism.

721

A FULLY-HUMAN HYPERIMMUNE POLYCLONAL ANTIBODY PRODUCT FROM TRANSCHROMOSOMIC BOVINES TO TREAT DENGUE INFECTIONS

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No specific therapeutic is available to prevent or treat classical or severe dengue infections. Passive immunotherapy with target-specific immunoglobulin (IgG) is effective in the treatment of toxin, venom and pathogen mediated diseases. Current target-specific immunotherapeutics are either polyclonal IgGs, derived from human or animal plasma, or monoclonal antibodies. Each production approach has limitations. Human derived IgG (or convalescent plasma) require the recruitment of convalescent human donors in sufficient numbers. Monoclonal antibodies require lengthy development periods and pose the risk of escape mutants. Both these approaches can be prohibitively expensive. Animal derived heterotypic-antibody IgGs can cause severe reactions. Transchromosomic bovines (Tc-bovine) provide an alternative method to economically produce large quantities of target-specific IgG with fully-human antibodies. Tc-bovines have had their repertoire of bovine antibody genes deleted, and instead carry a human artificial chromosome containing the full repertoire of human antibody genes. Tc-bovines can rapidly produce up to 600 grams per month of hyperimmune, multi-pathogen, fully-human immunoglobulin (hIgG) to prevent and treat human diseases. Tc-bovines were hyperimmunized with psoralen inactivated tetravalent dengue vaccine (serotypes 1-4) and a purified Tc-bovine hIgG (SAB-123) with tetravalent dengue virus neutralization titers up to 2560 was produced. Four groups of Cynomolgous monkeys (n=3) infected with 5E5 PFU of dengue 1 on day 0 were infused with 100 mg/kg of SAB-123 on days -1 (prophylactic), +2 or +1&3 (therapeutic) or negative control hIgG on day -1. Control animals infused on day -1 all had detectable viremia on days 2-6. Those receiving SAB-123 on day -1 and day 1&3 had no detectable viremia at any time. Those receiving SAB-123 on day 2 only had detectable viremia for one day prior to transfusion. Similar hIgG therapeutic products for two separate infectious disease indications have obtained FDA clearance for clinical testing. These results warrant human evaluation of this product for treatment of dengue infections.

722

CHARACTERIZATION OF CYD DENGUE VACCINE VIRUSES WITH HUMAN MONOCLONAL ANTIBODIES TARGETING KEY CONFORMATIONAL EPITOPES

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The Sanofi Pasteur CYD tetravalent dengue vaccine, which is now licensed in several dengue endemic countries, demonstrated significant efficacy in phase III studies with differences between serotypes. To characterize the quality and specificity of vaccine-induced antibody responses, we investigated the surface epitopes on each vaccine virus serotype using human monoclonal antibodies (MAbs). These antibodies have previously been shown to target conformational/quaternary epitopes, to be highly neutralizing, and either serotype-specific against DENV1 (1F4), DENV2 (2D22) or DENV3 (5J7) or cross-reactive against the 4 serotypes (1C19). A DEN4-specific antibody was not available at the time of the study. We monitored the binding of 1F4, 2D22, 5J7 and 1C19 with the vaccine monovalent lots included in Phase III clinical formulations using the following assays: Dot Blots, ELISA, Biacore® and PRNT. We used attenuated DENV as positive controls and some immature viruses or VLPs as negative controls for anti-DENV reactivity. Assays were set up and calibrated with cross-reactive anti-Envelope mouse monoclonal antibodies. In each assay, the CYD1, 2 and 3 vaccine viruses were found to be recognized by the MAbs with the expected specificity, and were strongly neutralized at levels comparable with those previously reported with wild type DENV. Biacore® further indicated a high functional affinity. Overall, these findings demonstrate that the CYD dengue vaccine viruses display key conformational and functional epitopes of wild type DENV. Future investigations will assess the neutralizing antibody response elicited by the CYD tetravalent vaccine against these critical epitopes, and how it relates with vaccine efficacy.

723

PERIODICITY (LONG-TERM AND SHORT-TERM CYCLES) OF DENGUE IN VENEZUELA

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Mosquito-borne viruses are becoming major public health problems throughout the tropical and subtropical regions of the world. In Venezuela (South America), despite control measures, transmission of dengue virus (DENV) has become perennial with three large epidemics in the past decade. The long-term pattern of this disease has involved not only a general upward trend in cases but also a dramatic increase in the size of epidemic outbreaks. Previous studies indicate that DENV multiyear cycles are the result of both extrinsic (e.g. climate variability) and intrinsic (e.g. herd immunity and host susceptibility) factors. Here, we first explored the periodicity of dengue incidence in time-series of data (24 years) from several regions of Venezuela using wavelet analyses (WA), a statistical approach specifically developed for non-stationary patterns. Significant cycles of 1- to 3-year periods were identified. Additionally, we determined whether disease epidemics were related to local climate variability and regional climate anomalies such as the El Niño Southern Oscillation (ENSO) using WA to identify time- and frequency-specific associations. Understanding the periodicity of dengue in Venezuela can give useful insights about arbovirosis outbreaks. Indeed, the years 2014 and 2015 were marked by two relevant outbreaks of the emergent viruses:

chikungunya and zika. Therefore, our findings may be used to forecast dengue and other vector-borne viral epidemics and to improve disease surveillance and control strategies.

724

USE OF THE DENGUE HUMAN CHALLENGE MODEL TO CHARACTERIZE THE ROLE OF HETEROTYPIC ANTIBODY IN PROTECTION AGAINST DENGUE INFECTION

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Dengue has become the most important mosquito-borne virus in the world resulting in nearly 400 million infections annually. There are four dengue virus (DENV) serotypes, each capable of causing the full spectrum of illness. This can range from an asymptomatic or mildly symptomatic infection to a life-threatening vascular leak syndrome. It is generally believed that infection with one DENV serotype confers long-lived protection against symptomatic re-infection with that same serotype but only short-lived (~3 months) protection against infection with a heterotypic DENV. We sought to test this paradigm using our controlled dengue human challenge model. Twenty-four flavivirus-naïve subjects were enrolled in a randomized, placebo-controlled, double-blind trial. The treatment assignments remained blinded until study day 270. Eighteen subjects received a trivalent mixture of the live attenuated candidate DENV vaccines rDEN1Δ30, rDEN3Δ30/31, and rDEN4Δα30. Eight subjects received placebo. Six months later, all returning subjects were given the DENV-2 challenge virus DEN2Δ30. Following receipt of the trivalent admixture 15 subjects developed rash (83%) and 11/18 (61%) had one or more DENV recovered from the blood. rDEN3Δ30/31 was recovered from 7 subjects, rDEN1Δ30 from 5 subjects, and rDEN4Δ30 from 2 subjects. Twenty-one subjects returned for challenge (15 trivalent recipients and 6 controls). Following challenge with DEN2Δ30, only 3 (20%) of those subjects who had received the trivalent mixture developed rash compared with 83% of the controls, indicating that the trivalent mixture imparted some protection against the challenge virus. The clinical, virologic, and serologic responses following administration of the trivalent admixture and following DENV-2 challenge will be presented. The role of heterotypic antibody and cellular immune responses will be discussed.

725

LONGITUDINAL ANALYSIS OF B CELL RESPONSE TO INFECTION WITH A DENGUE-2 CHALLENGE VIRUS

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The four dengue virus (DENV) serotypes are the leading cause of arboviral disease globally, with 390 million new infections annually, and more than 40% of the world's population at risk. Both serotype-specific and cross-reactive antibody responses are observed at the population level, but the temporal patterns of how such antibodies emerge on an individual basis after primary DENV infection are not clear. A better understanding of the antibody response to primary infection can elucidate how circulating DENV-specific antibodies afford protection versus lead to risk of disease enhancement in secondary heterologous infection or vaccination. To address this, we analyzed the clonal evolution of B cells from the early plasmablast stage to the late memory stage in subjects from a controlled DENV human infection model. In a representative subject infected with DENV2 challenge virus we used Immune Repertoire Capture (IRC™) technology to identify over 400 unique, natively paired antibody heavy and light chains in the plasmablast repertoire. At six-month post challenge, we isolated and immortalized memory B cells from the same donor, and found that approximately 0.7% of IgG+ memory B cells exhibited reactivity to DENV. Most of the response was DENV2-specific though cross-reactive responses were also observed. Understanding the kinetics of the humoral response in this primary infection model will increase our understanding of the B cell evolution in response to DENV infection, and reveal insights on how specific vaccine components may be tailored immunogenically to maximize protective effect while minimizing risk.

726

DENV PREEXISTING IMMUNITY EFFECT ON ZIKV INFECTION AND THE RELIABILITY OF DIAGNOSIS IN AN AREA WITH CO-CIRCULATION OF SEVERAL ARBOVIRUSES

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Previous studies have demonstrated the effect of cross-reactive sub-neutralizing antibodies in DENV severe clinical presentation. Since, DENV and ZIKA are closely related viruses, we tested whether there is any impact of previous DENV antibodies on ZIKV infection. To do this, we tested serum samples collected pre and post ZIKV epidemic and evaluate ZIKV neutralization capacity by PRNT50. On the other hand, there are several obstacles for accurate diagnosis of vector-borne viruses in endemic areas. The problem is significantly higher when several pathogens, with similar clinical characteristics, co-circulate in the same area. With the latest emergence of African arboviruses in South America, there is a need for reliable, cost-effective tools to help in differential diagnosis, especially in rural areas. Thus, we decided to compare the sensitivity and specificity of a rapid test for dengue virus infection (Dengue Duo-Antigen) versus a

molecular biology technique (qRT-PCR). We evaluated whether a particular test is better for differential diagnosis in an endemic region of Colombia where Dengue (DENV), Chikungunya (CHIV) and Zika (ZIKV) are often concurrently transmitted. We will show the sensitivity and specificity of qRT-PCR vs. Dengue Duo test, and discuss the impact of preexisting immunity on arbovirus transmission dynamics.

727

VECTOR GENOTYPE INFLUENCES DENGUE VIRUS INTRA-HOST GENETIC DIVERSITY IN MOSQUITOES

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During infection of their arthropod vectors, arthropod-borne viruses (arboviruses) such as dengue viruses traverse several anatomical barriers that are believed to cause dramatic reductions in population size. Such population bottlenecks challenge the maintenance of viral genetic diversity, which is considered critical for fitness and adaptability of arboviruses. Anatomical barriers in the vector were previously associated with both maintenance of arboviral genetic diversity and alteration of the variant repertoire. However, the relative role of random processes and natural selection, and the influence of vector genetic heterogeneity have not been elucidated. In this study, we used high-throughput sequencing to monitor dengue virus genetic diversity during infection of several genetic backgrounds of their mosquito vector. Our results show that initial infection of the vector is randomly founded by only a few tens of individual virus genomes. The overall level of viral genetic diversity generated during infection was predominantly under purifying selection but differed significantly between mosquito genetic backgrounds. Thus, in addition to random evolutionary forces and the purging of deleterious mutations that shape dengue virus genetic diversity during vector infection, our results also point to a role for vector genetic factors in the genetic breadth of arbovirus populations.

728

A CLOSER LOOK AT ADE AND OAS IN THE SECONDARY DENGUE PLASMABLAST RESPONSE

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Epidemiological studies have linked heterologous secondary DENV infections with increased disease severity, implicating pre-existing immunity in the form of antibody titers as a risk factor for severe disease. In this study, we focus on B cell responses generated during the acute phase of secondary DENV infection, and describe their potential to participate in antibody dependent enhancement (ADE) and original antigenic sin (OAS). We isolated plasmablasts from four Thai patients during ongoing DENV2 infection and generated 53 monoclonal antibodies by single-cell immunoglobulin gene expression. The antibodies were largely cross-reactive to two or more DENV serotypes, with a small subset exhibiting serotype-specific binding and neutralization activities *in vitro*. Interestingly, although all patients were infected with DENV2 at the time of the study, a majority of the antibodies generated from two patients displayed stronger neutralization of DENV1 than DENV2. These findings were echoed at the serum level, where a clear bias in neutralization was observed towards DENV1 compared to DENV2. This neutralization bias is strongly reminiscent of OAS. Additionally, a majority of DENV-neutralizing mAbs either moderately or potently enhanced DENV infection of U937 cells indicating that the potential for ADE is not limited to cross-reactive mAbs. Our studies provide basis for future work examining the impact of antibody responses on dengue immunopathology.

729

DISPLAY OF QUATERNARY EPITOPES RECOGNIZED BY DENGUE VIRUS NEUTRALIZING ANTIBODIES

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Dengue virus (DENV) is the causative agent of dengue fever and dengue hemorrhagic fever. DENV and its mosquito vectors are widely distributed in tropical and subtropical regions and the disease is endemic in over 100 countries. Dengue vaccine development is challenging because of need to protect against four antigenically distinct DENV serotypes and evidence that, under some conditions, specific immunity to the virus can enhance disease. Recent studies have led to the identification of epitopes on the DENV envelope (E) protein targeted by human neutralizing antibodies. Some epitopes are preserved on the monomeric E protein, while other epitopes are complex and require the assembly of higher order E protein structures required for virion assembly. Here we describe studies to optimize the display of quaternary epitopes on artificial surfaces. The ectodomain of DENV E protein was expressed as a soluble recombinant protein (recE), which was secreted from cells. RecE was purified from the culture media and conjugated to a solid matrix. Using a large panel of human and mouse derived monoclonal antibodies, we confirmed that the conjugated protein was properly folded. Moreover, by adjusting factors such as pH, salinity and protein density, we optimized the display of quaternary structure neutralizing epitopes known to be critical for inducing protective antibody responses. These results have implications for developing novel subunit vaccines displaying quaternary epitopes from flaviviruses.

730

RAPID ACTIVE SEROPREVALENCE SURVEYS AS A TOOL TO MEASURE DENGUE VIRUS DISEASE BURDEN IN RESOURCE-LIMITED SETTINGS

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Cost-effective surveillance systems capable of accurately detecting acute febrile illness (AFI) are necessary to evaluate the endemic burden of diseases such as dengue virus (DENV) and to estimate the potential effectiveness of vaccines. Cross-sectional seroprevalence surveys are commonly used for outbreak investigations, but they have not been validated as a timely, cost-effective alternative to active surveillance. We used a 2-stage cluster design (30 clusters of 7 households) to enroll children age 0-17 years in a rural, resource-limited region of Guatemala into two parallel surveillance systems to estimate the burden of AFI and DENV. In the prospective Participatory Syndromic Surveillance (PSS) arm, 207 households with 483 children (enrolled Apr-Sep 2015) were provided a wireless internet-connected smartphone with a symptom diary application and asked to submit weekly self-reports of fever. Subjects reporting 2+ days of fever were visited and offered DENV testing (PCR and IgM). In the Rapid Active cross-sectional Surveys (RAS), 377 children from 209 households (cycle 1), and 369 children from 210 households (cycle 2) from the same community were surveyed for self-reported fever within the preceding 7 days and offered testing for DENV by IgM regardless of symptoms, and by PCR if fever was present for 2+ days. In the PSS arm, 71 children reported AFI during 362 person-years of observation (19.6

cases/100 person-years), and 3 of 40 (8%) tested were DENV+. In RAS cycles 1 (Oct-Nov 2015) and 2 (Jan-Feb 2016), 74 (20%) and 53 (14%) children reported AFI in the preceding week and 3/13 (23%) and 6/29 (21%) tested were DENV+, respectively. In logistic regression models adjusted for sex, younger age was a significant predictor of AFI symptoms in the RAS cycles but not in the PSS subjects. Younger age was not associated with DENV+ AFI. Our data demonstrate a significant burden of AFI and DENV in the community. The more cost-effective RAS cross-sectional surveys provided more sensitive estimates of AFI incidence and DENV infection rates than the smartphone-based PSS active surveillance cohort, though further surveillance and data collection are needed.

731

NATURAL AND LABORATORY-DERIVED GENETIC VARIATION IN DENGUE VIRUS TYPE 2 AT ENVELOPE PROTEIN POSITIONS 202 AND 203 MODULATES ANTIGENIC AND IMMUNOGENIC PROPERTIES

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The four dengue virus types (DENV1-4) cause up to 400 million infections each year. There is antigenic variation within and among the dengue types, but the genetic determinants of DENV antigenic variation are poorly understood. Others have shown that a single mutation at K204R (at the dimer interface of envelope [E] domain II) in DENV1 prototype strains drastically alters neutralization by a monoclonal antibody that targets a cryptic epitope exposed through virion breathing. Using antigenic cartography with primary infection African green monkey (AGM) antisera, we have found that a single amino acid substitution at a neighboring position, E202K, dramatically increases neutralization by homologous and heterologous antisera. Further, the AGM inoculated with E202K had a response highly focused to the homologous strain and remained the AGM's only detectable neutralization titer five months post-inoculation, while the AGM inoculated with the wild-type strain had balanced neutralization of diverse DENV2 strains. We are testing for differential neutralization of DENV2 E202K compared with the wild-type strain using monoclonal antibodies directed at cryptic epitopes as well as for improved neutralization with extended incubation times, suggestive of virion breathing. We also analyzed available E sequences in GenBank for natural variation and found that while positions 202 and 204 are highly conserved within and across serotypes, position 203 is naturally variable and differs between genotypes of DENV2 as well as DENV4. Interestingly, strains with 203D, including American genotype DENV2 strains shown by others to be cross-neutralized by primary DENV1 antisera, cluster closer to DENV1 antisera on antigenic maps, while Asian DENV2 genotypes with 203N are more distant from DENV1. We are testing if this antigenic difference is caused by exposure of a cryptic epitope. Understanding the mechanistic basis of the antigenic and immunogenic effects of genetic variation at 202-204 may provide insights into the antigenic representativeness of laboratory-adapted strains as well as if virion flexibility is important to DENV antigenic evolution.

732

CO-INFECTION OF DENGUE VIRUS BY SEROTYPES 1 AND 2 IN A PATIENT FROM STUNG TRENG PROVINCE, CAMBODIA

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Dengue virus (DENV), the etiological agent for dengue fever and dengue hemorrhagic fever/dengue shock syndrome is a significant contributor to the morbidity and mortality rates in tropical and subtropical regions of the world, especially in Southeast Asia. There are currently four circulating clinical serotypes, and all of them have circulated in Cambodia during the past five years, with serotype1 (DENV-1) having historically been described as the predominating serotype and co-infection has not been commonly identified. Here, we describe a case of DEN-1 and DENV-2 co-infection that represents the first reported case of this serotype combination from Cambodia. The case was from Stung Treng Province, Cambodia, and was enrolled in a passive febrile surveillance study cohort administered by US Naval Medical Research Unit-2 (NAMRU-2). The case had classic dengue fever symptoms. Both dengue rapid test and serology were positive. Flavivirus screening was performed with Real Time-PCR and then confirmed as DENV-1 and DENV-2 co-infection by semi-nested PCR. The remainder of his hospital course was uncomplicated and recovered six weeks later without sequel, corroborating with previous reports. This case highlights the importance of dengue surveillance with serotyping. It demonstrates that dengue co-infection with different serotypes can occur naturally, and can go undetected if only rapid testing is performed. Absence of serotype-specific information in individual cases may impede clinical management, resulting in potentially unnecessary or detrimental treatment. With such high dengue incidence rates on national and regional levels, the availability of such information has substantial potential benefits to population health.

733

MAPPING HUMAN NEUTRALIZING ANTIBODY RESPONSES TO DENGUE VIRUS SEROTYPE 4

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Dengue viruses (DENVs) are mosquito-borne flaviviruses consisting of four serotypes (DENV1-4). Primary DENV infections develop protective type-specific neutralizing antibodies, only to the serotype of exposure that target predominantly the quaternary structure epitopes. While specific quaternary epitopes responsible for the neutralization of DENV serotypes 1, 2 and 3 have been well defined, very little is known about the molecular basis of serotype 4 neutralization by human antibodies. This is a significant gap because a successful dengue vaccine has to induce protective antibodies to all 4 serotypes. We aimed to characterize the human long lived plasma cell (LLPC) and memory B-cell (MBC) derived neutralizing antibody responses in people exposed to primary DENV4 infections. To characterize the LLPC-derived responses, specific populations of antibodies were depleted from naturally infected DENV4 immune

subjects and DENV4 NIH monovalent vaccine recipients. In parallel, MBCs from naturally infected DENV4 immune subjects were transformed with Epstein Bar virus (EBV) to produce human hybridomas secreting DENV4-specific hMAbs. Two type-specific DENV4 neutralizing hMAbs were isolated and epitope mapped using binding and neutralization assays with wild type and recombinant DENVs. Further, shotgun mutagenesis studies aided in mapping the critical residues for these monoclonal antibodies. Antibody depletion studies showed that the DENV4 immune subjects had type-specific antibodies that strongly neutralized DENV4 only. These serum properties were also reflected in two hMAbs that strongly neutralized DENV4 only. The epitopes of the two DENV4 antibodies were mapped to the EDI/EDII hinge region. Importantly, LLPCs and MBCs in the subject from which the mAbs were isolated targeted the same epitope regions. A significant proportion of the type-specific responses induced in the NIH DENV4 monovalent vaccine recipients were also directed to the EDI/II region. We will discuss the specific location of the DENV4 neutralizing site and also the implications of our work for natural infection and dengue vaccine induced antibody responses.

734

DETERMINING THE EFFICACY OF LARVIVOROUS FISH, COMMUNITY ENGAGEMENT, AND A NOVEL SLOW RELEASE PYRIPROXYFEN FORMULATION SUMILARV® 2MR ON DENGUE VECTORS (*AEDES AEGYPTI* AND *AEDES ALBOPICTUS*) IN CAMBODIA: A CLUSTER RANDOMIZED TRIAL

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Asia records 70 percent of the 390 million dengue infections occurring each year, and Cambodia has one of the highest per-capita incidence rates in the region. Due to the relatively high cost of the current vector control methods and documented insecticide resistance in Cambodia there is an urgent need to find alternative cost-effective solutions for *Aedes* vector control that are operationally feasible by the National Dengue Control Program. A cluster randomized, controlled superiority trial was developed to establish the effectiveness of an integrated vector management approach using guppy fish (*Poecilia reticulata*), a new slow release pyriproxyfen matrix (Sumilarv® 2MR), and community engagement through a clear Community for Behavioral Impact (COMBI) strategy. The trial based in Kampong Cham, Cambodia includes 30 clusters with approximately 200 households (1000 individuals) per cluster and runs from October 2015-October 2016. The clusters were randomly assigned with a 1:1:1 allocation through a public randomization process to one of three arms; (1) all interventions, (2) guppies and COMBI activities, and (3) control. The control area receives only interventions currently available through the government, which currently includes insecticide distribution and health education during outbreaks. To avoid spillover effects, clusters are at least 200 meters from the nearest household as *Aedes aegypti* in this region have an average flight range of 50-100m. The primary outcome is the population density of adult female *Aedes*, and will be evaluated through four entomological surveys. Secondary outcomes include classical Stegomyia indexes, coverage rates of the intervention, and changes in Knowledge, Attitudes, and Practices (KAP) indicators evaluated through monthly monitoring forms and baseline/endline KAP surveys. Polymerase chain reaction will be used to determine dengue virus rates in adult female *Aedes* mosquitoes. The results of the trial will be used to inform policy recommendations for Cambodia.

735

DENGUE OUTBREAK IN EAST DELHI, DELHI STATE, INDIA, 2015

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Dengue has emerged as a major vector borne public health problem in India. It is endemic in capital city-state, Delhi, which experienced its largest ever dengue outbreak in 2015. An outbreak investigation was conducted in the East Delhi Municipal Corporation (EDMC) area with objectives to characterize outbreak features and to assess risk factors. A case of dengue was defined as "any person residing in EDMC area and had fever (2-7 days duration) between 14th May and 18th December, 2015 and tested positive for IgM ELISA or NS1 antigen ELISA". We prepared a line list and conducted descriptive analysis. We analyzed larval survey data. A 1:2 case control study was conducted to assess risk factors. Controls were persons from case's neighborhood with no fever history. We collected data on living conditions, domestic mosquito breeding sites, vector control and personal protection measures. There were 1775 dengue cases including 8 deaths. Age group 10 to 14 years (273 cases, 16.5%) most affected. Males were 1038 (61%). Median age was 21 years (Range: 7 months to 88yrs). Attack rate was 44 per 100,000 population. NS1 antigen ELISA test was positive in 1169(66%) cases. There was circulation of DENV 2 and 4 sero-types. In August, *Aedes* mosquito breeding detected in 14 per 1000 house visits and Breteau index was 15. Risk of dengue was more in those lived in overcrowded houses (OR 2.02, 95% CI 1.2-3.2), stored water in containers (OR 1.8, 95% CI 0.8-3.9), dumped waste disposables around/over the house (OR 1.6, 95% CI 0.7-3.6), spent day time in work place or school (OR 1.6, 95% CI 0.9-2.8) and low education (OR 1.4, 95% CI 0.8-2.3). Less risk was among those used larvicide in desert cooler (OR 0.3, 95% CI 0.08-1.1), worn trouser and full sleeved clothes (OR 0.6, 95%CI (0.4-1.1) and covered windows with mesh (OR 0.8, 95% CI 0.5-1.3). Factors like increased *Aedes* mosquito breeding in key domestic sites, circulation of multiple DENV sero-types, overcrowding and low education led to the outbreak. Concurrent anti-larval and anti-adult measures are needed to control *Aedes* mosquito. Active community participation to monitor key breeding sites and personal protection advocacy are needed.

736

CORRELATION OF CLINICAL DIAGNOSIS AND DENGUE ASSAYS IN CAMBODIA OVER SIX YEARS

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Dengue fever is a flavivirus infection endemic to tropical regions, estimated to be responsible for 50-100 million infections annually. As a part of an ongoing febrile cohort study in Cambodia, febrile patients (>38C) presenting to regional health centers are enrolled and tested for a variety of febrile illnesses. During the period of Jan 2010-Feb 2016, specimens were tested for dengue with varying combinations of rapid test (NS1), PCR, or paired ELISA, with the follow-up performed at four weeks. Of the 19,823 enrolled patients, 1,572 were given a clinical diagnosis of dengue, but 4070 patients ultimately had at least one positive confirmatory test of NS1, PCR, acute IgM, or convalescent IgG. Positive results were obtained in 876 samples tested for NS1, 1272 for PCR, 1243 for acute IgM, and 2500 for convalescent IgG, resulting in 349 positive paired serologies. Clinical diagnosis had a sensitivity of 57.5% and specificity of 90.2%, but a PPV of 33.4 for correlation with NS1. NPV was 96.1. PCR performed similarly in relation to clinical diagnosis (Se 43.8%, Sp 94.8%, PPV 38.7, NPV 95.8; $p<0.001$). Analysis of a subset of 4667 patients for whom all tests were performed showed only marginal differences in comparison to the whole cohort. Clinical diagnosis performance decreased when used in

combination with the historical gold standard of paired serologies, with a sensitivity of 40.7%, specificity of 87.5%, and PPV of 17.1. NPV was 95.9 ($p<0.001$), and showed an overall positivity rate of 6.0%, as opposed to 7.3% for NS1 and 10.7% for conventional PCR. Distinct seasonality was observed for all measures. Serotyping was performed for 1271 samples. Predominance varied by year, but across the six years, serotype-1 was most common (62.3%), followed by serotype-2 (23.3%), type-4 (12.5%) and type 3 (1.9%). Dengue fever continues to be an illness with significant morbidity in Cambodia. Newer diagnostic tools such as rapid NS1 antigen tests and PCR should be used in resource-limited settings to supplement clinical diagnosis. Their improved accuracy may help reduce unnecessary antimicrobial use and improve quality of care.

737

REGULATION OF PROTEIN TRANSLATION IN MOSQUITO CELLS INFECTED BY DENGUE 2 VIRUS

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Dengue virus (DENV) is naturally transmitted between humans by *Aedes* mosquitoes. The virus generally replicates and amplifies to a high level of virus progeny population in mosquito cells which usually survive the infection. As many other viruses, DENV can modulate host protein translational machinery that benefits for viral infection. In order to explore its regulatory mechanism, we applied a technique of SUnSET which is a nonradioactive measurement of protein synthesis in C6/36 cells with DENV-2 infection. The result revealed a significant shutdown of newly synthesized proteins in C6/36 cells infected by DENV-2. As UV-inactivated DENV-2, it seems that virus replication after entry is required to trigger signals for protein synthesis in mosquito cells. It has been reported that the initiation of cap-dependent translation is a key step during the process of protein synthesis via the assembled eIF4F complex which targets 5'-cap of mRNA, and may be hindered by the phosphorylation status of eIF4E-BP, a component of the eIF4F complex. Expression level and phosphorylation of eIF4E-BP has been observed to reduce in C6/36 cells with DENV-2 infection for 24 h. It suggested that the eIF4E-BP is one important factor involving in host cap-dependent translation of mosquito cells, particularly in the status of DENV-2 infection. On the other hand, PERK is a signaling pathway which may be triggered by DENV infection, causing attenuation of protein translation, in virus-infected cells. In this study, the PERK inhibitor (GSK2606414) was implemented to DENV-2-infected C6/36 cells, resulting in recovery of protein synthesis, implying that this signaling pathway was rather likely involved in modulating protein synthesis. However, it remains to be work out for understanding how these two factors involving in protein synthesis work together.

738

RECENT SCIENTIFIC AND CLINICAL ADVANCES IN SANOFI PASTEUR'S DENGUE VACCINE PROGRAM

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The first dengue vaccine has been licensed by several dengue-endemic countries in Asia and Latin America for use in 9-45 or 9-60 year-olds. Licensure was supported by two pivotal phase 3 trials conducted in Asia, with 10,275 participants aged 2-14 years, and in Latin America, with 20,869 participants aged 9-16 years [NCT01373281 and NCT01374516 respectively]. Long-term safety follow-up studies, as recommended by the WHO, are currently ongoing and available data will be presented. Pooled efficacy results for the active surveillance period from both trials in subjects ≥ 9 years old demonstrated 65.6% (95%CI 60.7-69.9), 80.8% (70.1-87.7) and 93.2 (77.3;-98.0) efficacy against all symptomatic virologically-confirmed dengue (VCD) cases, hospitalized cases and severe dengue cases, respectively. During long-term follow-up in the third year of phase 3 and 2b trials, the pooled relative risk of hospitalized VCD cases among participants ≥ 9 years of age was 0.50 (0.28-0.89). In totality, these efficacy and safety data determined the currently licensed indication. Post-phase 3 investigations focusing on the quality of vaccine-induced responses: i) affinity and serotype-specific neutralization of antibodies, ii) infectivity and immunogenicity of the vaccine in a relevant *in vitro* tissue module (MIMIC), and iii) detection of key epitopes on the vaccine using clinically relevant human monoclonal antibodies, were initiated to understand the biological basis of these observed trial results. To complement findings, immune correlates derived from PRNT50 antibody neutralization data will be presented. Together, these data further support a vaccination strategy targeting high disease burden age ranges, combining routine vaccination with several catch-up cohorts at introduction would substantially reduce the burden of dengue disease in endemic regions.

739

IMMUNITY TO ZIKV, DENV AND CHIKV IN A NON-ENDEMIC HUMAN IMMUNE COHORT IN PORTLAND, OREGON

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Zika (ZIKV), dengue (DENV) and chikungunya (CHIKV) are the most important emergent and epidemic mosquito-borne viruses worldwide. These viruses often share the same vectors and frequently co-circulate. The determinants of natural human immunity to these viruses are not fully characterized, nor are the degrees to which immunity to one cross-reacts with the other viruses. Moreover, their co-circulation potentially complicates traditional serological diagnoses and poses a particular challenge for safe and effective vaccine design. Cohorts of naturally immune individuals are vital resources for defining the critical correlates of ZIKV, DENV and CHIKV immunity. While international immune cohorts are often used to study natural arboviral immunity, local immune cohorts offer advantages over internationally based cohorts, including: 1) recruited individuals are unlikely to be confounded by repeat infection over study periods 2) adult recruits can provide large volume serum and cell samples serially that can be processed locally and contemporaneously, 3) given time, a local cohort is expected to include donors with diverse exposures that vary over time, geography, and viruses beyond what would be found at any single international site. Here we report demographic, travel, medical and virus exposure data, baseline serum neutralization values, cross-neutralizing activity between ZIKV, CHIKV and DENV immune sera against each other as well as West Nile Virus, Yellow Fever Virus and Japanese encephalitis virus for a human immune cohort in Portland, Oregon. We characterize persistence of neutralization over time, evaluate neutralizing antibody decay and report results of virus specific B-cell frequency. Long-term we expect this cohort to provide high-value immune sera and cells for both dissecting components of protective ZIKV, DENV and CHIKV immunity and validating candidate targets and correlates of long-term arboviral immunity.

740

SPATIOTEMPORAL ANALYSES OF DENGUE HOSPITALIZATIONS CODED IN BRAZIL

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Dengue is the most rapidly spreading vector-borne viral disease and now a global health threat due to its presence in almost every tropical region and its alarming incidence increase within the last decade. Here, we have studied the temporal and regional epidemiological patterns of dengue in

Brazil in order to identify factors that were associated with higher numbers of hospitalizations from 1998 to 2013. We found that this number had increased in recent years, with a lower incidence in the southern part of the country. A marked seasonality was observed, with cases peaking during periods of the year in which mosquito abundance and activity were higher due to optimal levels of humidity and temperature. A novel contribution of our analyses is that the seasonality of dengue hospitalizations had a clear West-East gradient in Brazil. The analyses also revealed a higher proportion of children that were hospitalized due to dengue within the last 15 years, especially during strong outbreaks. These changes are likely to be a result of multiple factors, such as the accumulation of multitypic immunity in adults during the 20 years following re-introduction of dengue virus into Brazil in 1986 and thus the higher probability for children to be susceptible or monotypically immune, and the re-emergence of the more aggressive dengue virus strain DENV2 in 1990. Alternative explanations for the higher number of dengue outbreaks and hospitalizations and the higher proportion of children affected are fluctuations in serotype-specific transmission intensity, regional variations in circulating DENV serotypes, and the density of the vector population. Based on these results, we may speculate that the number of children hospitalized due to dengue is likely to increase in the southern part of the country within the next years. Our findings may allow health systems to improve control interventions and contribute to reducing dengue morbidity and mortality by using integrated vector control in conjunction with early diagnosis.

741

HYPOXIA ENHANCES ANTIBODY-DEPENDENT DENGUE

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Dengue virus (DENV) has been found to replicate in lymphoid organs, such as lymph nodes and spleen, as well as the liver in post mortem examination. These organs universally have significantly lower oxygen levels (lymphoid organs~0.5-4.5% O_2) compared to atmospheric air (~20% O_2) due to the vascular anatomy. How physiological oxygen levels in the organs where DENV replicates, affect DENV infection through hypoxia-induced changes in the immune response has, however, never been investigated. We report here that, compared to cells cultured at 20% O_2, infection of THP1 and primary monocytes at 3% O_2 (hypoxia) required 4-fold more antibody for complete neutralization. Furthermore, sub-neutralizing levels of antibodies produced 2-3 fold higher enhancement in DENV infection under hypoxic conditions. We show that these observations were mediated by the hypoxia-induced upregulation of FcγRIIA but not FcγRIIB expression. High-resolution microscopy shows that FcγRIIA directly mediates internalization of DENV immune complexes under hypoxic conditions. Mechanistically, the stabilization of hypoxia inducible factor (HIF1α) by hypoxia or chemically with desferrioxamine (DFX) under 20% O_2 conditions both upregulated FcγRIIA. However, DFX induced FcγRIIA expression only resulted in increased DENV immune complex attachment but not internalization into cells. In addition to the upregulation of FcγRIIA, a hypoxia driven but HIF1α independent increase in membrane ether lipid concentrations is required to synergistically increase internalization of DENV immune complexes for enhanced infection. Our findings thus indicate that the increased viral burden associated with secondary DENV infection is thus antibody-dependent but hypoxia-mediated and suggest a role for targeting hypoxia-induced factor for anti-dengue therapy.

742

VIREMIA AT PRESENTATION IS ASSOCIATED WITH LONG-LIVED ANTIBODY RESPONSES TO DENGUE VIRUS

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Infection with one of four related dengue virus (DENV) serotypes is thought to elicit life-long, serotype-specific immunity but only short-lived immunity to the remaining 3 serotypes. This theory has been derived from the study of typical, symptomatic DENV infections. In the current study, we evaluated the humoral immune response to DENV infections that were detected in febrile children clinically diagnosed with a non-dengue illness (referred to here as atypical cases). 2,892 acute-phase serum samples were tested for DENV by real-time RT-PCR (rRT-PCR), and viremia was quantitated using a serotype-specific DENV multiplex rRT-PCR. Samples were collected as part of an ongoing pediatric dengue cohort study in Managua, Nicaragua. As part of the study, patients had healthy annual serum samples tested using a specific Inhibition ELISA to detect total anti-DENV antibodies. These data were used as a measure of the long-term humoral immune response to DENV. 130 atypical cases tested positive for DENV, and 111 had Inhibition ELISA results from paired pre- and post-infection annual samples. 53 cases (47.8%) showed seroconversion or a >4-fold increase in titer in pre- vs post-infection samples (referred to as a positive Inhibition ELISA result), which was significantly lower than expected based on data from typical, symptomatic cases (79.7%; $p<0.01$). Viremia at presentation was significantly higher in atypical cases with positive Inhibition ELISA compared to cases with negative Inhibition ELISA [mean 7.6 (SD 1.5) vs 4.5 (SD 1.6) log10 copies/mL serum, respectively; $p<0.01$), and this remained significant in multivariable analysis ($p<0.01$). Atypical cases also appeared to alter the response to subsequent (secondary) typical, symptomatic cases. All 16 patients with two typical cases developed the expected rise in Inhibition ELISA titer following a second DENV infection (reciprocal titer ≥ 2560). However, in patients with an atypical primary case, only 1/8 patients developed the expected response ($p<0.01$). These data have important implications for understanding DENV immunology, estimating DENV incidence, and modeling virus transmission.

743

CHARACTERIZATION OF THE OVERWINTERING PROCESS OF JAPANESE ENCEPHALITIS VIRUS IN *CULEX* SPECIES MOSQUITOES

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Japanese encephalitis virus (JEV) is a mosquito-borne flavivirus endemic in the Asia-Pacific region and has been continuously regarded as a potential threat to human and veterinary public health in North America. Previously, we and others have identified potential competent vector species and amplification hosts of JEV in North America. These studies suggest that the establishment of enzootic transmission is highly likely in the event of its

introduction. A significant gap in determining the likelihood of establishing endemic transmission of JEV in North America is whether or not the virus can successfully overwinter in persistently infected animals. As observed with West Nile virus, successful overwintering allows the initiation of transmission in the spring and would ultimately lead to its establishment in North America. Whilst persistent infection of amplification hosts can serve as one mechanism for overwintering, persistently infected arthropods are also considered an important mechanism for overwintering. In this study, the overwintering process was investigated by maintaining orally infected American mosquitoes species at 16°C. Infectious virus was successfully recovered from infected mosquitoes demonstrating that JEV can lead to persistent infection at lower extrinsic temperatures. Therefore, we conclude that infection of arthropod vectors can serve as a potential overwintering mechanism for JEV in the event of its introduction in to the United States.

744

PROPOSED GUIDELINES FOR ADMINISTERING LIVE YELLOW FEVER VACCINE TO TRAVELERS

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The risk of acquiring yellow fever (YF) remains high in large parts of sub-Saharan Africa and South America. Travelers to endemic areas should expect that one in seven infected individuals will become symptomatic and 20 to 60% of symptomatic persons will succumb. Effective antivirals are not available. The principal control mechanisms involve mosquitoes, principally *Aedes aegypti* in the case of urban YF, and administration of the effective YF vaccine. The development of large urban slums in tropical areas facilitates the growth of *Ae. aegypti* in water filled containers making mosquito control difficult. In 2001 recognition of the rare possibility of vaccine-associated severe viscerotropic disease (YEL-AVD) with a case fatality rate of ~63% has made it necessary to consider the relative risks of acquiring YF and developing YEL-AVD. New guidelines for the administration of the live YF vaccine have been proposed. The guidelines begin with a risk assessment of prospective vaccinees in established groups associated with increased susceptibility to YEL-AVD: Males older than 55, women between the ages of 19 and 34 living in non-endemic areas of Peru, people of either gender older than 76, persons with a variety of autoimmune diseases and patients with thymomas. The risk assessment is then used in connection with an estimate of the risk of acquiring YF in the area of intended travel. The risk for a particular area is influenced by continent (the risk in Africa being substantially greater than the risk in S. America), current reports of YF activity and rainfall. In some instances in areas of S. America the risk of YEL-AVD may be equal or greater than the risk of YF. In situations in which the risks of both entities are high, the recommendation is don't travel. Although currently available information from official sources may be uninformative, judicious use of weather and map websites may be surprisingly helpful even for isolated rural communities in Africa and S. America. The decision to vaccinate with the live YF vaccine has become more complex, but the vaccine remains the most significant method for prevention of YF.

745

COMPREHENSIVE MUTAGENESIS OF HCV E1/E2 ENVELOPE TO EPITOPE MAP ANTI-ENV ANTIBODIES AND FUNCTIONAL RESIDUES CRITICAL FOR HCV INFECTIVITY

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To obtain epitope maps for anti-HCV Envelope (E1/E2) monoclonal antibodies (MAbs), we individually mutated 552 residues of HCV (H77 strain) E1/E2 to alanine. Each mutant was expressed in human cells and analyzed for its effects on MAb reactivity. This 'Shotgun Mutagenesis' approach offers the capability of mapping both linear and conformational epitopes, even for structurally complex proteins such as the oligomeric and glycosylated HCV Envelope protein. This approach identified critical amino acids required for the binding of dozens of MAbs, and has also been used to propose E2 disulfide bond cysteine pairs that are not resolved by the available E2 crystal structures. This approach has helped define the range of immunodominant structures on HCV E1/E2 and identify novel neutralizing antibody epitopes that can be used for the development of improved therapeutics, diagnostics, and vaccine candidates. In addition, to identify residues important for HCV infectivity we produced infectious HCV pseudoviruses from each mutant Env clone in the library. These pseudoviruses were used to evaluate each Env clone for infectivity on target cells. This allowed us to identify critical E1/E2 residues whose mutation eliminated HCV infectivity, identifying crucial HCV E1/E2 structural components that enable HCV infectivity.

746

ANALYZING THE IMMUNE RESPONSE TO ZIKA VIRUS: A REPORTER VIRUS PARTICLE (RVP) SYSTEM FOR SERUM AND ANTIBODY NEUTRALIZATION ASSAYS, AND A COMPREHENSIVE ALA-SCAN MUTATION LIBRARY OF ZIKV PRM/E TO EPITOPE MAP ANTI-ZIKV ANTIBODIES

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We have developed a reporter system, using pseudoinfectious reporter virus particles (RVPs), to facilitate analyses of the immune response to Zika virus (ZIKV) infection and anti-ZIKV vaccines. RVP-based assays provide a rapid reliable alternative to PRNT-based assays and are particularly suitable for high throughput screens of large panels of patient sera or isolated MAbs. ZIKV RVPs are replication-incompetent virus particles, antigenically equivalent to live viruses, containing the ZIKV prM and E envelope proteins, capsid protein, and a sub-genomic replicon encoding a reporter protein (luciferase or GFP). After infecting permissive cells, RVPs express their reporter protein, providing a convenient and reproducible quantitative assay for measuring the neutralizing capabilities of serum or individual monoclonal antibodies (MAbs). To further characterize the immune response to ZIKV infection, we have also developed a high-throughput strategy that enables the rapid identification of both linear and conformational antibody epitopes on ZIKV prM/E envelope proteins. We used Shotgun Mutagenesis technology to create a comprehensive library of 660 single mutations in ZIKV prM/E. The individual mutant expression plasmids were arrayed into 384 well plates and transfected into human cells to achieve native protein expression and folding. The immunoreactivity of MAbs to the prM/E variant in each individual well was quantified by high-throughput flow cytometry, enabling us to map a number of anti-ZIKV MAbs. The epitopes obtained are being correlated with MAb abilities to neutralize ZIKV *in vitro* and to protect against ZIKV infection *in vivo*.

747

EVALUATION OF SELECTED REAL-TIME RT-PCR PROTOCOLS AIMING AT THE BEST POSSIBLE MOLECULAR DIAGNOSIS OF ZIKA VIRUS INFECTIONS

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Zika is an emerging infectious disease resulting from Zika virus (ZIKV) infections and usually presenting itself by dengue-like symptoms. Clinical manifestations of Zika, dengue and chikungunya are somewhat similar, making it difficult to reach the diagnosis based only on clinical grounds. To make the diagnosis even more difficult, there is an intense cross-

reactivity between Zika and other flavivirus antibodies. Thus, the correct diagnosis of Zika is better achieved by molecular methods, but due to the short-lived and low titer viremia, molecular methods need to have a high analytic sensitivity. Real-time RT-PCR (rRT-PCR) is the best diagnostic approach available since it is possible to design protocols with no cross-reactivity with other flaviviruses. In this study, the analytic sensitivity of some published probe-based rRT-PCR protocols to detect ZIKV genome was evaluated. Ten-fold serial dilutions of ZikaSPH2015 strain, titrated in Vero cells, with a final virus concentration ranging from 1000000 to 1 virus/dilution was spiked in the serum of a flavivirus naïve healthy donor. ZIKV RNA from all dilutions, as well as from serum and urine samples obtained from patients, was extracted and amplified by rRT-PCR with five different pairs of primers (ZIKVA, B, C, D and E) and their respective probe. The analytic sensitivity of each rRT-PCR protocol was evaluated by the cycle threshold (Ct), where the lower the value the higher the sensitivity. Among all rRT-PCR protocols, ZIKVD and ZIKVC had the lowest and highest Ct values, respectively. Besides, ZIKVC detected only to 1000 copies/µL, while the other primers could detect virus in all dilutions. ZIKVD also had the best sensitivity when using patients' samples. ZIKVD rRT-PCR protocol consistently detected ZIKV genome in serum and urine samples while ZIKVA and ZIKVB were more reliable in urine, and ZIKVC and ZIKVE were usually negative in any sample. In conclusion, ZIKVD rRT-PCR is the best protocol to detect ZIKV genome in any sample. The presence of possible mismatches between the ZikaSPH2015 strain and the sequence used by Tappe et al to design ZIKVC primers might be the cause of its low sensitivity.

748

WHOLE GENOME SEQUENCING AND PHYLOGENETIC ANALYSIS OF ZIKA VIRUS ISOLATED IN INDONESIA

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Zika virus (ZIKV) was isolated from serum of a febrile patient in Jambi municipality, Sumatra, Indonesia during an outbreak of dengue-like illness in 2014. Although human ZIKV infections are normally associated with mild illness, WHO has declared the ZIKV outbreak a global public health emergency because of its strong association with Guillain-Barré syndrome and microcephaly. To understand the ZIKV evolution and its genetic characteristics, whole genome sequencing and phylogenetic analysis was performed on the ZIKV isolate (JMB-185). ZIKV RNA was extracted from cell culture supernatant and subjected to sequencing employing the Ion-Torrent Next-Generation Sequencing technology. The complete genome sequence of JMB-185 was assembled and aligned with all available ZIKV complete genome sequences retrieved from GenBank. To evaluate the evolutionary history of this isolate, we performed phylogenetic analysis using the Bayesian MCMC inference method. The molecular clock phylogeny analysis shows that the ZIKV JMB-185 strain, which shares common ancestry and time to the most recent common ancestor (TMRCA) with 2014 and 2013 Thailand strains around year 2008, has older lineage than the Polynesian and the Brazilian strains linked with microcephaly and is not closely related to those strains. The TMRCA result indicates that the ZIKV Jambi JMB-185 strain may have been in circulation in the South East Asia region, including Indonesia since 2008. We observed high nucleotide sequence identity and similarity between Indonesia, Thailand, and American strains. Further analysis including those of amino acid substitutions is in progress.

749

CLINICAL CHARACTERIZATION OF ACUTE ZIKA PATIENTS IN BRAZIL, VENEZUELA, AND EL SALVADOR IN 2015/16

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Latin America and the Caribean are currently facing an epidemic of three co-occuring arboviruses - Dengue, Chikungunya, and Zika. The multi-center observational IDAMS study is one of the few prospective studies capturing incident zika cases alongside with dengue and chikungunya. The study was initiated in 2011 and patients ≥ 5 years with undifferentiated febrile disease within the first 72 hours were enrolled in three sites in Brazil, Venezuela, and El Salvador. In 2015, 574 patients were recruited in total, among them 334 in Brazil (194 in Recife, 55 in Fortaleza, 85 in Rio de Janeiro), 72 in Venezuela, and 168 in El Salvador. The aims of the IDAMS study are to evaluate risk factors for severe dengue disease and validate the case definition for presumptive dengue in the absence of confirmatory laboratory testing. A broad range of clinical signs and symptoms and laboratory values are assessed daily during the acute illness, and at follow-up 1 week later. The study is currently ongoing and the inclusion criteria were adapted to presence of fever and/or rash in 2016. PCR for dengue , zika (and possibly chikungunya) viruses will be performed on all samples from 2015 and 2016, following strict protocols. Dengue is diagnosed by an algorithm including PCR, NS1, and IgM seroconversion. Preliminary results show a considerable proportion of zika-PCR-positive cases. We will describe the spectrum of clinical manifestations in zika patients and analyze clinical and laboratory parameters associated with zika vs. dengue at enrolment and over the course of the disease. We will carry out multivariable regression, stratified by age group, day of illness, and country, and assess the heterogeneity between the sites before pooling the data. Results to be presented will include patients recruited up to June 2016 and are expected to have considerable impact on the validation of the zika interim case definition issued by WHO.

750

DETECTION AND DIFFERENTIATION OF IGM RESPONSE TO RECENT EXPOSURE TO ZIKA AND OTHER VECTOR BORNE VIRUSES USING A MULTIPLEXED, BEAD BASED SEROLOGY ASSAY

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Since May 2015, South and Central America have experienced an epidemic outbreak of Zika Virus. Several travel related cases have already been identified in the United States. Zika infection during pregnancy has been implicated in the development of microcephaly in the developing fetus and is thought to be responsible for an increased incidence of Guillain-Barre syndrome. The expanding footprint of the disease, along with the devastating neurological effects associated with Zika Virus warrant a cost effective and specific diagnostic. In Zika infection, the detection of viral RNA in serum is transient and is only detectable for a few days post infection. Therefore, specific detection of IgM representing a recent infection from a vector borne virus as a companion to molecular detection is required in order to expand the diagnostic window. In areas

with co-circulating vector borne viruses, serological responses must not only be detected, but differentiated in order to provide accurate results. An effective test also must distinguish a recent infection from historical infections and vaccinated individuals. A serology based, multiplex microsphere assay was developed to detect and differentiate IgM response to Zika Virus, Chikungunya Virus, Dengue Virus (Serotypes 1-4), West Nile Virus, Yellow Fever Virus, Japanese Encephalitis Virus, Tick Borne Encephalitis Virus, St. Louis Encephalitis Virus, and Usustu Virus. Assay performance was established using clinical samples. The assay demonstrates required analytical specificity and is able to detect and differentiate each virus. The multiplex serological assay eliminates the need for sequential testing and complicated patient care algorithms. The multiplex format allows for simultaneous identification of IgM responses to a group of viruses that have historically been difficult to distinguish due to strong immunological cross reactivity and challenges with distinguishing old verses new infections.

751

MAPPING THE GLOBAL DISTRIBUTION OF YELLOW FEVER

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The spread and recent outbreaks of Zika, dengue, yellow fever and chikungunya viruses across the globe in 2015/16, highlight the need to reassess our understanding of these arboviruses, including their current distributions and potential for spread into new areas. Yellow fever is vaccine preventable, yet incomplete coverage means that it is widely distributed in the tropics of South America and Africa where infections cause an estimated 29,000 to 60,000 deaths annually. Epidemiologists have long been concerned about the introduction of yellow fever into large urban populations of naïve individuals where it can spread rapidly from human-to-human transmitted by *Aedes aegypti* mosquitoes. Earlier this year, the confirmation of yellow fever cases, imported from an outbreak in Angola, in areas of China with established vector populations, coupled with the depletion of vaccine stockpiles, raised concerns that yellow fever could gain an uncontrollable foothold in Asia. The disease has been conspicuously absent from Asia to date, despite multiple opportunities for introduction and the apparent presence of all components of a suitable transmission cycle, but no barriers to its introduction have been identified so vigilance is vital. We produced high resolution evidenced-based maps of the current distribution of yellow fever in Africa and the Americas. These maps were derived from a boosted regression trees model that used geo-positioned data on occurrences of yellow fever infection, environmental and socio-economic variables, as well as vaccination coverage data. The outputs were then used to predict areas of suitability for yellow fever transmission in South and South East Asia. This work furthers our understanding of the current global distribution of yellow fever and the potential for its spread in parts of Asia.

752

ACUTE ENCEPHALITIS SYNDROME IN ASSAM, INDIA: IMPORTANCE OF JAPANESE ENCEPHALITIS IN THE ADULT POPULATION, 2014-2015

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In India, thousands of cases of acute encephalitis syndrome (AES) are reported each year, predominantly among children. Japanese encephalitis virus (JEV) accounts for 13-18% of reported AES. From 2011-2015, Assam, a highly affected northeastern state, has reported over 1,000 AES cases annually. We conducted facility-based surveillance at Assam Medical College (AMC) hospital to characterize illness and evaluate potential etiologies of disease. Between Jan 2014-Dec 2015, serum and cerebrospinal fluid (CSF) samples were collected from each patient admitted to AMC with AES, defined as acute onset fever (>38°C) and ≥1 of altered mental status or seizures. Serum and CSF were tested for JEV IgM antibodies. A diagnosis of JEV was confirmed if JEV IgM was detected in CSF and probable if JEV IgM was detected in serum only. JEV-negative patients were evaluated for scrub typhus IgM, dengue virus IgM, and West Nile Virus (WNV) IgM in serum, and for bacterial/viral nucleic acids for *Streptococcus pneumoniae*, *Hemophilus influenzae*, and herpes simplex virus-1 (HSV) in CSF. Of 925 patients admitted with AES, 606 (66%) were male and 491 (53%) were ≥15 years old. CSF was collected from 772 (83%) patients and serum from 772 (83%). Among the 925, 329 (36%) were diagnosed with either confirmed (254, 28%) or probable (75, 8%) JEV. Of these 329, 174 (53%) were ≥15 years old, and 52 (16%) died. Among 596 JEV-negative patients, the following additional pathogens were detected in serum: scrub typhus IgM (67/485, 14%), dengue IgM (17/408, 4%). WNV IgM was not detected in any patient. CSF molecular testing in 254 patients indicated evidence of *S. pneumoniae* in 4 (2%) and *H. influenzae* in 2 (1%). Of 351 evaluated, HSV was detected in 6 (2%). JEV is the most common cause of AES in Assam, is associated with high mortality, and disproportionately affects adults, highlighting the need for ongoing adult JEV vaccination efforts in the region. The identification of additional treatable etiologies of illness, such as scrub typhus and pyogenic meningitis, has important clinical management implications, and underscores the need to employ a standardized laboratory testing algorithm for AES.

753

HOSPITALIZATIONS BY HEPATITIS C BETWEEN 1998 AND 2013 IN BRAZIL: AN INTRIGUING AMAZONIAN TALE

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Hepatitis C (HCV) infection is a public health problem of global dimensions, affecting 3% of the world' s population. In Brazil, between 2.5% and 4.9% of the population is infected with HCV. The Brazilian Amazon region has been reported to be hyper endemic for HCV and two other hepatotropic viruses (Hepatitis B and D). Here we explore the temporal patterns of incidence of hospitalizations attributed to HCV in Brazil per state and age group. We used hospitalization data from 1998 to 2013, from the nationwide administrative database of the Unified Health System (SUS). The records of hospitalizations coded as Hepatitis C were filtered using the codes B171 and B182 and aggregated in monthly time-series by state and age groups. Incidences were obtained using census-interpolated matrices. Visual inspection and spatio-temporal parameters were obtained using the analytical software epipoi. Between 1998 and 2013, 24210 patients were hospitalized with a principal diagnosis of HCV infection. As expected, the majority of new HCV cases (12038 patients) were recorded in Sao Paolo that has an estimated population of 44.39 million people. However, the incidence rate of HCV-related hospitalization was significantly higher in the region of the state of the Acre (mean incidence rate > 0.12 and linear trend < 16x10^-4) compared to all other Brazilian states. A total of 340 hepatitis C cases were recorded in the SUS database, the majority of which (186 cases) aged between 40 and 59 years. We detected worrying high incidence rates of HCV hospitalizations in the Western Brazilian Amazon Region. An increased prevalence of HCV among health care workers in Rio Branco located at the State of the Acre has been previously reported (Parana J *et al*, Am J Trop Med Hyg, 2007). If it is ruled out that the detection of HCV is exceptionally better in this region, then the infection control services in the hospitals of the State of

the Acre need to be urgently evaluated. Our findings indicate the need for the implementation epidemiological awareness, prevention and control programs for Hepatitis C in this region.

754

DESIGN AND VALIDATION OF A RAPID ASSAY FOR ZIKA VIRUS IN BIOFLUIDS AND INSECT VECTORS

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Understanding the dynamics of Zika virus transmission and formulating rational strategies for its control require diagnostic tools that are appropriate for resource-poor environments. We have developed a rapid and sensitive loop-mediated isothermal amplification (LAMP) assay that is highly specific for the Puerto Rican Zika virus isolate, PRVABC59, and related isolates in the Asian clade. The assay does not detect Senegalese or Ugandan Zika isolates, or dengue, yellow fever, West Nile, or chikungunya viruses. The conditions described for the PRVABC59-LAMP assay allow direct detection of virus in infected cells, mosquitos, serum and blood without reverse transcription or RNA isolation. Time to detection of a single infectious particle in blood is 60 minutes in laboratory or field settings. It offers rapid, specific, sensitive and inexpensive detection of the Zika viruses currently circulating in the western hemisphere. Results from this assay will be presented.

755

MAPPING GLOBAL SEASONAL SUITABILITY FOR ZIKA VIRUS TRANSMISSION

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Zika is a zoonotic mosquito-borne disease that became a global public health emergency in late 2015 when it spread throughout the Americas. While genetically similar to other globally distributed arboviruses such as dengue and chikungunya, Zika is of particular concern due to its link with neurological birth defects among infected pregnant mothers. Given this potential threat and the alarming rate of spread of the virus global predictions are urgently needed to assess where and when the virus may spread next with enough warning to deploy surveillance, control and diagnostic measures to protect those most at risk. Key insights into Zika seasonality can be gained through analysing the seasonal drivers of its principle mosquito vectors: *Ae. aegypti* and *Ae. albopictus* and their ability to transmit viruses. Here we combine habitat suitability analyses of these mosquito species with viral incubation period models to produce a series of global maps that depict when the arboviral season begins and ends around the world. This revealed broad areas of potential Zika transmission in temperate latitudes for long periods of the year, while also showing that the outbreak in some parts of South America may have been prematurely ended due to seasonal forcing instead of depletion of susceptible hosts. This could mean some populations in South America could potentially see a return of Zika sooner than previously estimated. The resulting predictions can be used for real time prediction of risk, better understanding past outbreaks and preparing appropriately for future Zika outbreaks.

756

WORKING WITH ZIKA AND USUTU VIRUSES *IN VITRO*

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Usutu (USUV) and Zika (ZIKV) viruses are emerging arboviruses of significant medical and veterinary importance. These viruses have not been studied as well as other medically important arboviruses such as West Nile (WNV), dengue (DENV), or chikungunya (CHIKV) viruses. As such, information regarding the behavior of ZIKV and USUV viruses in the laboratory is dated. Usutu virus re-emerged in Austria in 2001 and has since spread throughout the European and Asian continents causing significant mortality among birds. Zika virus has recently appeared in the Americas and has exhibited unique characteristics of pathogenesis, including birth defects and transmission. Information about the characteristics of USUV and ZIKA viruses are needed to better understand the transmission, dispersal, and adaptation of these viruses in new environments. Since their initial characterization in the middle of last century, technologies and reagents have been developed that could enhance our abilities to study these pathogens. Currently, standard laboratory methods for these viruses are limited to 2-3 cell lines and many assays take several days to generate meaningful data. The goal of this study was to characterize these viruses in cell culture to provide some basic parameters to further their study. Cell lines from 17 species were permissive to both ZIKA and USUV. These viruses were able to replicate to significant titers in most of the cell lines tested. Moreover, cytopathic effects were observed in 8 of the cell lines tested. These data indicate that a variety of cell lines can be used to study ZIKA and USUV and may provide an updated foundation for the study of host-pathogen interactions, model development, and the development of therapeutics.

757

PREDICTING INTENSITIES OF ZIKA INFECTION AND MICROCEPHALY USING TRANSMISSION INTENSITIES OF OTHER ARBOVIRUSES

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The World Health Organization has declared Zika Virus (ZIKV) a Public Health Emergency of International Concern due to the virus' emergence in multiple countries globally and the possible association of ZIKV with microcephaly and neurological disorders. There is a clear need to identify risk factors associated with ZIKV infection and microcephaly in order to target surveillance, testing and intervention efforts. Using data collected by surveillance systems, we modeled the correlation between transmission intensity of dengue and chikungunya, with reported microcephaly incidence in Brazilian states and ZIKV incidence in Colombia, as very few microcephaly cases have been reported up to now. We show that there is a strong correlation between the incidence of ZIKV in Colombian departments and the force of infection (but not the crude incidence) of dengue ($R2 = 0.41$, $p<0.001$). Furthermore, we show that there is also a strong correlation between the incidence of microcephaly in Brazilian states and the force of infection of dengue ($R2 = 0.48$, $p<0.001$). Because dengue and ZIKV are transmitted by the same vector, these associations provide further support to the supposition that ZIKV infection during pregnancy causes microcephaly, and raise questions about potential interactions between these two flaviviruses. In addition, they provide an opportunity to project the expected incidence of microcephaly in multiple dengue endemic locations across Colombia and the American continent. If the relationship between dengue FOI and microcephaly incidence seen in Brazil holds in Colombia, we should expect to see 387 cases (95%CI 166-621) of ZIKV associated microcephaly to be reported over the next 7 months. These results are being updated as the ZIKV epidemic progresses and will be expanded to include other countries in the

American continents where data on dengue and ZIKV is available. Detailed knowledge of dengue transmission should be used to target surveillance, testing and intervention efforts against ZIKV and other flaviviruses.

758

CORRELATION BETWEEN SABIN POLIOVIRUS SHEDDING AND SUBSEQUENT SEROCONVERSION IN INDIAN CHILDREN VACCINATED WITH MONOVALENT TYPE 3 ORAL POLIOVIRUS VACCINE (MOPV3)

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OPV (oral poliovirus vaccine) shedding in stool after vaccination indicates vaccine virus replication in the gut which results in an optimal immune response to OPV, as evidenced by few studies. The objective of the study was to evaluate the correlation between mOPV3 shedding on day 7 after vaccination and subsequent seroconversion in Indian children aged 6-11 months. A total of 300 infants aged 6-11 months who were seronegative to type 3 poliovirus (neutralizing antibody titre <8) and recruited from Vellore, India as part of a clinical trial evaluating the effect of azithromycin on the immunogenicity of mOPV3 given to healthy infants aged 6-11 months, were included in the study (CTRI/2014/05/004588). Serum samples were collected after 21 days of vaccination with mOPV3 to evaluate seroconversion (antibody titre ≥8). Neutralization test was performed on the serum samples according to the WHO protocol to determine antibody titres against serotype 3 poliovirus. Stool samples were collected 7 days after vaccination to determine Sabin 3 shedding using quantitative real-time polymerase chain reaction (PCR). A Ct value of 40 was used as a cut-off value for positive samples. 160 infants (53.3%) were found to shed Sabin 3 poliovirus on day 7 after vaccination while 140 infants (47.7%) did not shed. Of the 160 infants who shed vaccine virus, 136 infants (85%) seroconverted while 24 infants (15%) did not seroconvert. Of the 140 infants who did not shed vaccine virus, only 14 (10%) seroconverted in contrast to 126 infants (90%) who remained seronegative. Shedding of Sabin poliovirus and seroconversion were strongly correlated (Fisher's <0.001). To conclude, our study found a significant association between mOPV3 shedding on day 7 and subsequent seroconversion (after 21 days of vaccination) in Indian children.

759

CHIKUNGUNYA, ZIKA, AND DENGUE IN CALI, COLOMBIA: PRELIMINARY RESULTS OF EPIDEMIOLOGICAL AND GEOSPATIAL ANALYSES

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Vector born disease control is expensive, time consuming, and difficult to achieve. Here, we propose a new method of describing environmental risks in order to more efficiently control *Aedes* born disease in Santiago de Cali, Colombia, an area which is endemic for dengue and chikungunya and currently controlling an outbreak of zika. Geospatial video narratives are a new method of capturing street level data for environmental risks with expert and community based opinion. These data, consisting of local expert interviews and video, are coded and analyzed in a laboratory and compared across space and time as GIS map-layers. Epidemiological analyses are being conducted including geospatial components (geographically weighted regressions, kernel density analysis, hotspot analysis) in addition to traditional incidence data measures. Preliminary dengue and chikungunya data from October 2014 - April, 2016, obtained from the local ministry of health database, SIVIGILA, suggest two primary hotspots (areas with higher than expected case data) which are related to local environmental risks (open sewers, homeless population, lack of trash and sanitation services). Local partners are being engaged to provide expert opinion and guide the work in the neighborhoods most in need. We expect to present the final results of the analysis to community partners and the secretary of health of Santiago de Cali as they continue to control these neglected tropical diseases affecting the most vulnerable populations of the city. New data is currently being cleaned and analyzed and final results are expected by July, 2016.

760

A TRIAL TO ASSESS THE THERMOTOLERANCE OF AN INACTIVATED RABIES VACCINE

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Mass dog vaccination is the most efficient way to eliminate human rabies. This study provides the first robust data that the immunogenicity (a surrogate of protection) of an inactivated rabies vaccine (Nobivac® Rabies), stored at temperatures in excess of recommended conditions, is not inferior to that of the vaccine stored in cold-chain conditions. A non-inferiority study was carried out comparing the serological response at 4 weeks post vaccination in dogs inoculated with vaccine stored at elevated temperatures for different durations, with dogs vaccinated with the product stored according to label recommendations. The results showed that the effectiveness of the vaccine at stimulating antibody was not inferior to cold-chain stored vaccine when it was stored for up to 6 months at 25°C or for 3 months at 30°C. Despite being unlikely to result in changes to the authorized storage conditions of this product, the development of thermotolerant vaccines will increase delivery options. For example vaccines could be stored in remote communities with no electricity, thus allowing dogs to be vaccinated throughout the year rather than annually when campaigns pass through. As such, puppies born after a campaign could be vaccinated in a timely manner, reducing the rate at which the inter-campaign vaccination coverage decreases. This will be useful where the 70% coverage target, required for local elimination of the virus from the canine reservoir host, has not been reached. Thermotolerant vaccines stored in remote areas will also provide a human life-saving resource in emergency outbreak situations where rapid vaccination of the dog population is required to control the epidemic. We have not confirmed a 3-yr duration of immunity for the high temperature stored vaccine, however annual re-vaccination is usually practiced for all dogs presented during vaccination campaigns in Africa and Asia. As such this should not be a cause for concern. Given the recent tripartite (WHO, OIE, FAO) commitment to eliminating canine-mediated human rabies by 2030, these results are extremely timely.

761

RISK FACTORS FOR ANTIBODY LOSS AFTER HEPATITIS E VIRUS NATURAL INFECTION OR VACCINATION: RESULTS OF A MULTI-SITE COHORT STUDY

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Hepatitis E virus (HEV) is a vaccine-preventable emerging infection causing 20 million infections in developing countries per year. In South East

Asia HEV causes yearly outbreaks, with the majority of disease seen in adults. This is unexpected for an enteric pathogen, suggesting that HEV antibody persistence after exposure is not long-lasting. We revisited 170 subjects with a documented HEV infection from Bangladesh and China and 97 subjects vaccinated with the HEV239 vaccine during a phase III trial in China to retest their serum for anti-HEV antibodies 6 to 10 years after exposure. Overall, 22.1% (95%CI: 17.3-27.6%) no longer had detectable antibodies at follow-up. Antibody loss was greater among the naturally infected subjects compared to the vaccinated subjects, 24.1% (95%CI: 17.9-31.3%) versus 18.6% (95%CI: 11.4-27.7%), although not statistically significant (p=0.292). Among all the subjects, age at exposure was associated with antibody loss, with younger age increasing the risk of antibody loss (RR: 0.87 per 10 years, 95% CI: 0.76-1.00, p=0.057). Among the subjects from Bangladesh, each 10 year increase in age at infection decreased the risk of antibody loss by 50% across univariate and multivariate Poisson regression models (p<0.05). This age-dependent antibody loss could partially explain the wide body of cross-sectional seroprevalence data from SE Asia where a paucity of pediatric infections has been observed. In multivariate models, factors that increased the risk of HEV were generally associated with antibody persistence among the naturally infected subjects, suggesting repeated exposures over time contribute to antibody persistence. This pattern was not found in the vaccinated subjects. This is the first study to compare long term antibody persistence after HEV exposure in naturally infected and vaccinated individuals, exploring host characteristics. The development of a successful, subunit vaccine has increased the need to understand the duration of antibodies and protection after HEV infection and vaccination in order to implement the most cost effective disease control and vaccination strategies.

762

ANTIBODIES TO EBOLA IN INTERNATIONAL RESPONDERS TO THE WEST AFRICA EBOLA EPIDEMIC

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The 2014/5 Ebola (EVD) epidemic in West Africa (WA) resulted in a large international humanitarian and research response. Health care workers (HCWs) from WA were disproportionately affected, and a few international HCWs were infected. Since asymptomatic and pauci-symptomatic EVD has been described, we tested international returnees for EVD antibodies. An online consent and survey link were distributed using a snowball technique. Eligibility criteria included never having tested positive for Ebola virus, and not ever having received a filovirus vaccine. Oral fluid collection devices were posted to participants and returned using standard mail. Samples will be analysed using an IgG capture ELISA. Non-exposed controls in the UK will also be tested. Data are stored on a secure database, and analysed using STATA 14. A total of 270 individuals who travelled to WA during the 2014/5 Ebola epidemic completed an online survey. Of these, 155 (57.4%) were women. The majority (253, 93.7%) travelled to Sierra Leone; 13 (4.8%) to Liberia and 14 (1.5%) to Guinea. Roles included, but were not limited to; laboratory (95, 35.2%), clinical (71, 26.3%), epidemiologist/research (23, 8.5%), community engagement/burial (14, 5.2%) and water/sanitation/engineer (4, 1.5%). A total of 139 (51.5%) returnees spent time in the red zone, of whom 21 (7.8%) described direct physical contact with suspected/confirmed EVD patients or their children outside of the red zone, and 99 (36.7%), direct physical contact with survivors. A total of 56 (20.7%) experienced a febrile illness in WA, or within a month of return. Twenty-five (9.3%) had a negative PCR test for Ebola. Of 236 individuals who wore personal protective equipment (PPE), 22 (9.3%) had concerns regarding Ebola exposure during removal. Serological results will be available. A high proportion of international responders reported potential exposure outside of PPE, concerns about exposure when removing PPE, and reported a febrile illness during the incubation period for Ebola. Improvements in training and procedures, and consistency in PPE equipment and removal may mitigate some of this perceived risk.

763

MEN'S HEALTH SCREENING PROGRAM: EBOLA VIRUS DISEASE (EVD) SURVIVOR SEMEN TESTING PRELIMINARY FINDINGS — LIBERIA, 2015-2016

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Ebola virus (EBOV) RNA has been detected in semen of EVD survivors > 9 months after disease onset. However, information regarding duration and risk factors for sustained viral persistence in semen is limited. Liberia's Men's Health Screening Program, created to prevent EBOV sexual transmission, offers counseling and semen testing for EBOV RNA by RT-PCR. Males ≥15 years of age with proof of EVD survivorship (e.g., discharge certificate from an Ebola Treatment Unit) were enrolled in a study launched in July 2015. Participants are provided semen testing for EBOV RNA by RT-PCR; counseling on safe sexual practices; condoms with instructions on use; and referrals for healthcare services as needed or requested. In accordance with WHO guidelines, participants are eligible to graduate from the program after receiving two consecutive RT-PCR negative semen tests; with samples collected at least 2 weeks apart. As of February 2016, RT-PCR results were available for 307 participants. In total, 35 (11%) participants had at least one RT-PCR positive semen test. Of these, 21 (7%) participants were ≥ 12 months from ETU discharge at the time the RT-PCR positive semen sample was collected. The longest time after ETU discharge to collection of a semen sample that tested positive for EBOV RNA was 523 days. The median age of participants who had a RT-PCR positive test at program enrollment was significantly older than those who never had a RT-PCR positive test (p=.0031). Excluding participants who enrolled within 90 days from ETU discharge, men aged ≥35 years were more likely to have at least one semen sample test RT-PCR positive compared to men aged <35 years (p=.0024). Frequency of sexual intercourse was not associated with age (p=0.2208). We found persistence of EBOV RNA in semen up to 523 days from ETU discharge, far exceeding previous reports. Sustained viral RNA persistence in semen appears to be associated with older age, an association not previously reported. As frequency of sexual intercourse was not associated with age, differences in viral RNA persistence may be related to other age-related factors such as changes in semen composition or immune function.

764

HEPATITIS E INFECTION IN ARGENTINA, FROM IMMUNOCOMPETENT TO IMMUNOCOMPROMISED

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Hepatitis E virus (HEV) is an RNA virus that can cause hepatitis in an epidemic fashion. In immunocompetent individuals, infection with HEV

usually leads to silent seroconversion or to acute self-limited disease. In immunosuppressed individuals, HEV can develop into a chronic infection. Information about prevalence of HEV in immunocopromised subjects outside of Europe or North America is scarce. In this study we addressed the seroprevalence of HEV in immunocompetent and immunocompromised subjects in Argentina and associated risk factors. We performed third generation enzyme immunoassay for determination of IgG and IgM specific antibodies against HEV in 204 subjects infected with HIV, 81 subjects on hemodialysis (HD) and 58 solid-organ transplant recipients. HEV PCR was performed in all samples. Subjects on HD and transplant recipients were evaluated regarding social habits and potential risk factors. Results were compared to 433 HIV-negative, immunocompetent controls from our center. In our entire HIV-positive group we found 15 of 204 samples to be positive for HEV IgG (7.3%), compared to 19 of 433 samples (4.4%) in the control group. Interestingly, we found significantly lower CD4 counts on HEV-positive samples compared to HEV-negative samples (average CD4 count of 234 vs 422 mm^3, p=0.01) indicating that patients with lower CD4 counts were more likely to be HEV IgG positive. Eight out of 81 subjects (9.8%) on HD and 5 of 58 (8.6%) of transplant recipients were positive for HEV IgG. Half of HEV seropositive patients in the HD group had positive IgM for HEV. There was no association between HEV serostatus and consumption of pork, alcohol, potable water or history of blood transfusion. There was a weak, but significant, association between fish consumption and HEV positivity. Only 1 sample showed a positive PCR for HEV, within the HIV group. In conclusion, our study found an increased seroprevalence of HEV IgG in subjects infected with HIV, on HD and solid-organ transplant recipients in Argentina. However, the only significant difference compared to controls was on HIV-infected patients with low CD4 counts.

765

CHARACTERISTICS AND OUTCOMES OF PEDIATRIC PATIENTS WITH EBOLA VIRUS DISEASE (EVD) ADMITTED TO TREATMENT UNITS IN LIBERIA AND SIERRA LEONE: A RETROSPECTIVE COHORT STUDY

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This retrospective cohort study describes the clinical characteristics and outcomes of children <18 years with PCR-confirmed EVD among patients evaluated for suspected EVD in West Africa. Demographic, epidemiological and clinical data were collected systematically from patients with documented EVD admitted to five Ebola Treatment Units (ETU) in Liberia and Sierra Leone during 2014/2015. Standardized care was provided to all patients based on International Medical Corps protocols. The pediatric cohort was described in aggregate and stratified by age group. We analyzed associations between mortality and patient characteristics. Among the 128 pediatric cases admitted, 122 had PCR-confirmed EVD. Incidence in females (56%) and males (44%) was similar. The overall mortality rate was 57%. Stratified by age, mortality was 89% for <5 years, 43% for 5-9 years, 41% for 10-14 years, and 25% for 14-17 years (p<0.001). Mortality for children aged <5 years was significantly higher than children 5-17 years old (89% vs 38%, p<0.001). At triage, prominent features included fever (79%), anorexia (68%), and weakness (64%). Throughout the duration of illness, the most frequent features were weakness (92%), fever (85%), anorexia and diarrhea (both 80%). Of those presenting without fever (n=26), 73% developed fever after triage. Of those patients without fever throughout hospitalization, 3 of 6 (50%) died. Hemorrhagic features were present in 5% at triage and 45% anytime during admission. The mortality of patients that developed bleeding at any time during admission was significantly higher (58% vs 27%, p=0.002). The median length of stay was 9 days (range 1-31 days). Length of stay was significantly higher for those that survived compared to those that died (16 vs 6 days, p=0.001). In summary, pediatric EVD patients aged <5 years had significantly higher mortality. One out of every five children presented without fever. Prominent features of EVD in children included constitutional and gastrointestinal signs and symptoms. Hemorrhagic features developed in less than half of pediatric patients, but were associated with significantly higher mortality.

766

SALIVA FOR DETECTION OF ANTIBODIES FOR MEASLES, MUMPS AND RUBELLA TO CONFIRM VACCINE STATUS IN TEENAGERS

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Vaccines had resulted in the fall of prevalence of several childhood viral infections in the past decades, also affecting boosting exposure to environmental viruses. Previous concepts of lifelong immunity induced by live virus vaccine have been abandoned due to several outbreaks occurring in vaccinated young adults, obliging revaccination specially for preventing congenital rubella. Serology has been considered the main approach to measure individual protection but demands low adherence blood collection. Saliva could be an alternative for the detection of *Toxoplasma gondii* specific antibodies, due to IgG exuded from crevicular liquid. Saliva collection is noninvasive, simple and inexpensive, with high adherence for children and other protected groups. In this work, we show the development of ELISA for detection of specific IgG against measles, mumps and rubella virus, using a protein A IgG capture and biotinylated recombinant antigens as probes. Anti T.gondii IgG detection was used as control. Samples were collected in public high schools, during an exposition of transmissible diseases, with voluntary collection after parental approval. Vaccination was identified by individual vaccine files. Negative samples were obtained from discarded sera from Pediatric Center Lab, from children below vaccine age. All samples were tested on 384 wells plates, adsorbed with Protein A (10mg/ml), reacted with saliva IgG 10 x concentrated by clearing and ethanol precipitation. Commercial recombinant antigens from measles, mumps, rubella and *T. gondii* extracts were biotinylated and allowed to react to bound IgG, followed by avidin peroxidase and TMB reaction. All the assays were efficient for distinction of reactive sera, without false negative or false positive results as compared to unvaccinated young children sera. These tests could be easily transformed in high throughput assays, allowing the determination of individual vaccine status with consequent preventive measure as revaccination. Saliva IgG will enable vaccine control without the need for blood collection.

767

PRESENTING SYMPTOMS AND CHARACTERISTICS OF PATIENTS WITH EBOLA AND MALARIA ADMITTED TO EBOLA TREATMENT CENTERS IN SIERRA LEONE

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Discriminating between Ebola Virus Disease (EVD) and malaria is difficult when clinical testing is not immediately available. This retrospective study describes characteristics of patients admitted to Ebola Treatment Units (ETU) in Sierra Leone during the recent epidemic in West Africa with variable states of EVD and malarial infections. Data was collected from three International Medical Corps ETUs in which all patients received standardized care including Artesunate combination treatment (ACT). The population was stratified by infection status as malaria negative/EVD negative (m-/e-), malaria positive/EVD negative (m+/e-), malaria negative/EVD positive (m-/e+) and malaria positive/EVD positive (m+/e+). These groups were analyzed for inter-group variation in characteristics, symptomatology and outcomes. Among 1548 admitted patients, 753 patients had diagnostic test results available for EVD and malaria. There were 431 (57.2%) m-/e-, 180 (23.9%) m+/e-, 108 (14.4%) m-/e+,

and 34 (4.5%) m+/e+ analyzed in the cohort. Patients diagnosed with malaria had significantly younger median ages at 23 and 20 years for the m+/e- and m+/e+ respectively versus 32 years among both the m-/e- and m-/e- groups (p<0.001). Females accounted for a larger proportion of EVD cases than males (42% m-/e-, 42% m+/e-, 65% m-/e+ and 68% m+/e+; p<0.001). Patients were significantly more likely to have abdominal pain if they had malaria rather than EVD (49% m+/e-, 36% m-/e+; p=0.043). Presence of anorexia, diarrhea, bone pain, vomiting, cephalgia, dyspnea and abnormal bleeding were not significantly different between m-/e+ and m+/e- groups. Distinguishing between EBV and malaria before rapid diagnostic testing is available presents a difficult diagnostic challenge. Significant overlap exists in the clinical presentation of patients with variable EVD and malarial infection statuses. This may justify the continued use of ACT in an EBV epidemic. Better understanding of such characteristics among variably infected patients may enhance development of response protocols and care in future EVD epidemics.

768

CHARACTERIZATION OF SEVERE FEVER WITH *THROMBOCYTOPENIA* SYNDROME VIRUSES (SFTSV) FROM PATIENTS IN KOREA, 2015

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Introduction Severe fever with thrombocytopenia syndrome (SFTS) is emerging infectious disease characterized by acute febrile, thrombocytopenia and gastrointestinal symptoms. It is caused by SFTS virus (SFTSV), in the genus of Phlebovirus (family Bunyaviridae). Since the first reported case of SFTS in South Korea in 2013, we collected serum samples from hospitalized patients who experienced symptoms of SFTS. The major clinical symptoms and laboratory parameters of SFTS are fever, thrombocytopenia, leukopenia, and elevated serum hepatic enzymes, and SFTS patients usually die due to multiple organ failure. SFTSV was presumably transmitted by ticks, because it has been detected in Haemaphysalis longicornis ticks. Methods and Materials Total RNA extracted from serum was amplified with one-step reverse-transcriptase polymerase chain reaction (RT-PCR), designed to detect a portion of the viral N and Gc protein gene using specific primers for S or M segment. After analyzing aligned nucleotide sequences, we constructed the phylogenic tree based on partial S or M segment sequences. We tried to isolate viruses from patient by infection VeroE6 cells with the sera. Results We conducted RT-PCR with total RNA which is extracted from the patient sera. Among the 833 samples, seventy-nine samples are resulted in positive. The nucleotide sequences were assembled by the SeqMan program implemented in DNASTAR software (version 5.06; Madison, WI, USA) to determine the consensus sequences. Nucleotide sequence of the Korean strains showed 93 to 98 % homology to Chinese and Japanese strains. We also isolated 45 SFTSVs among the virus-detected 79 samples. Conclusion We examined the clinical specimen from the suspected case of SFTS in Korea. We detected 79 SFTSVs of 833 patient sera by RT-PCR, and isolated 45 viruses among them. Nucleotide sequences of positive samples were not only included in SFTSV by the phylogenic analysis but also formed the Korean strain group.

769

EFFICACY AND SAFETY OF A LOW-COST, HEAT-STABLE ORAL ROTAVIRUS VACCINE AGAINST SEVERE ROTAVIRUS GASTROENTERITIS IN NIGER

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Prevention of rotavirus disease through vaccination is a public health priority, and in 2009, the World Health Organization recommended rotavirus vaccination be introduced in all countries to reduce disease burden and mortality among young children. Two live oral attenuated rotavirus vaccines are globally licensed and WHO prequalified for the prevention of rotavirus gastroenteritis. Safety and efficacy of these vaccines has been established in high- and middle-income countries, but vaccination in sub-Saharan Africa, where there is the largest burden of rotavirus-related mortality, presents certain logistical and financial challenges. BRV-PV is a low-cost and heat-stable rotavirus vaccine manufactured by the Serum Institute of India, Limited whose introduction may help minimize the burden on already-strained national immunization programs throughout sub-Saharan Africa. We conducted a double-blind, placebo-controlled randomized phase III event-driven trial in Niger to assess the efficacy and safety of BRV-PV against severe rotavirus gastroenteritis in infants in Niger. Infants were randomized in a 1:1 ratio to receive three doses of BRV-PRV or placebo at approximately 6, 10, and 14 weeks of age. Facility and home-based surveillance is being conducted from 28 days post Dose 3 (gastroenteritis) and from the moment the first dose (serious adverse events) until 2 years of age. As an event-driven trial, the primary efficacy analysis was planned when 117 cases of severe rotavirus gastroenteritis are confirmed. Vaccine efficacy against severe rotavirus gastroenteritis and risk of serious adverse events, including hospitalization, intussusception and death will be presented. Evidence supporting the efficacy and safety of BRV-PV vaccine in an African setting would support the pre-qualification of and increased access to rotavirus vaccine across Africa.

770

CROSS-REACTIVE ANTIBODIES INFLUENCE IMMUNOGENICITY OF LIVE ATTENUATED VACCINES

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Epidemic viral diseases have become increasingly prevalent, as evidenced by the spread of ebola and zika. Among the different anti-viral strategies, vaccination, particularly with live attenuated vaccines (LAV), has seen exceptional success. However, due to previous exposure to pathogens and vaccines during the lifetime of an individual, many would have pre-existing cross-reactive antibodies that can potentially affect efficacy of live-attenuated vaccines (LAVs). To understand how cross-reactive antibodies affect LAVs, we designed a randomized trial design where subjects were subjected to Japanese encephalitis (JE) inactivated vaccine followed by the LAV yellow fever (YF) vaccine. We observed that YF vaccine immunogenicity is affected by the levels of cross-reactive JE antibodies. Transcriptional profiling of the patients highlighted that semaphorins and T-cell related cytokines were significantly correlated with YF immunogenicity. Further laboratory studies reveal that semaphorins that are expressed in Fc-receptor bearing antigen-presenting cells were directly upregulated by activating Fc-receptor signaling. That semaphorins have crucial roles in antigen presentation and T-cell responses suggest that cross-reactive antibodies can be exploited to improve LAV efficacy.

771

VERTICAL TRANSMISSION OF CYTOMEGALOVIRUS IN A RURAL MOZAMBICAN HOSPITAL

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Congenital cytomegalovirus (CMV) infection is the most prevalent congenital infection worldwide, varying the prevalence from 0.2% to 2%. This prevalence is higher in developing countries and among HIV infected newborns although it is decreasing among exposed but not infected

infants, following the increasing use of antiretroviral therapy for prevention of mother-to-child transmission (MTCT). We aimed to assess CMV vertical transmission prevalence and risk factors associated among newborns born in a rural Mozambican maternity. A cross sectional study was conducted on pregnant mothers attending Manhiça District Hospital at delivery. Blood cord samples in filter paper and placenta biopsy were collected for CMV detection measure by PCR. CMV seroprevalence and HIV status were also investigated in recruited pregnant women. One hundred and twenty mothers were recruited at delivery, mean age was 25,1 (±7,6) years and mean of gestational age at recruitment was 38,8 (±0,6) weeks. Prevalence of HIV infection among them was 27.5% (33/120) and only 28.3% were taking antiretroviral to prevent MTCT. One hundred and twenty three pregnancy outcomes were delivered. 5.8% (7/120) were premature, 1.7% (2/120) were stillbirths, 2.5% (3/120) born with malformations (2 with polydactyl and one with spine bifida) and 12.5% (15/20) had low birth weight. Data of three newborns were missing. CMV PCR was positive in 3 of 116 cord samples collected (2.6%) and only one child was exposed but not infected to HIV. 100% of them born asymptomatic at birth and at 6 months follow-up. Risk factors associated to vertical transmission of CMV were not found. We will present results of placental biopsies and maternal seroprevalence. Our results showed a higher prevalence of congenital CMV than studies in developed countries but lower than reports from low-income countries. Although it is an important cause of hearing loss completely neglected, it would be premature to consider newborn CMV screening in resource-poor settings because the disease burden from congenital CMV and the cost/benefit ratio of long term follow-up have not been defined.

772

IDENTIFICATION AND FUNCTIONAL CHARACTERIZATION OF MATING-DELIVERED MALE SEMINAL FLUID PROTEINS IN THE MALARIA VECTOR *AEDES*

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Anopheles gambiae mosquitoes are one of the primary vectors of human malaria, which represents a major public health burden globally. Reproductive success in these mosquitoes relies on a single mating, therefore characterizing their reproductive biology could offer a promising opportunity to interfere with their life cycle. *An. gambiae* male mosquitoes produce a complex cocktail of seminal fluids in their accessory glands (MAGs), including proteins (Acps), and lipids that are packed in a gelatinous mating plug and transferred to females during copulation. Female receipt of this plug induces large physiological and behavioral changes including refractoriness to further insemination, egg laying and activation of sperm storage mechanisms. We recently demonstrated that the steroid hormone 20-hydroxyecdysone (20E) in part mediates these change in females. However the identity and function of seminal fluid proteins in regulating the female post mating response remains largely unknown. We employed an *in vivo* stable isotope labeling technique coupled to mass spectrometry to identify male proteins transferred to females during mating. Female mosquitoes were labeled via feeding yeast containing stable isotope ^{15}N, which masked the female proteome, allowing identification of male specific proteins. First, the proteomic composition of unlabeled male reproductive tissues (MAGs and testes) was determined, which facilitated the spatio-temporal localization of male proteins within 5 female tissues (atrium, spermatheca, ovaries, hemolymph and head) at three time points after mating (3h, 12h and 24h). Notably we detected a total of 180 unique male proteins transferred to females, including 45 novel Acps. Male proteins transferred to the spermatheca maybe essential in coordinating sperm viability, and strikingly a MAG specific protein of unknown function was detected at all time points after mating in this tissue, suggestive of a stable, long term association with sperm. Ongoing functional knock-out analysis of this and other candidates are revealing the fundamental role of male transferred proteins in female reproductive success.

773

FBN30 IS A PATHOGEN RECOGNITION RECEPTOR AGAINST *PLASMODIUM* INFECTION IN *ANOPHELES GAMBIAE*

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Malaria is a worldwide health problem that affects two thirds of the world population. Invasion of *Plasmodium* through anopheline mosquitoes is an obligatory step for malaria transmission. Through genome-wide association studies, we identified significant association between genetic variations in FBN30 and the *P. falciparum* parasite infection in *Anopheles gambiae* mosquito populations from malaria endemic areas in Kenya. FBN30 sequence analysis shows that the fibrinogen-like domain (FBG) at the C-terminal is conserved across a range of mosquito species. In this study, we hypothesize that FBN30 works as a pathogen recognition receptor (PRR) in the defense against *Plasmodium* infection. Firstly, we expressed FBN30 in insect High Five cells and studied its biochemical features. The results show that insect cell expressed FBN30 is a secreted protein which can form a dimer through disulfide bond between two subunits which indicate four disulfide-bond linked homodimers to form a tetramer by non-covalent bond. In addition, we determined the cysteines, which are involved in the intra-chain and inter-chain disulfide bridge respectively. Secondly, we evaluated the expression efficiency of two FBN30 variants (FBN30(C/C) and FBN30 (T/T)) in mosquito cells, Moss55 and Sau5B. The results support that wild type mosquitoes in Kenya, with the genotype of FBN30(C/C), were more susceptible to *Plasmodium* infection than those with the genotype of FBN30 (T/T). The susceptibility is attributed to a lesser expression of FBN30 observed in mosquitoes with the non-synonymous mutation, which results in Phe10Leu in the signal peptide. Also we determined that FBN30 in *An. gambiae* only exists in hemolymph. Finally, ELISA and indirect immunofluorescence assay proved that FBN30 proteins bind to asexual and sexual stage of both *P. berghei* and wild type of *P. falciparum*. Based on all these data, we concluded that FBN30 is a PRR molecule in the mosquito innate immune system, which is critical for *An. gambiae* defense against *Plasmodium* infection.

774

IDENTIFICATION OF GLYCOSAMINOGLYCANS IN *ANOPHELES NEIVAI* AND *A. ALBIMANUS AND* ITS ROLE IN MALARIA TRANSMISSION

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Despite the advances in research related to malaria it is accepted and clear that interventions to limit transmission by the vector are not enough, therefore the search for new strategies for malaria control is necessary. The sexual cycle of *Plasmodium* or sporogonic cycle occurs in mosquitoes of the genus *Anopheles*. It has been reported that malaria transmission is due to glycosaminoglycans (GAGs) which are present in the epithelium of the midgut and salivary glands of the mosquito and its interactions with lectins in the parasite, it is thought that the presence of such molecules is necessary to promote maturation from ookinete to oocysts. Although some GAGs such as chondroitin sulfate and heparan sulfate have been identified in experimental models of interaction between host-parasite,

the real participation of those GAGs in malaria transmission is unknown. Therefore *An. albimanus* and *An. neivai* from the department of Chocó-Colombia has been taken as a natural model with the purpose of identify the GAGs present in its tissues and to establish its relationship in the transmission of malaria. Adult mosquitoes were collected and larvarian forms were cultured in the laboratory. All adult forms were used for identification by classical taxonomy and confirmed by BarCode technique. The midgut tissue of two sets of malaria vectors were obtained and were used for GAGs analysis by mass spectrometry. We will discuss about some subtle differences in the GAGs profiles between different species of *Anopheles*. This fact could explain the ability to permit or inhibit the *Plasmodium* parasites maturation and transmission by vectors. This knowledge will help to find out new strategies for blocking the transmission cycle.

775

DISCOVERING THE BINDING PARTNER(S) OF MOSQUITO MIDGUT FREP1 IN *PLASMODIUM* PARASITES

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We previously identified a mosquito midgut protein FREP1 in peritrophic matrix that can facilitate *Plasmodium* infection in *Anopheles gambiae* mosquitoes through binding to gametocytes and ookinetes. We proposed a FREP1-mediated parasites invasion model. This project aims to identify the parasite-expressed FREP1-binding partners (FBPs). Incubating insect cell-expressed recombinant FREP1 with *P. berghei* infected cell lysates pulled down several specific bands by anti-FREP1 antibodies. One of the bands was identified by mass spectrometry to be a 27 kDa, PEXEL motif-containing protein. This protein also has a trans-membrane domain. Insect cell-expressed this candidate protein confirmed its interaction with FREP1. The FBPs discovered in this report will improve our understanding of the molecular mechanism of FREP1-mediated *Plasmodium* invasion pathway, which can be targeted by novel approaches for malaria control.

776

NEW USES FOR AN OLD TECHNOLOGY TO CONTROL ZIKA VECTORS IN URBAN TROPICAL ENVIRONMENTS

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The recent epidemic of Zika virus transmission in the Southern Hemisphere has brought heightened awareness for the control of its vector *Aedes aegypti*. Dumping, removing or treating every water-holding container, especially in large urban neighborhoods, maybe impractical. The dense clouds of insecticide produced by the "old" thermal fog technology may now be advantageous for penetrating cryptic habitats. Compatible aqueous mosquito adulticide formulations for use in thermal foggers are commercially available that replace the dense fog with a fine mist. Therefore, we speculated "Could currently available aqueous larvicide formulations be applied by a thermal fogger and remain efficacious in cryptic larval habitats for the control of Zika vectors?" We initiated semi-field studies to determine penetration distance and associated *Ae. aegypti* larval mortality in a forest canopy (cryptic) environment using *Bacillus thuringiensis* var *israelensis* (*Bti*) (Vectobac WDG, AI 37.5%). Vectobac was applied at the maximum label rate with a hand held IGEBA TF 34 thermal fogger (fitted with water conversion kit) to empty 0.5-L clear plastic containers placed at 7, 14, 21, and 28m from application source at Camp Blanding, FL. Treated containers were returned to the laboratory where dechlorinated water and late second to early third instar *Ae. aegypti* were added to each container. The greatest amount of mortality (91-100%) occurred at 7m. We then operationally applied the *Bti* product, at maximum label rate, to a 0.2 ha urban tropical urban environment located in Key West, FL using the same larval evaluation bioassays above. Containers were placed randomly in cryptic areas within the area. Vectobac provided 99.9% larval mortality at 24h and 100% at 48h. Operationally, we found that thermal fog technology can be an effective tool for control of larval *Ae. aegypti* in cryptic tropical urban environments.

777

INFLUENCE OF BLOOD MEAL ON SUSCEPTIBILITY TO PYRETHROIDS IN *ANOPHELES GAMBIAE* FROM WESTERN KENYA

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Vector control is based on the use of insecticides. To ensure sustainable vector control we need to understand the other factors driving insecticide resistance and consequently threatening the sustainability of malaria vector control programs targeting the indoor resting mosquitoes. The aim of this study was to determine the influence of blood meal status on pyrethroid tolerance in field collected population of *Anopheles gambiae* from western Kenya. Field-collected mosquito larvae were reared to adulthood alongside the laboratory susceptible reference Kisumu strain. Female adults from the two populations were monitored for deltamethrin resistance using WHO bioassays at different gonotrophic stages. Metabolic assays were then performed to assay the level of detoxification enzymes. The WHO bioassay results showed increased resistance on younger female's (2-5 days old) field collected population with different gonotrophic status (mortality ranged from 36-83%). Older females (14-15 days) from the same population with different gonotrophic status showed reduced resistance to the same insecticide (85-98%). Biochemical estimations on younger females (2-5 days old) revealed significantly ($P<0.05$) higher levels of oxidase, non-specific esterase and glutathione-s-transferases activity in the blood fed and half gravid survivors *of An. gambiae* as compared to unfed survived individuals. For older females (14-15 days) of the same population, blood fed and half gravid survivors showed significantly higher oxidase and glutathione-s-transferases activity as compare to unfed and gravid survivors. Kisumu susceptible population showed 100% susceptibility with no significant elevation in enzyme activities following a blood meal. These results indicate that blood feeding status plays an important role in the toxicity of deltamethrin due to some physiological changes following feeding that confers increased tolerance to mosquitos.

778

NEW MATRIX-RELEASE FORMULATION, SUMILARV®2MR CONTAINING PYRIPROXYFEN FOR LONG LASTING CONTROL OF *AEDES AEGYPTI* LARVAE

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Aedes aegypti is a vector of human viral diseases, such as dengue, Zika, chikungunya, and yellow fever. Indoor and outdoor water storage containers are the main breeding habitats for this species in Southeast Asia, Central and South America, and also in some savannah areas of Africa. A new long lasting "matrix-release" formulation, SumiLarv®2MR,

containing 2% pyriproxyfen has been developed to control container-breeding *Ae. aegypti*. Pyriproxyfen is an insect growth regulator with a very low mammalian toxicity that inhibits the emergence of adult mosquitoes. Pyriproxyfen is recommended for use in drinking water by the World Health Organization (WHO). The key feature of SumiLarv®2MR is the controlled slow release of pyriproxyfen so that an effective concentration of active ingredient is maintained in treated water for at least six months after treatment. A simulated-field evaluation using plastic vessels containing 40 L water showed that the efficacy of SumiLarv®2MR lasted for at least 36 weeks, irrespective of the frequency of water replacement (half or full water replacement every week). A field trial conducted over a year in a rural village in Lao PDR where *Ae. aegypti* breeds throughout the year, with SumiLarv®2MR applied every 6 months to domestic water storage containers, resulted in a significant reduction in larval density. The long-lasting efficacy of SumiLarv®2MR demonstrated in these trials will reduce the number of treatments required per year and will therefore enable significant reductions in operational costs. These results are promising for the future long term control of *Ae. aegypti* and the diseases that this insect transmits. SumiLarv® is a registered trademark of Sumitomo Chemical Company Ltd.

779

INHIBITION OF ADULT EMERGENCE OF *AEDES AEGYPTI* USING A CONTROLLED RELEASE FORMULATION OF PYRIPROXYFEN (SUMILARV® 2MR) OVER SIX MONTHS IN CAMBODIA: A CLUSTER RANDOMIZED TRIAL

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Dengue is one of the most rapidly spreading mosquito-borne viral diseases in the world, and without a cure or widely available effective vaccine the best measures to control dengue are through vector control and the avoidance of mosquito bites. Pyriproxyfen (PPF) is a juvenile hormone analogue that interferes with the metamorphosis of juvenile mosquitoes and due to its favorable mammalian toxicity profile is ideal for use in vector control activities. A new slow-release PPF matrix release formulation (Sumilarv® 2MR) was developed and is recommended for six months (considerably longer than alternatives which reduces operational costs). While small scale field studies have been conducted, this is the first large scale field evaluation, where site-specific processes may modify the duration of residual effects relative to controlled experiments. Such large scale field trials therefore measure effectiveness, rather than efficacy, and are of strategic importance to control programs. A cluster randomized, controlled superiority trial was developed to assess the effectiveness of Sumilarv® 2MR. The trial based in Kampong Cham, Cambodia includes 96 sentinel containers from 20 clusters and runs from November 2015 to May 2016. The clusters were randomly assigned to an intervention or control arm. Within randomly selected households the small water jars most commonly used were selected as sentinel containers. Due to the large amount of water replacement the results likely indicate the lowest inhibition values in the household. The primary outcome was the percentage inhibition of emergence in monthly 250 mL water samples. Each lab assay was conducted with 25 lab reared third instar larvae. The beakers were monitored for larvae and pupal mortality and adult emergence once daily for eleven consecutive days or until the last adults emerged or all remaining pupae died. The amount of water replacement in water jars was also recorded to adjust the IE results from sentinel containers. The results can be used by the control programs to assess the long term efficacy and cost effectiveness of Sumilarv® 2MR under operational conditions.

780

STUDIES ON KNOCKDOWN RESISTANCE (KDR) MUTATIONS IN *AEDES AEGYPTI* AND *AEDES ALBOPICTUS* IN INDIA

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Knockdown resistance (kdr) is one of the mechanisms of insecticide resistance in insects caused by the reduced target site sensitivity i.e. voltage gated sodium channel (VGSC) rendering it less sensitive to DDT and pyrethroids. We evaluated insecticide susceptibility and its underlying kdr mechanism in eight *Aedes aegypti* and five *Ae. albopictus* field populations from India. Field population were collected from four different geographical regions of India viz. North, South, East and Central covering 18 districts of ten states. Adult bioassays revealed varying levels of resistance to DDT, permethrin and deltamethrin in all *Ae. aegypti* populations and susceptibility to pyrethroids in *Ae. albopictus* populations tested. Molecular screening for common kdr mutations, revealed the presence of five mutations viz. S989P, V1016G, T1520I, F1534C/L. Three PCR based assays; DNA sequencing, ASPCR, PCR-RFLP were used for genotyping of twelve global kdr alleles. Two novel mutations were observed, first at T1520 (ACC) residue where a C>T substitution at the second position of codon results in amino acid change to Isoleucine (ATC). Second mutation was an alternative point mutation at F1534 (TTC) residue where a substitution of T>C at the first position of codon results in an amino acid change to Leucine (CTC). No kdr mutation was observed in any field population of *Ae. albopictus*. ASPCRs were not accurate so three PCR-RFLP assays were developed. Representative samples of all genotypes were sequenced to validate the newly developed PCR based assays for *Ae. aegypti*. DNA sequencing data were in agreement with the genotyping results. Genotyping results showed that 989P is linked to 1016G and novel mutation 1520I was always found with 1534C allele. Present study confirmed the presence of DDT and pyrethroid resistance among *Ae. aegypti* populations in India and for the first time reported kdr mutations in this species from India including two novel mutations. Results of present study lead us to infer that, at least five kdr mutations (S989P, V1016G, T1530I, F1534C and F1534L) can be seen as a potential marker for DDT/pyrethroid resistance.

781

EVALUATING THE POTENTIAL OF REUSING LARVAL REARING WATER IN SUPPORT FOR THE STERILE INSECT TECHNIQUE (SIT) OR OTHER MASS PRODUCTION PROGRAM: EFFECT ON DEVELOPMENT AND QUALITY OF *ANOPHELES ARABIENSIS* MOSQUITOES

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The success of a mosquito mass rearing operation for sterile insect technique (SIT) or other release application relies on a reliable supply of water of sufficient quality for optimal larval development. An estimated 250 litres of water is required to raise 200,000 eggs in one larval rearing rack. Yet, many arid and/or seasonally arid countries face the difficulties of acute water shortage, deterioration of water quality, and environmental constraints. The reuse of water to rear successive generations of larvae is attractive as a way to reduce water usage and running costs, and help to make this control method viable. Therefore, we have initiated work at the IPCL to assess whether dirty water is a suitable rearing medium that could replace the clean dechlorinated water that is currently routinely used. Results indicated that reusing dirty water or using a 50:50 mix of clean and dirty water did not affect egg hatching. Moreover, no difference was found in time to pupation, larval mortality or sex ratio when first-

instar larvae were added to clean water, dirty water, or a 75:25, 50:50 or 25:75 mix of clean and dirty water and reared until emergence. When late-instar larvae were put back into their own rearing water, there was no effect on pupation rate, emergence rate or female longevity, though male longevity was reduced. When reared from first-instar larvae, however, dirty water decreased pupation rate, emergence rate, body size, and adult longevity. However no response variable differed significantly between recycled water (reverse osmosis and ultrafiltration) and clean water. This suggests that recycling dirty water necessarily restore overall performance or mosquito quality. Re-used larval-rearing water has no impact on egg hatching, development time or mortality of the immature stages of *Anopheles arabiensis*. However, dirty water is not suitable for the production of high quality adult mosquitoes. Recycling processes improve water quality and increase insect quality. These findings may have important implications for the implementation of the SIT in areas where clean water is a scarce or costly resource.

782

DISTRIBUTION AND FREQUENCY OF INSECTICIDE RESISTANCE IN *ANOPHELES GAMBIAE* S.L. POPULATION IN LIBERIA

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Liberia's malaria vector control program relies on insecticide-based interventions and may be compromised by the emergence, intensity, and spread of insecticide resistance among malaria vectors in the country. The distribution and frequency of *Anopheles gambiae* s.l. resistance to five insecticides were examined in six counties from June to December 2015. Three of the six counties included in this survey (Bong, Grand Bassa, Margibi) conducted indoor residual spraying (IRS) from 2009 to 2013, while in the other three counties (Nimba, Gbarpolu, Grand Gedeh), IRS was not done. Non-blood-fed, 3 to 5-day old, female mosquitoes reared from field-collected larvae were exposed to WHO insecticide impregnated and control papers. Four classes of insecticides were tested against *An. gambiae* s.l.: pyrethroid (deltamethrin 0.05% and alpha-cypermethrin 0.05%), carbamate (bendiocarb 0.1%), organophosphate (pirimiphos-methyl 0.1%), and organochlorine (DDT 4%). About 100 female *An. gambiae* s.l. were tested. Mortality was recorded after the 24-hour holding period. *An. gambaie* s.l. populations from all six sites were fully susceptible to pirimiphos-methyl (100% mortality rate). Full susceptibility to bendiocarb was observed only in Grand Gedeh. Probable resistance to bendiocarb was detected in four sites (Bong, Nimba, Margibi, and Grand Bassa), with 97%, 94%, 97%, and 94% mortality rates, respectively, while resistance to bendiocarb was detected in Gbarpolu (89% mortality). Tested mosquitoes were resistant to deltamethrin (22%-73% mortality) and to alpha-cypermethrin (5%-47% mortality) in all sites. The 24-hour mortality for DDT tested in five of the six sites was 2%-49%. Comparison of susceptibility of *An. gambiae* s.l. populations between counties with and without IRS did not show a significant difference ($p > 0.05$). Additional assays are needed over time to map the rest of Liberia and track insecticide resistance as interventions are scaled up.

783

IMAGING AND QUANTITATIVE MICROANALYSIS OF PYRETHROID INSECTICIDES ON THE SURFACE AND INTERIOR REGIONS OF LLIN FIBERS USING TIME-OF-FLIGHT SECONDARY ION MASS SPECTROMETRY

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Quantitative chemical mapping of permethrin and deltamethrin on the surface of and within polyethylene long-lasting insecticidal net (LLIN) fibers was carried out using time-of-flight secondary ion mass spectrometry (ToF-SIMS). This method uses a highly focused (0.3μm) Bi3+ primary beam to sputter the fiber surface, and ionic molecular fragments (secondary ions) from the top 1-2 monolayers of the fiber are extracted into a mass analyzer for chemical identification. By rastering the primary beam, a two-dimensional chemical map can be generated for a larger (e.g., 500μm^2) sampling area at submicron resolution. Subsurface regions were exposed for analysis by using a Cs+ beam to remove overlying material, thereby permitting three-dimensional mapping. Calibration standards for quantitative analysis were prepared by implanting known quantities of Cl- and Br- ions in polyethylene films and LLIN fibers. Limits of detection were determined to be 0.051 and 0.0088 weight percent for permethrin and deltamethrin, respectively. Both insecticides were observed as discrete deposits on fiber surfaces in as-received samples. The regeneration process was directly observed in samples that were washed and incubated, and the regenerated insecticide was more diffusely distributed on the surface than in the as-received samples. For permethrin-containing fibers, discrete high-concentration domains of insecticide were found within the fibers suggesting that they are supersaturated with insecticide at ambient temperatures. This method shows great potential for measuring the portion of bioavailable insecticide on LLIN fibers and directly observing the insecticide regeneration dynamics in "insecticide-incorporated" LLINs. It is also potentially applicable to the microanalysis of other active ingredients being considered for use in LLINs, such as piperonyl butoxide (PBO).

784

ANOPHELES ALBIMANUS MICROBIOTA AND LINKS TO INSECTICIDE RESISTANCE: A SHOTGUN METAGENOMIC SEQUENCING APPROACH

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Understanding the factors that contribute to insecticide resistance is needed to mitigate its threat to vector control, particularly in Latin America, where vector control is being intensified as part of regional malaria elimination strategies. Next Generation sequencing has been applied to investigate the role of mosquito microbiota in various mosquito behaviors and functions. We report here for the first time the results of shotgun metagenomic DNA sequencing to characterize mosquito microbiota in relation to insecticide resistance phenotypes. Following evidence of a link between insecticide resistance and insecticide detoxifying endosymbionts such as *Burkholderia sp.* in stinkbugs, we hypothesized that the mosquito microbiota may contribute to insecticide resistance. *Anopheles albimanus* from northern Peru were sequenced using the Illumina MiSeq platform. Resulting data were quality checked using PRINSEQ and Trimmomatic quality control tools, and taxonomic composition analysis was performed using GOTTCHA, Kraken and MG-RAST. Similar to other mosquito species, our data showed that *An. albimanus* microbiota was dominated by a single taxon: Proteobacteria at the phylum level, and *Enterobacter* at the genus level. Up to 50 bacterial genera, including *Burkholderia sp.*, constituted the remaining microbiota.

Our ongoing analyses will further identify the core microbiota and their functions in insecticide resistant and susceptible strains of *An. albimanus* from Peru, Guatemala, and Mexico.

785

A CLUSTER RANDOMIZED TRIAL TO COMPARE BENDIOCARB AND DELTAMETHRIN FOR INDOOR RESIDUAL SPRAYING ON BIOKO ISLAND, EQUATORIAL GUINEA

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Indoor residual spraying (IRS) - spraying the interior walls of houses with insecticide - has been used on Bioko for malaria control since 2004. The insecticide bendiocarb was used biannually from 2005 until 2012; and during this period prevalence of malaria and under 5 mortality dropped substantially. To reduce costs, biannual IRS with bendiocarb was replaced by an annual round of IRS with a long lasting formulation of deltamethrin in 2013, based on reported susceptibility of local vectors to both insecticides. A marked increase in malaria prevalence in children 2 to 14 years in the same year prompted a cluster randomised trial to be carried out to compare the effectiveness of bendiocarb and deltamethrin for IRS on Bioko and to investigate whether the rise in malaria prevalence was related to the change in insecticide. Twenty four clusters of houses were randomly allocated to receive IRS with either bendiocarb or deltamethrin in 2014. Approximately three months after the intervention, prevalence of malaria infection and levels of haemoglobin were measured in children aged 2 to 14 in each cluster. Prevalence of infection was lower in the bendiocarb arm (16.8%, 95% CI 11.1 - 24.7, N = 1374) than in the deltamethrin arm (23.2%, 95% CI 16.0 - 32.3, N = 1330) but this difference was not significant ($p = 0.390$), even after adjusting for confounders ($p = 0.119$). Mean haemoglobin was significantly higher in the bendiocarb clusters (11.6g/dl, 95% CI 11.5 - 11.8, N = 1326) than in the deltamethrin clusters (11.5g/dl, 95% CI 11.3 - 11.7, N = 1329), ($p = 0.049$ after adjusting for confounders). The results of this study are somewhat inconclusive by themselves, but they suggest that, on Bioko, bendiocarb may offer better protection against malaria infection than deltamethrin. Subsequent data on phenotypic and metabolic resistance to pyrethroids of local vectors would suggest that pyrethroid resistance renders deltamethrin IRS ineffective for malaria control on Bioko.

786

INSECTICIDE SUSCEPTIBILITY LEVELS OF *ANOPHELES GAMBIAE* S.L MOSQUITOES IN AWKA, NIGERIA

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Long-lasting insecticide nets (LLINs) and indoor residual spraying (IRS) are the main methods used for malaria vector control. However the success of these methods has been hampered by the development and spread of insecticide resistance in major malaria vectors. The emergence of insecticide resistance in *Anopheles* mosquitoes in Nigeria has enormous implications for vector control in the country. This study was therefore carried out to investigate the susceptibility status of *An. gambiae* s.l mosquitoes to the four main classes of insecticides used for vector control in Amansea community, Awka North LGA, Anambra State, Southeast Nigeria. Larval mosquitoes were collected from different breeding sites and reared in the insectary. Mosquitoes were identified morphologically and two to five day old adult female mosquitoes were used to conduct WHO susceptibility assays. Susceptibility assays were carried out against pyrethroids (0.75% permethrin and 0.05% deltamethrin), organochlorine (4% DDT), organophosphate (0.25% pirimiphos-methyl) and carbamates (0.1% bendiocarb and 0.1% propoxur) insecticides. All mosquitoes collected were identified as members of the *Anopheles gambiae* s.l. Susceptibility assays showed that the mosquitoes were completely susceptible to bendiocarb (100% mortality). Percentage mortality recorded for the other insecticides were as follows: DDT (1.3%), pirimiphos-methyl (15.6%), permethrin (26.3%), deltamethrin (38.8%) and propoxur (87.5%) respectively. The KDT_{50} recorded were bendiocarb (36.7 minutes), propoxur (39.8 minutes), deltamethrin (50.9 minutes), permethrin (91.4 minutes), Pirimiphos-Methyl (116.3 minutes) and DDT (119.1 minutes). On the other hand, the KDT_{95} recorded were bendiocarb (52.3 minutes), propoxur (62.3 minutes), deltamethrin (85.6 minutes), permethrin (171.1 minutes), Pirimiphos-Methyl (185.3 minutes) and DDT (170.1 minutes). The results show that there is very high frequency of insecticide resistance in the study area and calls for prompt implementation of resistance management practices in the area.

787

THE EFFECT OF IVERMECTIN ON THE AMAZONIAN MALARIA VECTOR *ANOPHELES DARLINGI*: LC_{50} DETERMINATION

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Current malaria vector control measures target mainly endophagic *Anopheles* vectors; yet malaria transmission in South America is dominated by exophagic and exophilic vectors, indicating the need to evaluate novel control tools effective against such vectors. Ivermectin mass drug administration has been shown to be lethal to wild *Anopheles* in West Africa and has the potential to target exophagic and exophilic vectors. Although lethal to numerous *Anopheles* vectors, there is no information on the effects of ivermectin on South American vectors. Here, we evaluated the impact of ivermectin on the dominant Amazonian malaria vector, *An. darlingi* through *in vitro* experiments. We estimated the lethal concentration of ivermectin that kills 50% (LC_{50}), 25% (LC_{25}) and 5% (LC_5) of mosquitoes in membrane feeding experiments. Varying concentrations of ivermectin were blood fed to 3-5 day old, laboratory-reared (F_{23}-F_{29}) *An. darlingi* mosquitoes; survivorship was monitored and recorded daily for 7 days post blood meal. Ten replicates with four to ten ivermectin concentrations per replicate, including a control, were tested across a range of 4 – 1000 ng/ml. The LC_{50}, LC_{25}, and LC_5 of ivermectin fed to *An. darlingi* was calculated as LC_{50} = 36.4 ng/ml [29.7, 42.7], LC_{25} =20.7 ng/ml [14.1, 26.1], and LC_5 = 9.2 ng/ml [4.5, 13.7] (n = 4333, 10 replicates). These LC_{50} values demonstrate that *An. darlingi* is susceptible to ivermectin concentrations found in humans post oral drug administration. Future *in vitro* experiments will determine if ivermectin inhibits the ability of *An. darlingi* to re-feed as was demonstrated in *An. gambiae* previously, and investigate whether ivermectin inhibits the development of *P. vivax* in *An. darlingi* as has been shown with *P. falciparum* in *An. gambiae* and more recently with *P. vivax* in *An. dirus*. *In vitro* ivermectin mosquito-lethal effects against *An. darlingi* would suggest that ivermectin mass drug administration could be a novel, powerful malaria vector control tool in Peru and other Amazonian countries.

788

USING STABLE ISOTOPES OF CARBON AND NITROGEN TO MARK WILD POPULATIONS OF *ANOPHELES* AND *AEDES* MOSQUITOES IN SOUTHEASTERN TANZANIA

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Marking wild mosquitoes is important for understanding their ecology, behaviours and role in disease transmission. Traditional insect marking techniques include using dyes, biological agents and tags but such techniques have various limitations like low marker retention and inability to mark wild mosquitoes at source. Stable isotopes are gaining wide spread use for non-invasive marking of arthropods, permitting greater understanding of mosquito dispersal and responses to interventions. We describe here a simple technique for marking naturally-breeding malaria and dengue vectors using stable isotopes of nitrogen (15N) and carbon (13C), and describe potential field applications. We created man-made aquatic mosquito habitats and added either 15N-labelled potassium nitrate or 13C-labelled glucose, leaving non-adulterated habitats as controls. We then allowed wild mosquitoes to lay eggs in these habitats and monitored their development in situ. Pupae were collected promptly as they appeared and kept in netting cages. Emergent adults (in pools of ~4 mosquitoes/pool) and individually stored pupae were desiccated and analysed using Isotope Ratio Mass Spectrometry (IRMS).*Anopheles gambiae* s.l and *Aedes* spp. from enriched 13C and enriched 15N larval habitats had significantly higher isotopic levels than controls (P=0.005), and both isotopes produced sufficient distinction between marked and unmarked mosquitoes. Mean δ15N for enriched females and males were 275.64±65.12 and 247.95±54.55, while mean δ15N in controls were 2.1±0.1 and 3.9±1.7 respectively. Similarly, mean δ13C for enriched females and males were 36.08±5.28 and 38.5±6.86, compared to -4.3±0.2 and -7.9±3.6 in controls respectively. In all cases, there were variations in standardized isotopic ratios between mosquito species. Enrichment of semi-natural mosquito larval habitats with stable isotopes of nitrogen and carbon resulted in effective marking of *Anopheles* and *Aedes* mosquitoes colonizing these habitats. This approach can significantly enhance studies on mosquito eco-physiology, dispersal, pathogen transmission and responses to control measures.

789

THE ROLE OF IMMUNE PATHWAYS IN *WOLBACHIA*-MEDIATED BLOCKING OF DENGUE VIRUS IN *AEDES AEGYPTI*

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The bacterial endosymbiont *Wolbachia pipientis* has been shown to protect its host against many pathogens, including dengue virus (DENV). DENV's main vector, *Aedes aegypti*, is not a natural carrier of the bacterium but a stable transinfection was achieved some years ago using the *Wolbachia* strain *w*Mel. Due to the protection conferred by *Wolbachia*, the symbiont is being developed as a means of vector control to reduce disease incidence long-term. Although, *Wolbachia*-induced priming of the immune system has been suggested as a mechanism conferring pathogen protection its role is poorly understood. Here we attempted to determine the individual contribution of several insect immune pathways to *Wolbachia's* protective ability. Using RNAi techniques, knockdown of genes representing each of the five major insect immune pathways (TOLL, Imd, Autophagy, JAK/STAT and RNAi) was achieved in *A. aegypti* cells (+- *w*Mel). Simultaneous knockdown of more than one gene was also performed to assess for multifactorial effects. Cells were challenged with a DENV-2 strain after the manipulation of host immune gene expression and DENV copy number was evaluated 5 days post-infection. We found that *w*Mel based blocking was dependent on 2 of the pathways; JAK/STAT and RNAi pathways. Both pathways are known to be involved in the antiviral response and are known to be manipulated by dengue virus infection. A weaker interaction was found between *Wolbachia* and the Toll and Autophagy, but not Imd pathways. Our results suggest that both the JAK/STAT and RNAi pathways are the main immune contributors to *Wolbachia*-mediated DENV blocking, since suppression of its effectors lead to a dramatic increase in the dengue virus titre.

790

COMPARISON OF MORPHOLOGICAL AND MOLECULAR IDENTIFICATION OF OUTDOOR ANOPHELINE MOSQUITO SPECIES IN AN AREA TARGETED FOR ELIMINATION IN SOUTHERN ZAMBIA

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Malaria was once a leading cause of morbidity and mortality in Macha, Choma District, Southern Zambia. However, in the past decade malaria incidence has declined and elimination strategies are now being rolled out. In conjunction with this decline, few numbers of the primary local malaria vector, *Anopheles arabiensis* caught indoors are still present in the vector population. Malaria cases are reported at health facilities with severe cases and deaths recently rising at Macha Hospital. To investigate the contribution that other anopheline species that forage outdoors make to malaria transmission, collections from homesteads within the catchment area of Macha Hospital were conducted. Standard morphology in conjunction with molecular tools were used for identification of mosquitoes caught between February 2015 and April 2015 from UV traps set outdoors next to animal enclosures. A total number of 1283 mosquitoes were collected of which 650 (50.7%) were anophelines. Morphological identifications revealed seven different species. Following DNA extractions of the abdomen, PCR was employed which amplified the intergenic-spacer -2 region of the nuclear rDNA. Of the 640 samples successfully analyzed, 11 different anopheline species were identified molecularly with the majority (53.8%) being *An. squamosus*. Morphological identification of specimens accurately identified 85% of *An. squamosus*, and 62% of the second been *An. arabiensis*. As malaria control targets and impacts the major malaria vectors in Zambia, surveillance of previously understudied species is important as they begin to constitute a larger proportion of potential vector collections. The use of molecular-based techniques for the identification of anopheline mosquitoes is vital to confirm identities, assign behaviors to particular species and accurately determine each species contribution to malaria transmission.

791

IS *ANOPHELES PALUDIS* A VECTOR OF MALARIA IN THE DEMOCRATIC REPUBLIC OF CONGO?

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Anopheles paludis was the most common *Anopheles* mosquito collected in human landing collections in Lodja, central DRC, during sentinel site collections in 2013 and 2014. *Anopheles paludis* had been previously collected in the neighboring province of Bandundu with sporozoite rates of 6%. To see if *An. paludis* was acting as a vector in Lodja, monthly human landing catches and pyrethrum spray catches were conducted over the course of 2015. Mosquitoes were identified to species and *Anopheles* mosquitoes were tested for presence of circumsporozoite

protein using ELISA. *An. paludis* was the most common I mosquito collected in 2015. *An. gambiae* s.l. and *An. funestus* were also collected. *An. paludis* displayed an early biting peak, with the highest numbers collected between 1900 and 2000h. None of 1366 *An. paludis* females tested positive for sporozoites. Despite high densities of anthropophilic *An. paludis* present in Lodja, it does not appear to be an important vector of malaria. Further work is needed to understand whether there are differences between *An. paludis* populations in Lodja and Bandundu.

792

DOMINANT ROLE OF *ANOPHELES FUNESTUS* GILES, IN A RESIDUAL TRANSMISSION SETTING IN SOUTHEASTERN TANZANIA

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Malaria is transmitted by more than one anopheline mosquito species, but the roles of these species vary in different epidemiological settings. We assessed the role of the two dominant *Anopheles* mosquito species in selected villages in Ulanga and Kilombero districts in south-eastern Tanzania. Monthly mosquito sampling was done in randomly selected households in three villages using CDC light traps, back pack aspirators and HLC between January -2015 and January-2016. Multiplex polymerase chain reaction (PCR) was used to identify members of the *An. funestus* group mosquitoes and *An. gambiae* s.l, to species level. Enzyme-linked immunosorbent assay (ELISA) was done to detect *Plasmodium* sporozoites in the mosquito salivary glands, and to identify sources of mosquito blood meals. The geographical distribution of infected *An. funestus* and *An. arabiensis* mosquitoes was determined by ArcGIS 10 (ESRI-USA) software. A total of 22,391 *An. arabiensis* and 4,802 *An. funestus* were collected. Among the *An. funestus* group mosquitoes, *An. funestus* s.s. predominated (76.6%), An. rivulorum (2.9%) and An. leesoni (7.1%) and unamplified samples (13.4%). About 86% of all infected mosquitoes were *An. funestus* s.s. while 14% were *An. arabiensis*. Overall, *An. funestus* group contributed to 93.4% and *An. arabiensis* contributed to 5.6% respectively of the annual EIR. In the *An. funestus* group, *An. funestus* s.s contributed to 96% of the transmission while An. rivulorum contributed 4%. Mosquito blood meal sources included: humans, 79.2%, bovine, 17.1%, dog, 2.4% and chicken, 1.2%. Infected *An. funestus* were distributed across the study area while infected *An. arabiensis* were confined to small part in the middle of study area. Though *An. arabiensis* are still the most prevalent of the vector species in the study area, ongoing residual malaria transmission could is predominantly mediated by *An. funestus*. The evidence from this study demonstrates that *An. funestus* have a significant role as the main driver of malaria transmission in these study villages. *An. funestus* could be responsible for resurging malaria transmission in communities where LLINs are widely distributed.

793

COMPARATIVE FLAVIVIRUS SUSCEPTIBILITY AMONG *AEDES AEGYPTI* STRAINS UNDER LABORATORY AND SIMULATED FIELD CONDITIONS

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The mosquito *Aedes aegypti* is the primary global vector of dengue virus and Zika virus. Previous investigations have demonstrated variable levels of virus susceptibility, and subsequently vector competence, due to genetic variability between *Aedes aegypti* strains. However, previous studies uniformly employed optimal laboratory mosquito rearing conditions that do not reflect the effects of environmental variability encountered by larvae under natural conditions. Our previous studies have shown significant differences in body size between field vs laboratory reared individuals of the same genetic background. Here, we characterized and compared differences in susceptibility of three *A. aegypti* laboratory strains and a recent *A. aegypti* field isolate from Trinidad to dengue virus (DENV2 JAM1409) and two isolates of Zika virus (Zika CAM, Zika MAL) under both optimized laboratory regimes and conditions simulating realistic, nutrient deficient field habitats. We present dissemination rates of each mosquito strain to each virus as well as quantify viral load in female *A. aegpyti* mosquitoes 14 days post infection. The implications of gene by environment interaction, and the role of phenotypic plasticity are then further discussed in the contexts of capitalizing on increasing our knowledge on heritable differences in flavivirus susceptibility to better inform disease prevention programs.

794

EVALUATING NEW TOOLS FOR MONITORING BRAZILIAN AND EAST AFRICAN MALARIA VECTORS

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To sufficiently monitor ongoing malaria transmission and evaluate whether interventions are achieving desired effects, adequate mosquito surveillance methods are essential. It is important that such tools are applicable in several environments and different countries, rather than just in specific localities. This study aimed to assess the BG-Malaria trap (BGM), which has recently been demonstrated as effective against the Brazilian malaria vector, *Anopheles* dalingi, as a practical method for monitoring malaria vectors also in east Africa. We compared BGM with BG-Sentinel trap (BGS) and Human Landing Catches (HLC) for sampling African malaria mosquitoes, using a set of two separate 3 by 3 Latin square experiments replicated 4 times each. The field study was conducted in rural Ulanga district, in southeast Tanzania for 12 nights (06:00pm - 06:00am). Separately we evaluated 5 different mosquito lures, all using BGM and parity ratios were assessed for all traps and compared. We collected a total of 1003 *Anopheles* mosquitos, almost half of whichit were caught by BGM (49.7%). HLC and BGS caught 34.0% and 16.3% respectively, with a significant difference between the methods (P=<0,0001). The mean numbers of *An. gambiae* and *An. funestus* caught in the BGM per night were19.0 [CI: 17.45 - 20.96] and 3.5 [2.22 - 5.49]. HLC caught 11.7 [CI: 10.09 - 13.58] and 1.3 [0.68 - 2.44] and BGS caught 6.0 [CI: 4.60 - 6.76] and 1.2 [0.62 - 2.24] respectively. The others *Anopheles* species corresponded to 2% of total mosquitos caught. Data on the comparative evaluation of the lures, and also on parity status of the mosquitoes is being analysed and will be presented at the meeting. The BGM trap was considerably more effective than BGS, a trap widely used for monitoring vectors, and performed better as HLC as well, which is considered the "gold standard" method for catching mosquitos. The results described here for BGM are promising and hence could be used as a surveillance tool for malaria vectors. More tests are underway to validate the results in other sites in the district and will be presented at the meeting.

795

EVALUATION OF A NOVEL POLYTETRAFLUOROETHYLENE (P.T.F.E) - BASED MEMBRANE FOR BLOOD-FEEDING MALARIA AND DENGUE FEVER VECTORS

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Blood feeding of female mosquitoes is an essential activity for colonization and maintenance of mosquitoes which are often required for research on vector-borne diseases. Common laboratory blood feeding strategies for rearing mosquito colonies use direct host feeding (DHF) such as on human arms or on live animals. The aim of this study was to evaluate the

artificial way of blood-feeding mosquitoes which is simple, affordable, sustainable and efficient in comparison to existing method DHF. We adapted and validated an artificial feeding (AF) method to replace DHF for the maintenance of *Aedes aegypti*, *Anopheles arabiensis* and *An. gambiae* s.s mosquito colonies. This blood feeding system uses fresh bovine blood as blood meal source, stored in vacutainer tubes containing the anticoagulant, ethylenediaminetetraacetic acid (EDTA). The blood was placed at the bottom surface of the disposable styrofoam cups and a simple membrane made of polytetrafluoroethylene (P.T.F.E) was stretched thinly over the bottom surface of the cup for mosquitoes to access the membrane and were fed for 20 min. Blood feeding rate, fecundity and survival rates of mosquitoes fed using the AF were compared to those mosquitoes fed using direct human arm feeding.The preliminary results show that *Ae. aegypti* mosquitoes had similar feeding rates on AF and DHF of about 100%. However the feeding rates of *An. arabiensis* and *An. gambiae* s.s on DHF had the highest rates compared to membrane feeding. Artificial feeding rates on *An. arabiensis* and *Anopheles gambiae* were (40%, 47.5%) and (52%, 48.5%) for the first and second replicate respectively while DHF the feeding rate was about 99% in malaria vectors for both replicates. The results show that bovine blood meal source has impact on the feeding rates of malaria vectors the *An. gambiae* s.s and *An. arabiensis* in comparison to *Ae. aegypti* species. Data of fecundity and survival will be presented at the meeting.The procedure can be adopted by laboratories due to its simple and affordable materials and design and it could be used as an alternative to the direct feeding method in different biological studies and studies on parasite transmission by vectors.

796

JAPANESE ENCEPHALITIS IN SIVASAGAR, ASSAM, INDIA, 2011-2014

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Japanese encephalitis (JE) is a vaccine-preventable cause of acute encephalitis syndrome (AES) with high (20-30%) case fatality rate (CFR). India has a high JE global burden;however, 50% JE cases are in Assam state. We estimated JE burden by time, place, and person in Sivasagar district, a high burden district within Assam, from 2011-2014. We reviewed records and reports of all AES patients who attended three hospitals in Sivasagar from January 1, 2011 to December 31, 2014. We evaluated AES/JE by time, place, and person, including disease trends and patients' vaccination status.In 2011-2014, Assam had 7,498 AES patients of which 647 (9%) were from Sivasagar (2011: 246; 2012: 140; 2013: 133; and 2014: 128). All were tested for JE virus and 314 (49%) were positive (2011: 128, 52%; 2012: 58, 41%; 2013: 64, 48%; and 2014: 64, 50%). During 2011-14, the majority of JE (291/314, 93%) and AES (313/333, 94%) patients were from rural areas and most JE patients were reported in July (65%). Median age of JE patients was 43 years (0.9-97 years) and AES was 29 years (0.1-85 years). Among JE patients, males (62%) were more affected whereas among AES, gender proportions were similar (males: 51%). Among 647 patients, 54 AES (16%) and 29 JE (9%) patients were vaccinated against JE. The CFR of AES and JE were similar (AES:38%; JE:38%) in Sivasagar and were both higher than the Assam average (AES: 17%; JE: 23%). JE/AES in Sivasagar with a low vaccination coverage and high CFR calls for urgent public health attention especially for rural areas and older persons. Analysis of risk factors and prevention strategies is recommended.

797

THE IMPACT OF SOCIODEMOGRAPHIC FACTORS ON *AEDES ALBOPICTUS* DISTRIBUTION AND ABUNDANCE IN ATLANTA, GEORGIA

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Since its invasion to the continental United States in 1985, the *Aedes albopictus* mosquito has become a well-established nuisance mosquito. Breeding in both natural and artificial containers, the distribution of this mosquito may be strongly influenced by humans. Although considered a secondary vector of certain arboviruses, recent outbreaks have caused its vector status to be reevaluated. Determining the sociodemographic factors which affect the distribution of this mosquito throughout distinct geographical locations is paramount in limiting future establishments and preventing the transmission of pathogens. A total of 142 houses were sampled across three neighborhoods with varying house values in Georgia, USA between July and August 2015. Non-parametric tests were utilized to determine if differences existed between container type and the three neighborhoods. The importance of house value was assessed within neighborhoods using simple linear regression. Finally, negative binomial generalized linear models with and without random effects were created in order to identify significant predictors of *Ae. albopictus* and container abundance across the three neighborhoods. The median number of rubber containers differed significantly between the low house value neighborhood and the high and middle neighborhoods (p-value = <0.001 and p-value= <0.001, respectively). None of the simple linear regressions yielded significant results; however, clear associations were present. The Akaike weights from the model which best accounted for number of mosquito-positive containers was 35.3% and IV instar *Ae. albopictus* larvae was 41.5%. House value was determined to be either the best predictor or one of the best predictors in the chosen models. The non-parametric tests and simple linear regressions suggest that house value does impact breeding site abundance and *Ae. albopictus* distribution. The multivariate hierarchical models support the hypothesis that the distribution of *Ae. albopictus* larvae depends, at least in part, on sociodemographic factors.

798

COMPARATIVE EVALUATION OF SIX OUTDOOR SAMPLING TRAPS FOR DISEASE- TRANSMITTING MOSQUITOES IN REFERENCE TO HUMAN LANDING CATCH IN RURAL TANZANIA

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There is a growing concern on how mosquito sampling methods can be safely performed in malaria endemic countries. Human landing catch is the best mosquito sampling method, it is labor intensive and exposes induvial to malaria transmission risks. This is an ongoing study which assesses the different mosquito sampling traps aiming to find an alternative for HLC in terms of effectiveness, densities, diversities and behaviors of disease- transmitting mosquitoes.Seven traps were introduced to sample mosquitoes. These were Mosquito Magnet (MMX), BG-Sentinel, Suna trap, Ifakara Tent Trap-C (ITT-C), M-Trap and M-Trap fitted with CDC Light trap. The traps were comparatively evaluated and calibrated with reference to the Human Landing Catch (HLC). A series of multiple 7*7 latin square experiments were conducted in 6 different villages over a total of 12 months, working in dry and wet season in each of the villages, to comparatively evaluate 6 different trap types against human landing

catches, the current gold-standard sampling tool. Seven position identified to each of this villages with the distance of 100m from one trap to another. The different traps rotated to the seven positions, that at the end of a 7 day rotation, each trap type had been to each of the seven locations at least once. The experiment were replicated 3 times for a total of at least 21 nights, start from 18:00hrs to 06:00hrs. The outcome measure will be the comparisons of effectiveness of different traps in terms of capturing high density and diversities of outdoor host seeking mosquitoes relatively to the reference method (HLC).A total of 62317 of all female mosquitos were collected for six villages for both seasons wet and dry, where BG-Sentinel n=5571 (8.94%), HLC n=13909 (22.32%) ITT-C n= 3775 (6.06) MMX n= 4468(7.17%) M-Trap n= 8429 (13.52) M-Trap with CDC Light trap n=12011(19.27%) and Suna trap n=14003 (22.47%) The result is showing there is no significant different between HLC, Suna trap and M-trap fitted with CDC for all total number of female mosquitoes but there is a different between HLC, against M-trap, BG-Sentinel, MMX and ITT-C in the first round.

799

CHARACTERIZATION OF SURFACE LAYER MICROBIAL COMMUNITIES OF *ANOPHELES GAMBIAE* COMPLEX LARVAL HABITATS IN BURKINA FASO

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Afro-tropical malaria vectors of the *Anopheles gambiae* complex represents a remarkable example of adaptive radiation thought to be driven by ecological divergence operating predominantly on the larval stages. Some ecological factors involved in larval niche partitioning, such as different ability to escape predators or tolerate abiotic stress, have been identified. Little is known, however, about the role in this process of environmental microbial communities occurring in the larval habitats. Thus, we tested the hypothesis that members of the *An. gambiae* complex are preferentially associated with different microbial communities.To this aim, we sampled *An. gambiae* s.l. larvae and the surface layer of 63 randomly chosen water collections in the village of Goundry (Burkina Faso). The microbiological profile of each site was obtained by PCR amplification using consensus primers flanking the V6-V8 region of bacterial 16S rDNA, and subsequent sequencing by Illumina Miseq. Paired-end reads were taxonomically analysed using the two bioinformatic pipelines BioMaS, for identification at species level, and QIIME, for OTU based analysis. The relative frequencies of mosquito species occurring in the larval sites (i.e. An. arabiensis, *An. gambiae*, and *An. coluzzii*) were associated to the inferred bacterial composition by Canonical Correspondence Analysis (CCA).Preliminary analysis of a subsample of 37 breeding sites based on 1,620 molecularly-identified mosquitoes (45% *An. coluzzii*, 38% An. arabiensis, 17% *An. gambiae*) showed that bacterial composition accounted for 6% of the total variance in larval relative frequencies. The first two canonical axes, which accounted for ~75% of the explained variance, separated the three species and associated bacterial communities.The results indicate that microbial communities occurring in larval habitats can be informative about the composition of sympatric species of the *An. gambiae* complex, supporting the hypothesis that particular environmental bacteria may represent an ecological marker of niche partitioning for these malaria vector species.

800

A DRAMATIC DECLINE OF MALARIA TRANSMISSION IN AND AROUND IFAKARA, A RAPIDLY GROWING TOWN IN SOUTHEASTERN TANZANIA, SINCE 2000

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Ifakara is a small but rapidly growing town of about 60,000 people in South-eastern Tanzania. A steady but high increase of population in Ifakara area has resulted in a rapid urbanization of the area, which in turn has had a negative impact on malaria transmission. In 2003, Ifakara had an estimated annual entomological inoculation rate (EIR) of 29. Our study aimed at determining changes in malaria transmission over the past decade. A total of 110 households were randomly sampled from across the five wards of Ifakara area. Mosquito collection was done between June 2015 and January 2016, using CDC light traps indoors, and Suna® traps outdoors. Comparison of indoor and outdoor mosquito density was done using the Human Landing Catches (HLC). *Anopheles* mosquitoes were morphologically identified, and analysed for *Plasmodium* sporozoites. Blood fed mosquitoes were also examined for blood-meal sources. A total of 2658 *Anopheles* mosquitoes were caught from 800 trap nights and 80 Human Landing Catches, including: 2,131 *Anopheles gambiae* sensu lato, 355 *Anopheles funestus* group, and 172 *Anopheles* coustani. Of all the malaria vectors, 85% were collected only from two wards, which were the most rural of the 5 Ifakara wards. All the *An. gambiae* s.l. were identified as An. arabiensis, and 95% of the *An. funestus* were identified as *An. funestus* funestus, the rest being An. rivulorum. Enzyme-linked immunosorbent assays were performed on 2,658 *Anopheles* mosquitoes and only one was found positive, which was an *An. funestus* caught outdoors by HLC in Katindiuka ward. *Plasmodium* sporozoite rate was calculated as 0.04% in all *An. gambiae* and *An. funestus* combined, and 2.8% in just the *An. funestus*. Overall mean nightly biting rates by malaria vectors were 3.02 mosquitoes per night, thus the EIR was estimated as 0.128. In conclusion, the EIR in Ifakara has dropped by over 99% in just over a decade, compared to what was observed in previous reports. The on-going transmission is concentrated in only a small and more rural section of the Ifakara area, which could be readily targeted with improved control measures towards local elimination.

801

SPATIAL SEGREGATION OF VECTOR MOSQUITOES IN URBAN BALTIMORE, MD

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Understanding processes that govern vector mosquito coexistence can help predict disease risk and guide public health interventions. Theoretical and empirical ecology indicate that under resource-limiting conditions in a constant environment, competition between species should result in the exclusion of the inferior competitor. Multiple vector mosquito species coexist in southwest Baltimore, MD, where vacant lots containing high densities of water-filled trash containers are interspersed within a matrix of maintained lots with fewer containers. One hypothesis that may help explain the coexistence of *Culex* mosquitoes with the superiorly competitive *Ae. albopictus* in Baltimore is a colonization-competition tradeoff, which predicts species coexistence in an environment with ideal habitats when an inferior competitor in a metapopulation can escape exclusion by having a superior ability to colonize vacant or sparsely populated patches. In this study, we tested the prediction that there would the highest abundances of *Cx.* species and *Ae. albopictus* in vacant lots compared to intervening occupied lots. We placed 5 oviposition traps in each of 6 vacant lots and 6 randomly selected sites in intervening

maintained lots in early and late mosquito season in paired city blocks in southwest Baltimore. Resident containers at each site were also surveyed for mosquito larvae during the late season session. In the early season, *Cx.* species and *Ae. albopictus* made up 80.8% and 15.2% of the 2619 total collected larvae, respectively. 75% of early season larvae came from abandoned lots. In the late season, *Culex* species and *Ae. albopictus* comprised 16.8% and 83.2% of larvae, respectively. 45 resident containers were identified with the majority (36) found in vacant lots. 51% of the resident containers contained larvae, with 87% of the larvae coming from abandoned lots. Resident container data and early season ovitrapping support the prediction that vacant lots support both higher abundances of larval habitat and abundances of *Culex* and *Ae. albopictus* compared to maintained lots, and should be targeted for mosquito reduction interventions in the future.

802

EXPERIMENTAL PERTURBATIONS OF *CULEX RESTUANS* POPULATIONS AND THEIR EFFECT ON WEST NILE VIRUS TRANSMISSION BY MEMBERS OF THE *CX. PIPIENS* COMPLEX

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In the eastern United States, the mosquito species *Culex restuans* and members of the *Culex pipiens* complex transition in abundance and epidemiological importance as vectors of urban arboviruses. *Cx. restuans* is most active during periods of West Nile virus (WNv) reemergence (early season) and the *Cx. pipiens* complex is most active during periods of peak WNv transmission (summer) and is considered the primary vector of WNv in the U.S. Previous studies have suggested evidence for early season enzootic WNv transmission by *Cx. restuans*, yet an empirical connection between the two species' transmission networks has not been established. We designed a semi-natural treatment-control experiment to test the hypothesis that the seasonal reemergence of the enzootic WNv transmission cycle is linked to the presence and blood feeding activities of early season *Cx. restuans* populations. To test this hypothesis, from March 21st to June 1st, 2016 we applied a rotating combination of methoprene (8.62%) and Bacillus thuringiensis (Bti) (10.31%) larvicides in road-side catch basins and storm drains in two urban parks in Atlanta, GA at temporal intervals corresponding with peak abundance of *Cx. restuans* populations. We then monitored WNv infection in the enzootic cycle during and post treatment by 1) testing blood samples obtained from the resident passerine birds for evidence of WNv antibodies and 2) testing all *Culex* spp. mosquitoes collected with aspirators, gravid traps, and CDC light traps for WNv infections. These surveillance methods were paired with collections from two untreated parks in Atlanta. Preliminary Before-After Control Intervention analyses show that *Cx. restuans* breeding populations were successfully suppressed in the treatment parks relative to the controls; however there was no effect of larval control on adult *Cx. restuans* population abundance. Avian WNv antibody seroprevalence and *Culex* spp. mosquito WNv infection data are currently being processed.

803

PRESENCE OF SPECIES WITHIN THE *ANOPHELES GAMBIAE* COMPLEX IN THE DEMOCRATIC REPUBLIC OF CONGO

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Anopheles gambiae s.l. is the primary vector of malaria in the Democratic Republic of Congo, however, there is little data on the species from this complex present in the country. This paper presents the species collected (as determined by PCR) between 2004 and 2011 in 16 locations across the country. The two species from the *An. gambiae* complex that were detected were *An. coluzzii* and *An. gambiae* s.s. *An. gambiae* s.s. was predominant in eastern DRC, whereas *An. coluzzii* was the main species found in several locations in Bandundu. The species were also found in sympatry in several locations (Kinshasa, Kisangani, Lodja). These results provide a basis for future work, which is needed to accurately describe the distribution of the *An. gambiae* complex species in DRC.

804

MAPPING PAST, PRESENT, AND FUTURE CLIMATIC SUITABILITY FOR INVASIVE *AEDES AEGYPTI* AND *AE. ALBOPICTUS* IN THE UNITED STATES: A PROCESS-BASED MODELING APPROACH

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Rapid changes in the distributions of the mosquitoes, *Aedes aegypti* and *Ae. albopictus* in the continental United States alter the potential for local transmission of dengue, chikungunya, and Zika viruses. All three viruses have caused major disease outbreaks in the Americas recently with infected travelers returning regularly to the U.S. Recent outbreaks of Zika, dengue, and chikungunya have proven that these diseases are capable of invading and being transmitted within the ranges of *Ae. aegypti* and *Ae. albopictus,* mostly in warm and tropical regions. The expanding range of these mosquitoes and discovery of new populations within the U.S. raises questions about whether recent spread has been enabled by climate change or other anthropogenic influences. In this analysis, we used daily average temperatures for the United States to model *Ae. aegypti* and *Ae. albopictus* population growth rates using a stage-structured matrix population model in order to understand past and present habitat suitability of these vectors, and to project future habitat suitability under IPCC climate change scenarios. We applied our model to the continental U.S. using temperature data on a 4-km grid. Our results indicate that, as expected, much of the southern U.S. is suitable for both *Ae. aegypti* and *Ae. albopictus* year-round; however, a surprisingly large proportion of the U.S. in addition to the southern states is suitable for positive population growth for much of the year. While the amount of suitable habitat in some regions of the U.S. has contracted within the past 50 years, the range of suitable habitat for both *Ae. aegypti* and *Ae. albopictus* has expanded in many regions across the country. Additionally, IPCC CMIP5 model projections of future climate change suggest that climate change will reshape the range of *Ae. aegypti* and *Ae. albopictus* in the U.S., and potentially the risk of the viruses they transmit. Understanding the range of these mosquitoes should be considered a high priority for public health officials and vector control agencies.

805

ABUNDANCE OF *AEDES ALBOPICTUS* AND PRESENCE OF DENGUE VIRUS IN PROXIMITY TO A PINEAPPLE PLANTATION IN COSTA RICA

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The role of *Aedes albopictus* in the transmission of dengue viruses (DENV) in the Americas is unknown. Considering that development of larvae can occur in bromeliads, the aim of this study was to determine the abundance of *Ae. albopictus* at three sites in proximity of an organic pineapple plantation, as well as to evaluate the presence of DENV. For adult collections, CO_2 traps were placed for 20-24 hrs in forested areas and houses adjacent to the plantation, as well as houses >1 km from the plantation. Pineapple plants were evaluated for mosquito larvae or pupae (8 clusters of 40 plants), as well as containers in and around 27 houses adjacent and 29 far from the plantation. Pools of *Ae. albopictus* were analyzed by RT-PCR, and DENV serotype was identified by sequencing. Adult mosquitoes at all sites included mainly *Anopheles apicimacula*, *Culex coronator*, *Cx. quinquefasciatus*, *Cx. nigripalpus*, *Cx. inflictus*, and *Ae. albopictus*. Biodiversity index (Shannon-Wiener) was higher in forested areas (1.39) than in houses adjacent (1.1) or far (0.75) from the plantation. *Cx. nigripalpus* and *Cx. quinquefasciatus* were the most abundant species in the forested area and houses, respectively. Adult *Ae. albopictus* were more common in forested areas and adjacent houses. Only 2 mosquito larvae were collected from pineapple plants. *Ae. albopictus* was the most frequent species in containers from houses; although house (HI) and container (CI) indices were higher in houses farther from the plantation (HI: 51, CI: 49) than those adjacent to it (HI: 41, CI: 29). DENV-2 and DENV-3 were detected in 2 of 20 pools of *Ae. albopictus* heads, and DENV-1 in 2 of 74 pools of larvae. Results suggest that nearby forested areas, which provide a natural habitat, may be the preferred sites for *Ae. albopictus*. However, the abundance and types of breeding sites in these forested areas is still to be determined. Moreover, results confirm that local *Ae. albopictus* harbor DENV, and that horizontal transmission is occurring. Further investigations will be required to determine the source of DENV and weather mosquitoes in forested areas are biting humans, monkeys, or other animals.

806

FLIGHT APTITUDE OF TETHERED MOSQUITOES AS A MEASURE FOR LONG DISTANCE MIGRATION BEHAVIOR

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Malaria kills over 500,000 people every year in sub-Saharan Africa. During the dry season in the Sahel, surface water required for larval sites disappears from this vast region, halting mosquito reproduction and bringing malaria transmission almost to a standstill. Recent studies have suggested that both *Anopheles gambiae* s.s and *An. arabiensis* (but not *An. coluzzii*) persist in this region by migrating from distant locations, where breeding occurs year round, though direct evidence for long-distance migrating malaria vectors to date is scant. In many insects worldwide, windborne long-distance migration occurs seasonally, facilitating exploitation of renewed resources far beyond their locomotor capabilities. In this study, our aim was to measure flight behavior in stationary-tethered, wild mosquitoes, and evaluate if flight intensity exhibits bimodality, consistent with "weak" and "strong" flying mosquitoes. Additionally, we evaluated the seasonal variation in flight behavior and compared it to the expected migration time of each species. Mosquito flight sound was recorded individually over 10 hour experiments. This assay enables us to characterize flight behavior of individual mosquitoes controlling for species, sex, and physiologic state. In the laboratory, this assay revealed the effect of age on flight aptitude. Preliminary results on data obtained using the assay on wild mosquitoes in Mali since July 2015 suggest that flight aptitude exhibits a wide variance among species and over seasons. Flight aptitude was elevated a few weeks after the first rains, consistent with results obtained using our free-flight assay (see poster), and in agreement with season-specific long-distance-migration. Comprehensive analysis of year-long data will be presented.

807

THE RELATIONSHIP BETWEEN ENTOMOLOGICAL INDICATORS OF *AEDES AEGYPTI* ABUNDANCE AND DENGUE INFECTION

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Routine entomological monitoring is a method to identify individuals at risk of dengue virus (DENV) infection. Using longitudinal entomological and serological data from Iquitos, Peru, we estimated the six-month risk of DENV for several *Aedes aegypti* monitoring indicators to determine whether *Ae. aegypti* abundance measures are associated with human DENV infection. Entomological survey data were linked with 8,157 paired serological observations taken approximately six months apart. Indicators of *Ae. aegypti* density were calculated from cross-sectional entomological data and linked by date to serological observations. Risk ratios estimating the association between *Ae. aegypti* abundance at the household and block levels and the six-month risk of dengue virus (DENV) seroconversion were obtained using log binomial models in a generalized estimating equation to control for repeated measures and clustering due to household membership. Risk ratios estimated using cross-sectional data were compared to risk ratios estimated from density measures calculated with entomological data collected up to 12 months prior to the start of the seroconversion interval. Cross-sectional *Ae. aegypti* densities (adult and immature mosquitoes) were not associated with an increased risk of DENV seroconversion. Larval and pupal measures showed no difference in risk. Incorporation of up to 12 months of prior entomological data into density estimates resulted in plausible risk ratio estimates for adult stage measures at the household and block levels, with adjusted risk ratios ranging from 1.02 (95% CI: 1.01,1.02) to 1.39 (95% CI: 1.17, 1.66), which was the strongest association with DENV seroconversion. Our results are likely due to the temporal variability in adult *Ae. aegypti* measures that result in areas with low levels of infestation being misclassified as having no entomological risk.

808

ADEQUACY OF DIETARY DIVERSITY FOR YOUNG CHILDREN IN THE DOMINICAN REPUBLIC AS A FUNCTION OF CHILD AGE AND HOUSEHOLD WEALTH

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Dietary diversity is one critical domain indexing the adequacy of children's diets. However, research to date has not adequately examined deficits in dietary diversity by child age despite marked changes in diets and dietary needs that occur over the first several years of life. Such information may guide the tailoring of nutrition education by child age. The aim of this study was to determine the extent and nature of deficits in dietary diversity as a function of (i) age of young children and (ii) household wealth in the

Dominican Republic. Data obtained from maternal interviews on 24-hour dietary recall were extracted from the Dominican Republic Demographic and Health Survey data from 2013. Dietary consumption was classified as per the World Health Organization-Minimum Dietary Diversity Indicator (WHO-MDDI) and findings stratified by six-month age bands and household wealth quintiles (examining those currently breastfeeding and not-breastfeeding separately). Despite recommendations to avoid complementary foods in the first six months of life, substantial minorities of children less than six months of age were consuming dietary items other than breast milk or infant formula. Among non-breastfeeding 6-11 month olds, 59.7% met the recommended minimal WHO-MDDI score of four. This increased to 81.8% for those 12-17 months of age, with no subsequent consistent trend upwards with increasing age. Eggs and vitamin-A rich fruits and vegetables were the WHO-MDDI food groups, which had the most infrequent use across age groups for both those currently breastfeeding and not breastfeeding. Promotion of these food groups, particularly in the 6-11 month age period may improve dietary diversity. Unexpected was little to no difference in mean and minimal dietary diversity by wealth quintile. This may suggest nutrition education initiatives aimed at improving young child dietary diversity may not need specific tailoring by wealth strata although examination of food cost affordability by wealth strata would be important to further evaluate this recommendation.

809

IS INTESTINAL PARASITE INFECTION ASSOCIATED WITH OBESITY? AN ECOLOGICAL ANALYSIS IN MEXICO

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Obesity is a worldwide healthcare challenge. Recent studies have shown an association between both viral and bacterial infection with obesity. However, studies on the association between intestinal parasites and obesity are scarce. The aim of this ecological study is to evaluate the association between the approximated probability of infection with *Ascaris lumbricoides* or intestinal protozoa (all reported intestinal protozoa excluding *Entamoeba histolytica* or *Giardia lamblia)* in 2000, 2006 and 2012 with BMI for age z-score (BMIz) in 2012 in Mexico. For this purpose, we used publicly available individual-level data for BMIz in 2012 and state-level data on the incidence of infection with *A. lumbricoides* or intestinal protozoa in 2000, 2006 and 2012 as a proxy for probability of infection. A higher approximate probability of infection with *A. lumbricoides* in 2012 was associated with a lower BMIz in 2012. In contrast a higher approximate probability of infection with intestinal protozoa in 2012 was associated with a higher BMIz in 2012. A higher approximate probability of infection with *A. lumbricoides* and intestinal protozoa in 2000 and 2006 were associated with a higher BMIz in 2012. In summary our results suggest that there may be species specific effects of intestinal parasitic infection that may have both, short and long term consequences on health. Further research is needed to confirm these ecological associations and study the possible mechanisms. These findings have important implications for Mexico, given the context of a high incidence of parasitic infection and emerging obesity epidemic.

810

TEAMING UP AGAINST MALARIA: THE STORY OF SENEGAL'S SUCCESSFUL PARTNERSHIP

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Senegal's National Malaria Control Program has a long tradition of consultation with its partners. The effective partnership of government, nongovernmental organizations, donors, and communities, coordinated by strong local leadership, has enabled Senegal to achieve significant progress in the fight against malaria in the past decade. Several coordination mechanisms have helped to democratize the national response to malaria and have led the non-public sector to invest in the implementation of policies in a manner and at a level never seen before. A strong National Coordination Committee has ensured effective pooling of resources and the steady coordination of activities. In addition to activities implemented in collaboration with its traditional partners, the NMCP is also strengthening its longstanding partnership with research and training institutions. Communities have been an integral part of the NMCP's action plan since 2005. To leverage the momentum toward malaria elimination, Senegal strives to couple treatment and prevention efforts with a systematic drive to mobilize resources and catalyze the leadership of not only domestic and international governing bodies and policy-makers but communities as well to increase levels of support in the malaria fight. With campaigns such as Zero Malaria Starts With Me, the NMCP launched an inclusive national movement in favor of malaria elimination throughout communities in Senegal. Networks of community champions will be trained to raise awareness among their communities on malaria prevention and treatment and contribute to the national effort toward malaria elimination. In order for Senegal to sustain its intensive malaria control and elimination activities and achieve economic benefits, the country needs diverse and robust partnerships, both domestic and international, to ensure long-term, reliable financing for its program. Our poster presentation aims to shed light on the tremendous progress Senegal has been able to achieve thanks to strong partnerships with like-minded institutions and individuals who share the same objective: malaria elimination in Senegal.

811

EVALUATION OF A MOBILE HEALTH APPROACH TO A LLIN UPTAKE INTERVENTION AMONG PREGNANT WOMEN: THE HATI SALAMA STUDY

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The Hati Salama (HASA) cluster-randomized controlled trial aims to increase malaria awareness among pregnant women using mhealth technology in Tanzania. HASA utilized an electronic system whereby nurses issue vouchers to pregnant women, allowing them to redeem a Long Lasting Insecticidal bednet (LLIN) at a retailer for a highly subsidized cost. A RCT was selected to test efficacy of SMS behaviour change communication messages aimed to increase the uptake of LLINs in areas of Tanzania identified as malaria hotspots with overall low uptake of LLINs. HASA was implemented in 97 antenatal health facilities; where 49 clinics were assigned to the control group (no targeted SMS messages sent to beneficiaries) and the other 49 in the intervention group (targeted messages sent to beneficiaries). A major study objective is evaluating the process of distributing the vouchers and understanding why they were not redeemed (approximately 30%). The investigators utilized a post-study phone survey to speak directly to those who did not redeem. The most common responses were: I went to redeem but the shop did not have any bednets; I cannot afford the co-payment; I do not live close enough to a shop that accepts the vouchers; I went to the shop but the retailer did not know how to redeem the voucher; and I did not know how to redeem the voucher. These responses demonstrate significant barriers that deter women from obtaining life-saving bednets. The process evaluation showed significant insights - the nurses revealed a large learning curve in the technology to distribute the vouchers; a trained nurse was consistently away from clinic, leaving the others overwhelmed; overall network

connectivity across Tanzania remains low, leaving women waiting for prolonged periods to receive vouchers or not at all; and many women do not have their own mobile phone. The responses collected from the participants and nurses are extremely valuable in understanding why highly subsidized bednets are not redeemed. It is imperative for donors to critically appraise these program shortcomings and barriers as many can be improved or overcome, thereby increasing bednet uptake in malaria hotspots.

812

PUBLIC HEALTH INTERVENTIONS TO PROTECT AGAINST FALSIFIED MEDICINES: A SYSTEMATIC REVIEW OF INTERNATIONAL, NATIONAL AND LOCAL POLICIES

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Falsified medicines are deliberately fraudulent poor quality drugs that pose a direct risk to patient health and undermine healthcare systems, causing global morbidity and mortality. We aimed to produce a comprehensive overview of healthcare and pharmaceutical policies that could be deployed at international, national, and local scales to reduce the burden of falsified medicines in low and middle income countries (LMIC). We identified 660 studies in a systematic search of the PubMed, Web of Science, Embase, and Cochrane Library databases, of which 203 met title/abstract inclusion criteria and were categorised according to their primary policy focus: international; national; local pharmacy; internet pharmacy; and drug analysis tools. 84 were included in the qualitative synthesis, along with 108 articles and website links retrieved through secondary searches. On the international stage, we discuss the need for accessible pharmacovigilance (PV) global reporting systems, international leadership and funding incorporating multiple stakeholders (healthcare, pharmaceutical, law enforcement), and multilateral trade agreements that emphasise public health. On the national level, we explore the importance of establishing adequate medicine regulatory authorities and PV capacity, with drug certification requirements and screening along the supply chain. Local healthcare professionals can receive training on medicine quality assessments, drug registration, and pharmacological testing. Finally, we discuss novel techniques for drug analysis which allow rapid identification of fake medicines in low-resource settings. Innovative point-of-purchase systems like scratch-off mobile phone verification codes allow consumers to check the authenticity of their medicines. Such technology will be increasingly relevant to LMIC as mobile phone coverage expands, offering opportunities for integration with other "mHealth" initiatives. In summary, we describe how anti-falsifying strategies that target different levels of the pharmaceutical supply chain can be combined to protect against falsified medicines.

813

CONTRIBUTION OF REMOTELY SENSED DATA FOR MALARIA RISK SURVEILLANCE IN MADAGASCAR

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In the Central Highlands (CHs), the history of malaria was marked by deadly epidemics due to the existence of unstable malaria. Disease surveillance using remote sensing becomes crucial because of environmental and climate change. It allows a better understanding of the vector behavior and disease transmission patterns. This decision tool is not yet adopted by the Malagasy National Malaria Control Program. There are no regular updates of Malagasy land cover maps and number of weather stations are not sufficient. This study aims at updating environmental and climate data. Malaria risk presents many variations at spatial scale. Second objective is to compare the relevance of information obtained from remotely sensed data with two different spatial resolutions (SR). Satellite images from Spot 5 and Landsat 8 sensors were used to classify land cover in Ankazobe district and CHs. The SR of each image was increased using pan-sharpening method. Object based image analysis method was used to classify images according to wetness, vegetation index. Climate data were acquired from NOAA and MODIS sensors. Kappa index (KI) quantifies the concordance between classification and ground truth and was used to evaluate the accuracy of the land cover classification. Two maps with five land cover classes were obtained, including rice fields, wetland, water bodies, bare soil, wood. The first three classes were highlighted because these are the preferred breeding sites of the mosquito responsible of transmission in CHs. First map is to Ankazobe district with KI of 87%. CHs are represented by the second map with a KI of 84%. The correlation between both classified images is 86%. To cover CHs, Landsat 8 needs five scenes against 40 for Spot 5 and its data processing takes less time. A 79% correlation was obtained by comparing the temperature map with the thermal band of Landsat 8 images. Landsat 8 is preferred because of its free access, satisfactory SR, wide study area and its less time consuming process. In limited resource countries like Madagascar, where national updated geographic data are rare, this allows integrating recent information in malaria risk assessment.

814

DISTANCE LEARNING: REVITALIZING ANESTHESIOLOGY TRAINING IN RESOURCE-LIMITED ETHIOPIA

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Ethiopia has a significant paucity of available healthcare workers for its population of 94 million. There are an estimated 0.027 physicians per 1,000 people and specifically, 0.02 trained medical anesthetists per 100,000 people. Despite the increasing number of medical schools, there are not enough physician instructors at the schools. Furthermore, there is a lack of availability and standardization of post-graduate training. Modalities of e-learning have been shown to be successful when used to impart medical education in other resource-limited countries. The Emory University and Addis Ababa University (AAU) Departments of Anesthesiology have formed a collaboration with the intent of improving the AAU Anesthesiology Residency program, the only post-graduate training program for anesthesiology in Ethiopia. An initial educational needs assessment identified areas in the existing training program that require improvements. Interviews with faculty and residents led to study topics. In this pilot study, we describe how the current classroom-based curriculum is augmented by the introduction of interactive educational sessions and distance learning in the form of video lectures. Video lectures cover topics based on areas identified by local residents and faculty. Interactive sessions include journal clubs, problem-based learning sessions, and oral board review-type sessions. Assessment of the additions of the newly introduced blended learning technique are conducted via pre- and post-tests on the topics presented. An expansion of educational resources and modes of didactics are needed. Incorporating interactive and distance learning educational sessions into the existing didactic structure leads to improved trainee satisfaction.

815

IMPROVING PREGNANCY OUTCOMES: ALLEVIATING STOCK-OUTS SITUATION OF SULFADOXINE PYRIMETHAMINE IN BUNGOMA, KENYA

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WHO recommends intermittent preventive treatment of malaria in pregnancy using sulfadoxine pyrimethamine (IPTp-SP) to be provided at antenatal care (ANC) clinic. Ministry of Health (MOH) used to procure SP until 2013 when health services were devolved to counties and procurement of became the responsibility of county governments. This presented a major challenge as counties had not factored SP in their budgets. Consequently, counties experienced SP stock-outs from October 2014. In Bungoma County the number of pregnant women receiving IPTp dropped by 51% from 7,845 in October 2014 to 3,865 in February 2015. To alleviate the situation (MOH) at national level requested counties to procure SP. Advocacy efforts with Bungoma County by the Maternal and Child Survival Program focused on prioritization of SP procurement at least once every quarter. As a result of this intervention, Bungoma County procured SP from February to July 2015. The county advised health facilities to procure additional SP doses if the supplied stocks ran out. The procurement led to a 117% increase in the number of pregnant women receiving IPTp; from 3,865 in February to 8,404 in July 2015. The fiscal year ended in June 2015 and no funds were available to procure additional SP until October 2015. This contributed to a 33% decrease in the number of pregnant women receiving IPTp from 8,404 in July to 5,672 in October 2015. As a response to support counties, MOH at national level procured 2.24 million SP doses in November/December for 14 MIP-focus counties which were received at health facilities in February 2016. In conclusion, Bungoma County applied feasible mitigation measures including county-level procurement of SP, supplemented by additional procurement at health facility and national levels. This is a practice which is replicable in other counties to ensure continued availability of SP to protect pregnant women from effects of malaria in pregnancy.

816

MALARIA RISK ASSESSMENT USING MULTI-CRITERIA EVALUATION TO IDENTIFY PRIORITY AREAS FOR INDOOR RESIDUAL SPRAYING IN THE CENTRAL HIGHLANDS OF MADAGASCAR

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The malaria control strategy appropriate for the Central Highlands (CH) of Madagascar differs from the rest of the country. Indoor residual spraying (IRS) with insecticide is only implemented in the CH and Fringe regions of Madagascar due to vector behavior and efficacy of IRS. Two models of malaria risk gradient were performed in 2014 and in 2015. Malaria risk gradient was calculated for inhabited areas the CH, using Multi-Criteria Evaluation (MCE) of factors associated with malaria transmission (land cover, altitude, climate, and population data). MCE was performed by weighted linear combination method, to obtain the gradient of risk. Factor weights were determined by pair-wise comparison based on literature review and expert knowledge. In our study, fuzzy set theory was used to perform the factors' weighting. Change of risk magnitude between the two consecutive years was calculated to assess areas which shift from one risk category to another risk category within the year. The mapping of risk magnitude showed wide variation across the CH. Malaria risk gradient was categorized in five groups: very low, low, moderate, high and very high for the mapping. Risk magnitude between 2014 and 2015 showed an increase, with 1.3% and 7% risk for low risk groups and high risk groups, respectively. We observed a decrease of 1.1% for areas in the very low risk group, 2% for areas in the moderate risk group and 1.3% for areas in the very high group. However, risk status remained unchanged for 87.4% of the areas in CH. It is crucial to focus IRS efforts according to the risk gradient to improve its effectiveness, targeting only areas with the most need while optimizing available resources. Integrating data on previous intervention, malaria prevalence data from national routine surveillance or existing malaria early warning system in a Multi Criteria Decision Analysis may help improve the prioritization process for applying IRS to specific areas.

817

RESISTANCESIM - DEVELOPMENT AND FEASIBILITY STUDY OF A SERIOUS GAME TO IMPROVE UNDERSTANDING OF INSECTICIDE RESISTANCE MANAGEMENT IN VECTOR CONTROL PROGRAMS

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Insect vectors transmit many human pathogens, and the cornerstone of most vector-borne disease control programs is the use of chemical insecticides. Unfortunately, the development and spread of insecticide resistance threatens the continued efficacy of these interventions, and is poised to create a public health disaster in the context of malaria control. Considerable efforts to develop new active ingredients and interventions are underway. However, it is clear that strategies to mitigate the impact of insecticide resistance must be deployed now, both to maintain the efficacy of currently available tools as well as to ensure the sustainability of new tools as they come to market. Although best practice guidelines for insecticide resistance management (IRM) have been disseminated by the World Health Organization, the lack of understanding of IRM has been identified by Rollback Malaria's Vector Control Working Group as the primary gap in the translation of evidence into policy. We developed a serious game called ResistanceSim to fill this gap. The first part of the development process convened stakeholders to define the learning objectives, target audience, and the role of mathematical models in the game. A series of learning domains were identified, and a set of specific learning objectives for each domain were defined to be communicated to vector control programme personnel. A simple "game model" was proposed as a way to produce realistic game behaviour while also capable of running in real-time. The second part of the development process was to engage software developers to map the defined learning objectives to game elements. A game design document was produced that guided the development process. An internal beta-testing phase was organized to identify any bugs in preparation for final release. The third part of the development process convened stakeholders to define the most effective strategies to roll out the tool. Finally, a feasibility study was conducted among members of the target audience in Zimbabwe. We used questionnaires and focus group discussions to evaluate users' perceptions of the tool and to identify areas of improvement.

818

THE IMPACT OF LOCAL DISTRIBUTORS ON THE QUALITY OF MEDICINES IN RESOURCE-LIMITED SETTINGS: FIELD-BASED RECOMMENDATIONS

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The Sustainable Development Goal 3.8 aims at universal health coverage, including "quality and affordable essential medicines and vaccines for all". But the increasing globalization of pharmaceutical production, coupled to the lack of resources of regulatory authorities in low- and middle-income countries (LMICs), makes it difficult to thoroughly assess the quality of medicines manufactured, imported or distributed in these territories. In these contexts, the pharmaceutical distributors and wholesalers play a key-role in defining the local qualitative standards. In a previous study, we had showed the weaknesses of some international procurement agencies, in particular concerning the capacity to select pharmaceutical products based on stringent quality criteria, and to re-evaluate them regularly. As a follow-up to that study, we now looked at the compliance with WHO standards of about 30 private local distributors of medicines and medical products, located in different sub-Saharan Africa countries. The evaluation was conducted according to a set of standardised criteria, inspired by the WHO Model Quality Assurance System for Procurement Agencies (MQAS). Our preliminary findings confirm the existence of significant weaknesses, especially concerning the selection criteria for procured products and the capacity to reassess them on an ongoing basis, which means that the potential for exposure to sub-standard medicines is high. Also, clients' complaints systems may be weak or absent, which limits the possibility to identify post-marketing quality problems and to implement batch recalls if needed. To efficiently fight the plague of poor-quality medicines, there is a urgent need to improve the quality systems of private local distributors in sub-Saharan Africa. To achieve this objective, the use of harmonized evaluation tools based on the WHO Model Quality Assurance System for Procurement Agencies should be encouraged for regulatory supervision, audits and self-assessments.

819

COMMUNITY EXPERIENCES WITH BEDNET DISTRIBUTION AND INDOOR RESIDUAL SPRAYING CAMPAIGNS FOR MALARIA PREVENTION IN RURAL GHANA: A QUALITATIVE INVESTIGATION

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Malaria remains one of the primary causes of morbidity and mortality in the Upper-East Region of Ghana (Ghana Statistical Service 2011). Control methods for malaria in rural areas include long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) campaigns. This research study seeks to understand the experience residents had with LLIN distribution and the IRS campaign for possible explanations of why transmission has not significantly reduced in Nangodi (World Malaria Report, 2015). Data was collected in the town of Nangodi. Researchers conducted interviews with semi-structured interview guides. Participants were sampled based on district, and included community members (46), insecticide sprayers (3), and healthcare providers (2). Participants were interviewed in Nabte and English, using a translator if needed. All interviews were recorded, transcribed, and analyzed manually for key themes. Data were cross-checked between the three authors. Information on malaria interventions was intermittent and incomplete amongst participants. Issues that came up frequently included ineffective communication of timing and instructions for spraying and worn bednets not being replaced in a timely manner. Community members who lived far from the main road reported poor bednet access and fewer visits from IRS sprayers. Community members experienced adverse events including skin irritation, stomach aches, stains on walls, and loss of fowl. In conclusion. although residents reported adverse effects from spraying, they were initially hesitant to address adverse events. Almost all would accept IRS if offered again. Residents provided important design suggestions for the campaign, such as reducing potency of the chemical and provision of face masks. Additionally, residents reported varying knowledge about the IRS campaign and many barriers to accessing new bednets. Quality and uniform distribution of information and bednets must occur in order to reduce transmission rates. Further research is needed to understand determinants of access and how best to improve current LLIN and IRS campaigns.

820

PEPFAR 3.0: OPPORTUNITIES FOR ENHANCED NCD CARE WITH DIFFERENTIATED MODELS OF HIV SERVICE DELIVERY

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Since its creation more than a decade ago, PEPFAR has provided millions with life-saving ART and improved access to prevention, care, and treatment services that have drastically reduced HIV/AIDS related mortality. PLHIV can now enjoy lifespans on par with people not living with HIV. As this population ages, many also find themselves affected by a variety of non-communicable diseases such as CVD, diabetes, and a variety of cancers. Thus the treatment services provided to PLHIV could begin to change to meet these evolving needs. With the introduction of PEPFAR 3.0 and the 90-90-90 goals, HIV programs now find themselves providing even greater levels of testing, testing yield, and ART, often within the context of a flat budget. This has led to the creation of a variety of innovative solutions and approaches to both testing and treatment of PLHIV. At the forefront of these innovations are differentiated models of service delivery. Instead of offering a one size fits all approach to PLHIV care, the focus is now moving to service delivery that is tailored to the specific patient, context, and health systems within a country. As we rethink the package of services offered, and how these services are delivered to the population, there exists a great opportunity to incorporate NCD services. Multi-month prescriptions for ARVs can provide benefits to patients and providers by reducing burdens on clinics and lessening work time lost to doctor visits; however, these benefits are lessened if PLHIV continue to have monthly visits to fill their NCD medications. Multi-month prescription services require robust supply chain systems which ensure timely delivery of medications while avoiding stock outs. Looking at current developments in HIV service delivery, we identify areas and activities where the integration of NCD services would provide more holistic treatment while also working towards the 90-90-90 goals by ensuring better uptake and adherence.

821

THE GLOBAL HEALTH SERVICE PARTNERSHIP: AN ACADEMIC-CLINICAL PARTNERSHIP TO BUILD CAPACITY IN NURSING AND MEDICINE IN SUB-SAHARAN AFRICA

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Thirty-one countries in sub-Saharan Africa (SSA) have a critical shortage of health care professionals, limiting healthcare delivery, response to emerging needs, and teaching and retention of future professionals. As a result, nursing and medical schools are increasing enrollment, further increasing demand on limited faculty. To address this shortage, the Global Health Service Partnership (GHSP; Seed Global Health/Peace Corps) places US nurses and physicians at institutions in SSA, working with local faculty to strengthen health education and delivery. A mixed method evaluation examined feasibility, outcomes and impact in GHSP's first 2 years (quantitative measures of productivity; qualitative data from stakeholder interviews). Between July 2013-June 2015, 69 GHSP educators were deployed to 13 universities in Malawi, Tanzania, and Uganda. They provided 85,612 service-hours, taught 300 courses to 7,219 trainees, participated in curricula revision and development (including new post-graduate MSN and MMed programs), enhanced the teaching infrastructure, and made academic-clinical linkages to facilitate practice improvement projects. Qualitative data revealed the largest impact on students (the provision of quality, consistent education, particularly clinical supervision; value added to the learning environment; and increased student confidence and empowerment). Impact on faculty was significant in the areas of workload reduction, mentoring, and value added to the learning environment. Taken together, these data suggest that an innovative, locally tailored and culturally appropriate academic partnership is feasible and generated new knowledge and best practices relevant to capacity strengthening for nursing and medical education. Key features of GHSP include the intentional pairing of US/African educators, emphasis on faculty supervised clinical instruction, and a sustained commitment over time. Continued evaluation will inform optimizing classroom and clinical pedagogy in resource-constrained settings and improve the health and wellbeing of populations who suffer a high burden of disease.

822

ANTIMICROBIAL ACTIVITIES OF SIX SELECTED MEDICINAL PLANTS AGAINST *STAPHYLOCOCCUS AUREUS*

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The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. For a long period of time, plants have been a valuable source of natural products for maintaining human health. Therefore, such plants should be investigated to better understand their antimicrobial properties, safety and efficacy. The study evaluated *in vitro* antimicrobial activities of aqueous and ethanol fractions of six selected medicinal plants, including *Eugenia cryophyllata*, *Psidium guajava*, *Alchornea cordifolia*, *Cinnamomum zeylanicum*, *Zanthoxylum xanthoxyloides* (*fagara*), and *Tridax procumbens* against 19 clinical isolates and a standard strain of *Staphylococcus aureus* using a modification of the agar diffusion method. The potency of 16 formulations from five of the medicinal plants that showed significant antimicrobial activity was also evaluated. The ethanol fractions inhibited the growth of the test organisms with zones of inhibition ranging from 4.0-20.5 mm averaging 8.6 mm whereas that of the aqueous fractions ranged from 4.0-20.5 mm averaging 7.4 mm. Formulations from the combinations of *Alchornea cordifolia* and *Eugenia cryophyllata* showed inhibition zones ranging from 9.0-16mm and 7-16 for the aqueous and ethanol extracts respectively. There was significant difference between the ethanolic and aqueous plant extracts against *S. aureus* used in this study. Minimum inhibition concentrations (MICs) of the herbal preparations used against the control strain (*S. aureus* ATCC® 29213™) and the clinical *S. aureus* isolates showed that, both the aqueous and ethanolic extracts of *Alchornea cordifolia* and ethanol extract of *Cinnamomum zeylanicum* produced the lowest MICs of 2 mg/ml. Considering the fact that these extracts are crude, it could be inferred that they possess antibacterial activities that need further investigations to identify the compound(s) responsible for the antibacterial activities.

823

CREATING A HOLISTIC FRAMEWORK FOR UNDERSTANDING THE FULL IMPACT OF THE ZIKA VIRUS ON MOTHERS, INFANTS, HEALTH SYSTEMS AND SOCIETY

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In February of 2016, the World Health Organization (WHO) declared Zika Virus as a Public Health Emergency of International Concern under the International Health Regulations. Zika is projected to take a huge toll on the health and wellbeing of people across the Americas as well as globally, given international climactic and transport pathways for dispersion. Zika now presents a new challenge, with implications beyond generalized mild infections, suggesting considerable risk in pregnancy of a clear malformation in the child and longer-term effects on proper brain development and this will have important effects on costs of health and social care. The most likely impact on the majority of households is that someone, usually the mother, will become a long-term caregiver, often taking women out of the workforce and creating extra strain on household finances leading to a greater risk for financial shocks that tend to lead to poverty. In many low- and middle-income countries, the increase in women in the workforce has been a stabilizing factor in household welfare, pulling many households out of poverty. Mothers forced to become full-time caretakers could see that progress reversed. Alternatively the threat of having a malformed child can lead the mother to search for a way to terminate her pregnancy. In countries with strong anti-abortion policies, unsafe abortions could negatively affect maternal mortality. If

a period of high fertility is followed by a sudden period of low fertility, after which the rate picks up again, there will be a risk for the balance of dependents in future generations. Tax and public service provision systems rely on there being a good match between working age populations and dependents, such as the young and the retired. Using widely available data sets containing data both fertility rates, formal and digital incidence trends of Zika, unsafe abortions, health care utilization, female workforce participation and income and poverty levels we developed a simple epidemiological-economic model to estimate the likely long term impact on the health, health care costs social welfare and poverty of households across the Americas.

824

USING AMR DATA IN DATA-RICH COUNTRIES IN COMBINATION WITH UNIVERSAL 'MEDIA' DATA TO MAP ANTIMICROBIAL TRENDS AND DRIVERS ACROSS DATA-POOR AREAS OF AFRICA

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The World Health Organization has highlighted antimicrobial resistance (AMR) as a "major global threat" to public health. Resistance is growing in every region of the world, including resistance to 'last resort' antibiotics. In the United States, an estimated two million illnesses and 23,000 deaths are attributed to antibiotic resistant bacteria or fungi each year. The key drivers of AMR are largely known; antimicrobial overuse or misuse, suboptimal dosing, including from substandard and falsified drugs, suboptimal diagnostics and vaccination. Despite this knowledge, the complexity of the interaction between these factors and how each affects the scale of the problem in different contexts is heavily reliant on the health care system, rules around prescription and use of antibiotics, and the prevalence, mix and concentration of disease burden from bacterial diseases in each country. In addition to this context specific complexity, the countries that suffer most from bacterial infections are often those that are most data-poor in terms of both routine laboratory testing of patients and systematic data collation on the existence and trends of AMR drivers. To better assess the risk of AMR across countries where the impact could be most devastating requires a more novel approach to generating real time estimates of both the prevalence and rate of growth of AMR. We have developed a data collection and analytic framework that utilizes three distinct sources of routine data, but which mixes data from data-rich settings and data-poor settings, using a method of triangulation to extrapolate gaps in prevalence and growth of AMR in data-poor countries. These data sources are: 1) data on annual incidence of AMR in data rich countries from official government surveillance reports; 2) digital real-time reports acquired from HealthMap Resistance Open, which aggregates online news, social media and hospital reports of AMR events; and 3) data on key AMR drivers from routine healthcare and pharmacy data, and local drug policy and regulation data. We show how this framework could be used to monitor real time trends in AMR and its relationships to key drivers of AMR.

825

ZIKA VIRUS-RELATED PHOTO SHARING ON PINTEREST AND INSTAGRAM

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Communicating accurate, accessible and actionable information to diverse populations is a key component of emergency responses against the Zika virus outbreak. Public health agencies are engaging the public using fast-growing photo-sharing social media sites such as Pinterest and Instagram. In this cross-sectional study, 616 Pinterest photos (keyword: "zika" AND "virus"; the maximum number of photos that we were able to retrieve via web scraping) and 616 Instagram photos (#zikavirus; randomly selected from 9370 Instagram photos retrieved via Instagram Application Programming Interface) were retrieved on April 3, 2016. Two trained individuals manually coded photos based on their relevance to Zika virus, words embedded, language and their content categories (any category that applies). Among our samples, 47% (290/616) of Pinterest photos and 23% (144/616) of Instagram photos were relevant to Zika virus. Words were embedded in 57% (164/290) of relevant Pinterest photos and 100% (144/144) of relevant Instagram photos. Among the photos with embedded words, more Instagram photos were in Spanish and Portuguese (77/144, 53%) than Pinterest (14/164, 9%) ($P<0.0001$). There were more Zika virus-related photos on Instagram than on Pinterest containing prevention information (59/144, 41%, versus 41/290, 14%, $P<0.0001$), issues relevant to effects on pregnancy (27/144, 19%, versus 32/290, 11%, $P=0.04$) and deaths associated with this infection (4/144, 2%, versus 0/290, 0%, $P=0.01$). Given the relatively international demographics of Instagram (vis-à-vis Pinterest that is more American), it is reasonable to suggest that Latin American users were more concerned about Zika virus prevention and its impact on pregnancy outcomes. In conclusion, Pinterest and Instagram have similar representation of Zika virus-related photos. Health communicators may use both Pinterest and Instagram as similar platforms to disseminate Zika virus information to the public.

826

IMPROVEMENT OF CHILD NUTRITIONAL STATUS IN THE DEMOCRATIC REPUBLIC OF CONGO OVER TIME: SERIAL CROSS-SECTIONAL ANALYSIS OF THE DEMOGRAPHIC AND HEALTH SURVEY

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In the Democratic Republic of Congo (DRC), prolonged conflict, high levels of infectious disease and severe poverty take a toll on the health and development of children. Approximately one in seven children die before reaching the age of five and approximately half of these deaths are due to malnutrition and nutrient deficiencies. To assess the change in child health status over time, we utilized nationally representative, cross-sectional data from the 2007-2008 and 2013-2014 DRC-Demographic and Health Surveys. Data from those under 5 years of age included but was not limited to height, weight, anemia and health outcomes among 3,951 children in the first and 8,552 children in the second wave of the survey. According to child anthropometric measures, a significant improvement ($p<0.0001$) was observed between 2007 and 2013 for stunting (height-for-age, 55% and 46%), wasting (weight-for-height, 27% and 13%) and underweight (weight-for-age, 38% and 27%). Such improvements varied

by province (percent change range: stunting, 4% to 45%; wasting, -12% to 69%; underweight, -13% to 68%) and place of residence (with the greatest improvements observed in urban compared to rural settings). Additionally, while significant improvements (p<0.0001) were observed for anemia between 2007 and 2013 (hemoglobin count <11 g/dl, 71% and 50%, respectively), little change was observed in moderate to severe estimates during this time (hemoglobin count <10 g/dl, 2007: 23%, 2013: 21%). While location of residence (urban/rural) was not associated with anemia, estimates did vary by province (percent change range: 15% to 53%). As poor growth performance during infancy and early childhood is commonplace in many developing countries, it is important to track how these measures change over time. Malnutrition is a major contributor to impaired intellect, increased mortality and susceptibility to infection, thus such surveillance allows for the identification of vulnerable subgroups to whom intervention efforts may be targeted to improve the health and development of children.

827

CAN WE TARGET MOBILE POPULATIONS WITH AN INTEGRATED DISEASE CONTROL APPROACH? A QUALITATIVE STUDY ON RISK BEHAVIOR FOR MALARIA AND HIV ALONG THE THAI-CAMBODIA BORDER

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The border zone in Oddor Meanchey province in Cambodia is characterized by high human mobility, as economic opportunities such as logging, farming and plantations attract migrants. Malaria parasite resistance has been detected in this region, requiring active case detection and management. High prevalence rates for HIV also occur among high-risk groups such as female entertainment workers, active around the border casinos and catering to loggers and the military. This qualitative study, carried out in collaboration with the national control centers of Malaria (CNM) and HIV (NCHADS), aimed to characterize the vulnerabilities of different types of mobile groups in order to propose integrated control activities. Between December 2014 and 2015, semi-structured and open-ended interviews (N=195) were conducted in Oddor Meancheay with theoretically and snowball-sampled informants. Four main categories of mobile populations were identified whose activities exposed them to malaria and HIV: (i) military men, (ii) local farmers, (iii) rural migrants working on plantations, (iv) female entertainment workers. The first three categories of people support their families by forest and farm work, which increases the risk of malaria since during nightly logging or overnight farm stays people lack malaria preventive measures. Additionally, income produced by these activities is partly spent in entertainment places, both in temporary camps in the forest and around casinos along the border, where alcohol consumption and drug use lead to unsafe sexual practices. Tests and treatment for HIV and malaria from the public sector or the Village Malaria Worker are less popular than those bought in the private sector, where privacy and quality are expected. As such, many HIV and malaria cases go undetected or are not followed up by adequate treatment. As certain risk behaviors tie these mobile populations together, integrated HIV and malaria control activities are feasible, potentially through the active detection of at-risk individuals by both HIV and malaria programs in collaboration with the military and private medical sectors.

828

BANGLADESH OBSERVED NATIONWIDE MEASLES-RUBELLA VACCINATION CAMPAIGN

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Bangladesh observed nationwide Measles-Rubella (MR) vaccination campaign from 25 January to 13 February 2014. This three weeks rolling campaign supplemented and complemented the routine immunization effort to achieve and maintain high population immunity against measles and rubella as well as to provide a second opportunity for measles-rubella (MR) vaccination among the susceptible children. About 52 million children aged 9 months- <15 years were targeted for MR vaccination through this campaign. The key objective of the MR campaign was to achieve at least 95% coverage of measles and rubella to develop high population immunity against these two diseases. 52,745,231 children aged 9 months to <15 years were enlisted to vaccinate with MR vaccine through educational institutions and community EPI sites. School going children were vaccinated in educational institutions and non-school going children were vaccinated in community sites. There were about 157,983 community sites and 158,555 educational institution sites to give MR vaccine. For successful outcome, efforts were given on advocacy and communication. All vaccinators received training on MR vaccination and campaign activities. Orientation programs were organized for volunteers and school teachers in view to seek their cooperation during campaign. The national EPI team and partner organizations observed that the presented administrative coverage data were good at national level and for most of the districts and city corporations. As per micro-plan 53,644,603 (101.7%) had been vaccinated, among them 32,933,783 (62.4%) children were vaccinated in educational institutions and 20,710,820 (39.3%) children were vaccinated in community sites. MR campaign was conducted safely and minimum number of AEFI happened and reported.

829

MOTHER AND CHILD PAIRS DOUBLE BURDEN OF MALNUTRITION IN THE SAME HOUSEHOLD, IN BENGO PROVINCE, ANGOLA

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The coexistence of undernutrition and over nutrition, known as double burden of malnutrition, presents significant threats to human health, especially in countries that are undertaking rapid economic development. In this countries, factors as the diet transition from high carbohydrates, low fat diet to a diet with refined grains, meat, oils and dairy products, all high fat content is happening. The present study, included in a cross sectional study in Bengo Province Angola, aims to identify modifiable factors associated with the occurrence of mother overweight and child underweight in the same household. A total of 622 mother child pairs were included in the analysis. From the total of 622 mothers, 6.4% were underweight (BMI < 18.5), 60.3% were normal weight and 23,3% were overweight (BMI 25-30) and 10% were obese (BMI >30). In what concerns the children, wasting was observed in 20,1 % (WHZ score under -1), stunting in 61.9% (HAZ score under -1) and underweight in 42,9 % (WAZ score under -1). Concerning mother/child pairs, 11,1 % of the pairs revels a relation of overweight mother/underweight child, 32,2% normal weight pair, 22.2% overweight mother /normal weight child, 28.1% normal weight mother /underweight child and 3.7% were both underweight. The pairs overweight mother / underweight child (n=200), and normal weight mother and child (n=69) were included in a case control analysis. Overweight mothers tend to be older, and with lower frequencies attending secondary or university studies. The study did not report any association between dual burden of malnutrition and mother employee, age and gender of the child, household size or Household month income. A significant association was observed in the multivariate analysis with place of birth, with an apparent risk reduction with home born. The

present study confirms the existence of dual forms of malnutrition in the same household in Angola. Further studies are necessary to understand the factors responsible for this coexistence. Moreover, this information is vital in the preparation of food intervention programs addressed to the adoption of proper and healthy behaviors.

830

JOINING THE HALVES: A PRIVATE PUBLIC PARTNERSHIP TO MAKE ROUTINE HEALTH REPORTING ATTRACTIVE TO PRIVATE SECTOR PROVIDERS IN UGANDA

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An estimated 50% of fever cases seek care from private-for-profit (PfPs) providers, highlighting their significant role in serving the population in Uganda. Although all PfPs are mandated to report health data, there are challenges with non-availability of private sector friendly tools and this has hindered regular reporting of health data by PfPs, resulting in limited knowledge of the private sector contribution to disease control and surveillance. As part of the UNITAID-funded project to expand malaria rapid diagnostic tests (mRDTs) use in Wakiso, Uganda, 289 participating outlets were trained and availed with a user friendly individual-level data register to strengthen routine monitoring. The register was developed in collaboration with the District Health Team (DHT) and was compatible with the Health Management Information System (HMIS). Reporting outlets were incentivized by awarding certificates of recognition during district-led provider meetings. The creation of this tool, improved capacity for reporting on HMIS malaria indicators was achieved, with 10 (3.5%) outlets reporting at baseline in August 2014 and 210 (72.7%) outlets reporting in June 2015. The collaborative involvement fostered a partnership between the PfPs and the DHT and provided a platform for PfPs to transition to directly report into the HMIS. Customizing data collection tools to the private sector, administering trainings that highlight the benefits of reporting, promoting public-private partnerships and provision of non-monetary incentives has demonstrated improvement in compliance of reporting essential routine health indicators. Strategies for expansion will be presented.

831

IMPACT OF MEASLES MASS-VACCINATION CAMPAIGN AMONG THE CHILDREN UNDER FIVE YEARS OLD IN THE DEMOCRATIC REPUBLIC OF THE CONGO

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Measles is a highly contagious viral infection. Initial signs and symptoms typically include fever >40°C, rash, cough, runny nose, and inflamed eyes. Measles continues to be a leading cause of vaccine-preventable deaths worldwide, most of which occur in resource-limited countries. In 2014, an estimated 17,574 measles cases were reported in the African region. In the Democratic Republic of Congo (DRC) epidemics of measles have been reported since 2010 due to postponed supplementary immunization activities (SIAs). Since, provincial level SIAs have been conducted in each province.Using dried blood spots collected during the 2013 Demographics and Health Survey (DHS), we assessed the seroprevalence of anti-measles IgG among children children 6-59 months of age in the DRC. We overlaid SIA data obtained from DRC's Expanded Programme on Immunization (EPI) to determine if there were correlations between vaccination campaigns and measles immunity, and calculated the Correlation Coefficient.
In provinces that experienced an SIA in 2013, 77% of children were seropositive for measles, while in provinces with no SIA, only 59% of children were seropositive. The correlation coefficient is 0.420.Our findings suggest the importance of a strong routine immunization program coupled with frequent SIAs. While we were unable to determine whether Repeated occurrences of large-scale outbreaks in DRC suggest the need to reevaluate and modify DRC's measles prevention and control strategies to meet regional elimination goals.

832

SEVERE MATERNAL MORBIDITY AND ASSOCIATED FACTORS IN TWO LARGE HOSPITALS IN THE ASHANTI REGION, GHANA

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Ghana's maternal mortality ratio is one of the highest in the world. To achieve significant reduction in maternal mortality there is a need to go beyond analyzing mortality and explore the risk factors of maternal morbidity. Studying Severe Maternal Morbidity (SMM) enables faster quantitative analysis and makes it possible to obtain in-depth information on the affected woman herself. The research was therefore conducted to determine factors contributing to severe maternal morbidity in Suntreso and Kumasi South Government hospitals in the Ashanti region of Ghana. A case control study was conducted at the Suntreso and Kumasi South Government Hospitals of Ghana between January 2015 and June 2015. WHO near miss classification system was used to identify maternal near-miss. Univariate analyses of categorical variables were expressed as frequencies and proportions. Factors independently associated with severe maternal morbidity were determined by multivariate analysis with a significance level of 5%. Among 2,238 pregnant women, 15 maternal near miss (MNM), 7 maternal deaths (MD) and 71 potentially life-threatening conditions (PTLC) were identified. The maternal mortality ratio was 229.6 cases/ 100,000 LB with a mortality index of 31.8%. The most diagnosed potentially life threatening condition was postpartum hemorrhage (57.7%). Risk factors of severe maternal morbidity identified were preterm delivery (<37 weeks) [aOR 7.8 95%CI (3.0 - 20.2)], caesarean section in current pregnancy [aOR 9.7 95% CI (3.1-30.2)], and anemia during the current pregnancy [aOR 8.1 95% CI (2.9 - 22.2)]. Factors associated with SMM were preterm delivery, caesarean section in current delivery and anemia in current pregnancy. The use of herbal preparation during pregnancy though not associated with SMM was used by almost half of the study participants.

833

FACTORS AFFECTING REPORTING RATES OF COMMUNITY VOLUNTEERS IN AN MHEALTH INTERVENTION REPORTING DATA THROUGH CELL PHONES

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In lower-income countries, many mHealth solutions have been devised to empower health workers and facilitate information systems. For example, in Zambia, cellular phones are used to transmit information on malaria trends as well as water and sanitation access using community volunteers. mHealth solutions, and particularly those using volunteers, are attractive to global health programs for a number of reasons including the rapid transmission and availability of data as well as the cost-efficiency of using community volunteers to generate and send data. The strength of these mHealth information systems lies in the reporting rates of the data and the opportunity to gather data from community-level. Little research however is available on what influences reporting rates. From our experience in mHealth we have seen systems with <15% reporting each time period,

but also other systems with >85% reporting each time period. We utilize information system data from the WASH information system in Zambia to determine factors associated with reporting rates first at the district level, and then by community volunteer. We measure the impact of seasonality, remoteness (an indicator of cell coverage), increasing the number of data elements reported and follow-up from national-level staff. These analyses are planned for April 2016, with results available by May 2016. In this presentation we will discuss what factors influence reporting rates for mHealth surveillance solutions and the implications for global health programs utilizing mHealth.

834

IMPACT OF COMPUTERIZED PHYSICIAN ORDER ENTRY (CPOE) SYSTEM IN PREVENTING PRESCRIPTION ERRORS IN ADMITTED PEDIATRIC PATIENTS IN A DEVELOPING COUNTRY

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Prescription errors could account for up to three-quarters of iatrogenic deaths especially in the pediatric settings. Computerized Physician Order Entry (CPOE) with decision-support analysis has shown to be effective in reducing errors in resourceful settings. In resource-limited setting, the implementation of CPOE is challenging. This study evaluates the impact of CPOE in preventing prescription errors in a low-income setting.In this quasi-experimental study, patients admitted to the pediatric department Mayo Hospital Lahore, Pakistan were studied. The main intervention was a CPOE system with decision support analysis that was installed and piloted over the period from January to May 2014. Prescriptions of patients admitted in the period from seven months before the implementation and after the implementation were assessed by a resident. Physician only prescriptions continued to be issued in the post-implementation period and were included in the comparison. The final analysis compared the physician errors per 100 patient days between the physician only and the CPOE system.The sample included 2156 patients, 1103 in the pre-implementation phase and 1053 in the post-implementation period. The error rate for physician only prescriptions was 29.4 per 100 patient days. The error rate for CPOE system (3.9 per 100 patient-days) were nearly seven times less than the physician only system (Incidence Rate Ratio = 0.13; 95% Confidence Interval = 0.11 - 0.13). The error rate in physician only prescriptions was almost two times higher for patients who died than those who were discharged (47.4 vs. 23.5 per 100 patient-days). The error rates were also higher in patients in the CPOE group who died compared to those discharged (6.0 vs. 3.5 per 100 patient-days).CPOE system is feasible and effective in a resource-limited pediatric setting. Healthcare stakeholders in the resource-limited settings might need to prioritize CPOE like systems to prevent iatrogenic deaths.

835

LINKING HOUSEHOLD AND POINT-OF-CARE DATA TO ESTIMATE COVERAGE OF APPROPRIATE MANAGEMENT OF CHILDHOOD ILLNESS IN SOUTHERN PROVINCE, ZAMBIA

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Studies of indicator validity have demonstrated that household surveys may inaccurately estimate coverage of health interventions, including treatment of key child illnesses. Where household surveys are insufficient to estimate coverage, there is need for new methods for generating accurate measures of health intervention coverage for measuring global progress. Linking population-based data with point-of-care (POC) information on service readiness and quality of care (QOC) has been proposed as a means of generating more accurate coverage estimates. A 2016 survey was prospectively designed to collect temporally and geographically proximate population-based care-seeking data and POC readiness / QOC data to estimate coverage of appropriate management of child illness. Mothers of children <5 years old in Southern Province, Zambia were randomly sampled. Reported care-seeking events were ascertained in each household using a questionnaire modeled off the Zambia Demographic and Health Survey. Information on service readiness and QOC for child curative services was collected within 6 weeks of the household survey from all significant POCs in the study area, including public, private, informal, and traditional providers. Service readiness was assessed using a survey tool modeled off the WHO service availability and readiness assessment (SARA). Care-seeking data were collected for 537 children in urban areas and 547 children in rural areas. Service readiness and QOC data were collected for 75 POCs. Household and POC data were combined using an exact-match linking method, which assigned each illness episode a value for likelihood of appropriate treatment based on the POC readiness / QOC score of the reported source(s) of care. These values were then used to generate coverage measures for appropriate management of child illness. Analogous coverage measures were generated using 4 common geographic linking methods, and compared against the estimates generated through the exact-match method to assess bias introduced through geographical linking methods. Implications and recommendations for coverage measurement will be discussed.

836

USING THE KEMRI-CDC HEALTH AND DEMOGRAPHIC SURVEILLANCE SYSTEM TO DEMONSTRATE THE CHANGING NEONATAL MORTALITY RATE BETWEEN 2003 AND 2012 IN RURAL WESTERN KENYA

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Understanding the burden and causes of neonatal mortality is necessary for making progress towards Millennium Development Goal #4. We examined the overall rate, trends, epidemiology, and factors associated with neonatal mortality in the Western Kenya Health and Demographic Surveillance System (KHDSS), 2003 to 2012. The KHDSS, located in Bondo and Siaya Counties in rural Western Kenya, covers an area of 371 sq. km and a population of 159,000 people. Village reporters notify about all births and deaths. Standardized verbal autopsy questionnaires were completed by trained interviewers and the cause of death was assigned by a panel of three clinical officers, using ICD10 codes. Neonatal (≤28 days) and early neonatal (≤7 days) mortality rates were calculated per 1,000 live births. We explored factors associated with neonatal death using logistic regression to compare deaths to non-deaths among neonates. During 2003-2012, there were 52,385 live births, 899 neonatal deaths and 634 (70.5%) early neonatal deaths. The overall neonatal mortality rate (NMR) was 20.1 per 1000 live births (95% confidence intervals [CI] 19.7 -25.2) and early NMR was 14.4 per 1000 LB (95%CI 11.5-18.2). Neonatal deaths represented 18.3% of infant deaths. Higher maternal age, secondary education, and higher SES were significantly less common among neonates that died compared with those that survived. Verbal autopsies were done for 533 (71.3%) of neonatal deaths. Overall, the leading causes of death were neonatal sepsis (39%), pre-maturity (18%), and respiratory distress (10%). The leading causes of death in the first week of life were neonatal sepsis (28.8%) and pre-maturity (18.6%). Medical care was sought prior to death for 106 (11.8 %) neonates overall, including 45 (7.1%) of those that died in the first week of life. The burden of neonatal

mortality in Western Kenya is high, and the majority of neonatal deaths occur in the first week of life. Most neonates die without having received medical attention for their illness. Deaths from neonatal sepsis might be reduced through community-based management to ensure earlier access to antibiotic treatment.

837

STUDENTS AS AGENTS OF CHANGE: EXPERIENCES FROM SUDAN IN UPDATING NATIONAL SCHOOL CURRICULA TO INCLUDE TRACHOMA MESSAGING

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Beginning in 2014, the Sudan national trachoma control program (NTCP) began developing methods to incorporate trachoma messaging into their respective national and regional school sanitation guidelines. The aim was to focus attention on school-based programming that would result in schools and students becoming more involved in sanitation campaigns in the community thereby becoming agents of change within their homes and communities. In the Republic of Sudan, the NTCP has worked to engage the Federal Ministry of Health (FMOH) and the Federal Ministry of Education (FMOE) in an effort to coordinate the development of school health education materials. As part of this process, The Carter Center and NTCP developed school health education materials, while the National Centre for Curriculum and Education Research and FMOE revised and approved the trachoma curricula. Together, the FMOH, FMOE, NTCP and The Carter Center produced teachers' guidelines for basic and secondary schools on how to deliver information related to trachoma control. In 2015, 72 state coordinators, education inspectors and school hygiene coordinators were trained on how to be trainers of others. An additional 2,000 teachers were trained on the trachoma curricula and over 105,000 trachoma curricula and 1,900 manuals have been distributed in basic and secondary schools. Though the implementation of these activities are currently limited to the areas in which The Carter Center is assisting the FMOH and NTCP, it is hoped that they will provide a model that can be expanded to other parts of the country and used as an example for neighboring endemic countries that seek to integrate trachoma messaging into their own curriculum.

838

GENERATING AN ELECTRICITY ACCESS MAP ACROSS AFRICA

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For many health outcomes, there is a strong relationship with socioeconomic status. Demographic and Health Surveys (DHS) collect information about household indicators related to wealth in different countries across time. One such indicator is access to electricity. Unfortunately, due to the varying periodicity and spatial coverage of these surveys, the information they contain is not representative at a regional scale. Fortunately, unlike other indicators such as access to drinking water or education, access to electricity can be indirectly measured via remote sensing. National Oceanic and Atmospheric Administration satellites have been capturing images of nighttime light globally since 1992. While nighttime light images do not immediately translate into electricity access of households, we define a model to learn the association between both. We use information from 73 DHS surveys, light intensity extracted from inter-calibrated night light images and population density. We follow a Bayesian model based geostatistics approach, and use integrated nested Laplace approximation (INLA) to make inference on household's electricity access in Africa. Results show that access to electricity has a positive log-linear relationship with nighttime light intensity and population density. The model is used to predict the probability of a household having electricity annually from 2000-2015 across Africa at 5km resolution. These freely available maps describe the story of electrification across Africa and will be provide insight, in future work, on the link between infectious diseases and socioeconomic resources in the region.

839

VOTING FOR BETTER HEALTH IN DEPRIVED COMMUNITIES

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The Global Health Exchange Fellowship was a pilot project, designed to make global health real through experiential learning for UK and Kenyan trainees in family medicine/general practice and public health.Using Qualitative research methods, a health needs analysis was performed in two deprived areas- a rural Maasai community in Kenya and an inner city in the UK. Health issues identified were categorised into themes, which were prioritised by the community using an innovative voting methodology developed by the fellows. The same methods were applied in both countries.The voting method allowed each community a voice, in prioritising their health needs. Using the Capability Approach sustainable solutions were sourced within the community. Findings were presented to local health authorities to inform local resource allocation, improve health and reduce inequalities.The fellows learned a great deal about global health challenges in both high and low income countries. A methodology of community voting was established, providing insight to the true health needs of each community.This methodology provides new understanding from the perspective of two communities on global health, including social determinants of health. There is remarkable potential for its widespread use. Similarities in themes in areas of deprivation in low and high income countries is noteworthy. In Kenya, access to healthcare was voted as the number one priority. Had we taken an epidemiological approach, we may have found ourselves tackling specific diseases. However, the voting method identified the health issues that were much closer to the true health needs of the community. This was particularly important in Kenya, where there was no data available for our community. In the UK there is a wealth of data, therefore this project sought to address the "Why" and the "How", thus developing sustainable strategies to address health needs, whilst encouraging community ownership through Sen's Capability Approach.

840

A MULTI-'OMIC SYSTEMS BIOLOGY APPROACH TO IDENTIFYING HOST AND PARASITE FEATURES THAT CONFER RESILIENCE TO MALARIA INFECTION

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The Malaria Host-Pathogen Interaction Center (MaHPIC) and the Host Acute Models of Malaria to study Experimental Resilience (HAMMER) Project are intertwined systems biology investigations that are generating a wide array of biological, clinical and multi-omic data sets of diverse *Plasmodium* species in their non-human primate (NHP) and human hosts. Using NHP models, the MaHPIC project is investigating changes in host

immune status, erythrocyte phenotype, and metabolic state alongside changes in parasite abundance, stage, and gene expression through the use of clinical and multi-omic measurements. Using both mathematical modeling and data integration approaches, we are identifying multi'-omic profiles that are associated with the observed variation in the severity of malarial disease in the animal cohort studies. These studies involve *Plasmodium cynomolgi* and *P. coatneyi* infections of *Macaca mulatta* (rhesus monkey) to model *P. vivax* and *P. falciparum*, respectively; as well as *P. vivax* infections of the New World monkey species *Aotus nancymaae* and *Saimiri boliviensis.* Further, we are conducting high-resolution metabolomics analyses in plasma from human clinical cohort studies led from South America, Southeast Asia and Sub-Saharan Africa. Upcoming work will focus on characterizing multi-omic profiles of infection by *P. knowlesi* in two different host species with differing susceptibility to malaria disease, *M. mulatta* and *M. fascicularis*. The aim of this work is to identify host features that confer resilience to malarial disease. Altogether, we aim to identify novel host and parasite factors involved in malaria disease progression in NHPs, and translate these findings to what is observed in humans. The MaHPIC and HAMMER projects include an extensive bioinformatics infrastructure to generate and release datasets for use by the broad scientific community.

841

MOLECULAR DISSECTION OF THE *PLASMODIUM* SPOROZOITE SURFACE GAPDH FOR MALARIA LIVER INVASION

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Malaria, caused by parasites of the genus *Plasmodium*, is among the most devastating parasitic diseases worldwide. The bite of an infected *Anopheles* mosquito releases less than 100 sporozoites in the skin but after liver infection up to 10,000 merozoites per sporozoite are produced and released into the circulation. Therefore, the pre-liver hepatic stages represent a severe bottleneck in parasite numbers and constitute a prime target for induction of sterile immunity. To infect the mammalian host, parasites must leave the circulation in the liver by preferentially traversing Kupffer cells that together with endothelial cells, line the liver blood vessels (sinusoids). Previously we have identified CD68 on the Kupffer cell surface as a receptor for sporozoite traversal. We now report that *Plasmodium* GAPDH on the sporozoite surface serves as a ligand that interacts with CD68. Current experiments seek to define GAPDH domains involved in this interaction. Such domains are potential epitopes for the development of a pre-erythrocytic vaccine.

842

PFEBA-175 MEDIATED ROSETTING ENHANCES GROWTH OF *PLASMODIUM FALCIPARUM* AND OVERCOMES INHIBITORY ANTIBODIES: IMPLICATIONS FOR SEVERE MALARIA

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Severe malaria is defined by high parasetemia and anemia and results in the majority of fatalities due to *Plasmodium falciparum* infection. The mechanisms that enable high parasite burdens in patients resulting in severe malaria and death are poorly defined. Here, we show that PfEBA-175 shed from parasites during invasion to form infected RBCs (iRBCS) facilitates the recruitment of uninfected RBCS (uRBCS) to form rosettes. This recruitment increases parasite growth resulting in a defined fitness advantage that enables high parasitemia observed in severe malaria. This is the first demonstration of growth enhancement directly due to rosetting. We show that rosette formation is dependent on PfEBA-175 engagement of the receptor Glycophorin A and identifies a novel role for the PfEBA-175:Glycophorin A interaction in addition to RBC invasion. We propose that rosetting allows for invasion of adjacent uRBCs from one iRBC, bypassing the need for daughter merozoites to search and identify uRBCs to invade in the bloodstream. This may be one mechanism by which parasites achieve the high parasite burden seen in severe malaria. Rosetting also reduces the time in which the merozoite can be targeted by the immune system resulting in immune evasion. Further, we show that rosetting overcomes the effectiveness of antibodies known to inhibit growth. The results emphasizes the need to include PfEBA-175 in anti-malarial combination therapies that aim to block rosette formation and enable the function of neutralizing antibodies. Lastly, these data demonstrate that shed proteins may confer additional functions to enhance parasite survival and opens new avenues of research for severe malaria.

843

PLASMODIUM VIVAX INFECTIONS AMONGST DUFFY-NEGATIVE INDIVIDUALS IN THE DEMOCRATIC REPUBLIC OF THE CONGO: POSSIBLE ACQUISITION FROM APES

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The World Health Organization estimates that 10% of global malaria cases and deaths occur in the Democratic Republic of the Congo (DRC) annually. *Plasmodium falciparum*, *P. ovale*, and *P. malariae* account for the great majority of cases. However, little is known about *P. vivax* infection in the DRC. We selected 618 dried blood spots in the 2013-2014 Demographic and Health Survey of the DRC, a large population-based survey. Four cases of *P. vivax* infections were identified by PCR, each in a geographically different survey cluster. Using these as index cases, we tested all the samples from the four clusters. With this approach, an additional ten cases of *P. vivax* were identified. Among the fourteen *P. vivax* cases, nine were coinfected with *P. falciparum*. To assess host susceptibility to *P. vivax*, we PCR-amplified and sequenced each host's Duffy antigen/chemokine receptor gene (DARC) for the single point mutation in the GATA motif that represses the expression of the Duffy antigen. All fourteen hosts infected by *P. vivax* were Duffy-negative. This finding is consistent with a growing body of literature that suggests that *P. vivax* can infect Duffy-negative individuals in Africa. Next-generation sequencing of the mitochondria of four of these infections suggests that at least one of these infections contains strains of both human and ape origin. Currently, we are exploring the origins of these *P. vivax* infections using phylogenetically informative regions on six loci that have been previously used to distinguish non-human ape from human *P. vivax* strains. These results suggest that both human and ape strains of *P. vivax* exist within the DRC and the apes may represent a potential reservoir of *P. vivax* for at least some human infections.

844

CEREBROSPINAL FLUID CYTOKINE AND CHEMOKINE LEVELS AND NEUROCOGNITIVE FUNCTION IN UGANDAN CHILDREN WITH CEREBRAL MALARIA

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Inflammation appears to play an important role in cerebral malaria (CM), but little is known about which inflammatory factors contribute to development of CM or neurocognitive sequelae after CM. Assessment of cerebrospinal fluid (CSF) cytokines and chemokines in children with CM may provide the best available measure of the central nervous system (CNS) inflammatory process in these children. We measured CSF and plasma cytokine levels of 15 pro- and anti-inflammatory cytokines, chemokines and growth factors in 146 Ugandan children with CM and 10 North American control (NAC) children. Overall cognition, attention and associative memory were tested in children with CM 12 months after the CM episode. CSF levels of all factors were significantly higher in children with CM than in NAC. In children with CM, CSF and plasma values correlated significantly for 11 of 15 factors, suggesting that these proteins cross an impaired blood-brain barrier (BBB) into the CNS. CSF interleukin-8 (CXCL-8/IL-8) and monocyte chemoattractant protein 1 (CCL-2/MCP-1) levels were higher in CSF than plasma. Increased CSF IL-8 levels were the only factor associated with mortality (P=0.05). In children <5 years, increased CSF granulocyte-colony stimulating factor (G-CSF), IL-1 receptor agonist (IL-1ra) and MCP-1 levels correlated with worse cognitive ability, while in children ≥5 years, increased IL-1ra, MCP-1, macrophage inflammatory protein (MIP-1) and CCL5/RANTES correlated with worse cognitive ability (P value range, 0.006-0.04). No association was seen for any CSF protein with attention or associative memory. Children with CM have increased levels of pro- and anti-inflammatory cytokines and chemokines, likely due to passage from plasma to CNS across an impaired BBB, except in the case of IL-8 and MCP-1, which appear to be produced in the CNS. The association of increased CSF IL-1ra and MCP-1 with worsened cognitive scores in all ages suggests that these factors may be involved long-term brain injury in children with CM, but further study in other cohorts is needed, as these factors were not strongly associated with cognitive impairment.

845

A NOVEL FACS TECHNIQUE TO MEASURE AUTOPHAGY IN *PLASMODIUM FALCIPARUM*

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Autophagy is a normal homeostatic process by which cells degrade waste. The purpose of this research project is to develop a novel FACS technique to measure autophagy in *Plasmodium falciparum*. At least 26 homologs of established autophagic proteins exist in *P. falciparum* and recent publications suggest that autophagy occurs in the parasite in response to stressors; however, a robust assay has not yet been developed for the measurement of autophagy in this parasite. A recently developed assay to measure autophagy in mammalian cells involves the use of FACS to measure autophagosomes and lysosomes using pH-specific dyes. LysoID specifically stains lysosomes by staining low pH. Although lysosomes have not been specifically identified in *P. falciparum*, LysoID appears to stain the food vacuole—the acidic compartment of *P. falciparum*—as confirmed by confocal imaging. In other organisms, CytoID stains autophagosomes by staining intermediate pH and using Atg8/LC3 as an anchor. Previous studies have observed upregulation of autophagy in *P. falciparum* following a 6-hour amino acid starvation period. We incubated 3D7 for 6 hours in amino acid-free media, and found that intraerythrocytic ring-stage parasites stained with LysoID had significantly higher mean fluorescence intensity (MFI) than those that were incubated in complete media (979.3 vs. 555.5, p<0.001). In other systems, an increased LysoID signal alone can be used as a marker of autophagy induction. CytoID stained starved intraerythrocytic parasites with slightly greater intensity than normal parasites, though this was not significant (MFI 63.0 vs. 58.1, p=0.27). This project demonstrates the potential for using FACS to measure autophagy in *P. falciparum*.

846

DEVELOPMENTAL CYCLE AND TISSUE SEQUESTRATION OF *PLASMODIUM VIVAX* TRANSMISSION STAGES IN THE NON-HUMAN PRIMATE MODEL

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The recently launched global effort to eradicate malaria has been stimulated by the dramatic decrease of the disease incidence in sub-Saharan Africa. While *Plasmodium falciparum* is the deadliest of human malaria parasites, *P. vivax* is a major cause of malaria morbidity within and outside of Africa. *P. vivax* is now a major focus of the ongoing elimination agenda, with particular emphasis on development of *in vitro* culture systems and understanding of key biological features, such as latency and transmission, as a basis for better diagnosis and new interventions. Though not well studied it is believed that transmission stages, or gametocytes, of *P. vivax* take 48 hours to develop and are present in circulation throughout their cycle. They appear in blood circulation 3-5 days after the first asexual parasites are detected microscopically, and therefore transmission can occur well before the patient is symptomatic. The goal of the present study was to develop diagnostic markers to characterize the *P. vivax* transmission cycle in the *Aotus* non-human primate model and for future field studies. Comparative transcriptional analysis of *P. falciparum* versus *P. vivax* gametocytes demonstrated a conserved cascade of stage specific gene expression until maturity despite significantly different cycle length. A subset of conserved gametocyte stage-specific markers was successfully validated by quantitative Real-Time PCR (qRT-PCR) and antibody assays in peripheral blood samples from infected *Aotus* monkeys. Systematic investigation of different tissues from infected monkeys indicated an enrichment of gametocytes in the bone marrow and sub cutaneous fat by a multiplex qRT-PCR assay with our stage-specific markers. To investigate possible tissue specific sequestration of *P. vivax* gametocytes during infection, detailed histological analyses are ongoing to determine localization of specific parasite stages across the organs.

847

BIOENERGETIC CHARACTERIZATION OF MUTANT *PLASMODIUM FALCIPARUM* STRAINS RESISTANT TO MITOCHONDRIAL INHIBITORS

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The *Plasmodium* mitochondrion, in particular the ETC enzymes, has been considered as a promising drug target and there have been many reports of antimalarial agents targeting cytochrome *bc1* complex and DHODH. However, there are little bioenergetic studies regarding resistant

strains against mitochondrial inhibitors and fitness cost of the mutations. Previously, we developed a robust bioenergetic assay protocol utilizing an Extracellular Flux Analyzer that enables simultaneous investigation of mitochondrial respiration and glycolysis of *Plasmodium falciparum* in a physiologically relevant microenvironment with readout of an oxygen consumption rate (OCR) and extracellular acidification rate (ECAR). Using this assay protocol, we observed OCR increase by substrates of the ETC complexes, such as succinate, glycerol-3-phosphate and dihydroorotate in saponin-freed schizont stage parasites. In this study, we first compared OCR responses to the ETC substrates between Dd2 and mutant strains, BTZ^R, ATV^R and IDI-5994^R which are resistant to cytochrome *bc1* inhibitors, benzothiazepine, atovaquone, and IDI-5994 respectively. As a result, we found that all resistant strains had smaller OCR elevation compared to their parental Dd2 and that the degree of OCR response decreased in the following order: IDI-5994^R, BTZ^R and ATV^R. IDI-5994^R strain has mutation in Qi site of cytochrome *b*, while $BTZ1^R$ and ATV^R strains have mutations in Qo site, and therefore our observation might suggest that Qo site mutations have more impact on electron transfer to cytochrome *c*. Interestingly, ECAR readout showed that glucose increased glycolytic activity more slowly in BTZ^R and ATV^R strains than in IDI-5994^R and Dd2 strains. In addition, glucose titration revealed that ECAR elevation reached a plateau with 4mM of glucose in BTZ^R strain, while 8mM is required in Dd2. These observations might indicate that BTZ^R strain enhances its dependency to glycolysis to overcome fitness cost caused by the mutation. Further bioenergetic profiling of various strains including DHODH mutant strains will be discussed.

848

DEVELOPMENT OF A NOVEL MOUSE MODEL FOR PREGNANCY MAINTENANCE DURING MATERNAL MALARIA INFECTION

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Placental malaria, a severe clinical manifestation of *Plasmodium falciparum* infection observed in pregnant women, is a major cause of pregnancy loss, neonatal mortality, and severe maternal illness. Mouse models for malaria infection during pregnancy are vital to understanding the mechanisms underlying these outcomes. Here, we describe a novel mouse model for pregnancy maintenance during maternal malaria infection utilizing outbred Swiss Webster mice. When infected with *P. berghei* or *P. chabaudi* in early gestation, most mouse strains will abort their pregnancies at mid-gestation. However, outbred Swiss Webster mice infected with *P. chabaudi* AS in early gestation carry their pregnancies to term, providing a model for pregnancy maintenance during maternal malaria infection. Furthermore, as previously observed in non-pregnant mice, the gut microbiota of pregnant Swiss Webster mice influences the severity of malaria infection. Mice with 'susceptible' gut microbiota develop higher parasite burdens compared to mice with 'resistant' gut microbes. Despite the severe infections observed in 'susceptible' mice, these mice do not abort their pregnancies although litter sizes at term are reduced. Overall, this model provides a tool for exploring the mechanisms of embryo and fetal survival during maternal malaria infection, as well as the influence of the gut bacterial community on the severity of malaria infection in the context of pregnancy.

849

INVESTIGATING THE ROLE OF ACS5 IN *PLASMODIUM FALCIPARUM* FATTY ACID METABOLISM

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The remarkable plasticity of the *Plasmodium falciparum* genome allows for adaptation in response to selective pressures and challenges efforts to combat this important human pathogen. Evidence of the adaptive nature of this genome includes the expansion and recent positive selection of the acyl Co-A synthetase (ACS) gene family, which includes four orthologs predicted to activate exogenous fatty acids (FAs) and play important roles in fatty acid scavenging as well as nine paralogs with unknown function. The evolutionary and functional significance for the expansion of the PfACS9 ortholog to nine paralogs, including PfACS5, is unknown. In this study we sought to functionally characterize these molecules to understand their biological role in the parasite. Using the CRISPR-Cas9 gene editing system, we successfully knocked out ACS5, a member of this expanded family. The ACS5 knockout (KO) line shows reduced growth *in vitro*. This phenotype is exacerbated by limiting growth conditions to 45% glucose supplemented with minimal fatty acids. Here, we explore this growth defect and the functional role of ACS5 in the parasite using molecular and biochemical approaches. We compare changes in the FA profile of the ACS5 KO line and its 3D7 parent. Using an LC-MS/MS approach, we profile the metabolome of the ACS5 KO and the 3D7 parent, and identify changes in key lipid species in the KO. Using these approaches, we are exploring the role of ACS5 in downstream metabolic pathways, including desaturation and elongation pathways, as well as phospholipid biosynthesis. We hypothesize that the expansion and recent positive selection of the PfACS gene family are the consequence of metabolic pressures driving parasite evolution. Therefore, understanding FA metabolism will give us insight into key metabolic pathways that might serve as potential targets for novel antimalarials.

850

METABOLIC CONVERSION OF CARBOXY-PRIMAQUINE, A MAJOR METABOLITE OF PRIMAQUINE, TO POTENTIAL HEMOTOXIC INTERMEDIATES

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Plasmodium vivax malaria has broader geographical distribution than falciparum, forms dormant hard to kill hypnozoites liver stages, which can activate weeks to years after primary infection causing relapsing malaria episodes. Primaquine (PQ) is the only drug approved for prevention of malaria relapse. PQ also has activity against stage V mature gametocytes of *Plasmodium falciparum* and a key transmission-blocking agent. However, PQ causes severe hemolytic anemia in individuals with genetic deficiency of enzyme glucose 6-phosphate dehydrogenase (G6PD). Metabolism of PQ through oxidative deamination pathway generates high plasma levels of carboxyPQ (CPQ). This pathway determines characteristic pharmacokinetic properties of PQ and is primarily responsible for its short half-life. This is considered as a major therapeutic limitation of PQ, which requires a 14 days long treatment for malaria radical cure. The plasma levels of CPQ remain high even after 24 hours of treatment with PQ. CPQ has been considered to be a non-toxic and inactive metabolite of PQ. However, recent studies suggested further metabolism of CPQ through CYP mediated pathways generating potential hemotoxic metabolites. CPQ with pooled human liver microsomes (HLM) generated marked hemolytic toxicity response *in vitro*. Further, CYP profiling analysis showed that CYP1A2 and CYP2B6 were the major CYPs, which can elicit the *in vitro* hemotoxic response to CPQ, with CYP3A4 and CYP2D6 having less prominent effects. Incubation of CPQ with pooled HLM resulted in more than 35% depletion of CPQ within 2 hours. Hydroxy CPQ (m/z 291) and quinone-imine (m/z 289) were identified as the major metabolites. Interestingly, metabolism of CPQ with pooled HLM was not enantioselective. The studies confirm further metabolism of CPQ through CYP mediated pathways, which generated reactive quinone-imine CPQ and hydroxyl CPQ, the potential hemolytic metabolites. The studies indicate interesting differences in CYP mediated pathways for metabolism of PQ and carboxyPQ. These results warrant further evaluation of CPQ for the potential to cause red cell damage in G6PD-deficient individuals.

851

PHOSPHORYLATION OF *PLASMODIUM* EUKARYOTIC INITIATION FACTOR 2α IN RESPONSE TO ARTEMISININ THERAPY

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Artemisinin and its derivatives are the most potent anti-malaria drugs. Nevertheless, artemisinin monotherapy is associated with accumulation of dormant ring stages and with recrudescence of *Plasmodium* infection, which is considered a treatment failure. The molecular mechanism(s) leading to parasite dormancy are under investigation. Here we report that dormancy is associated with phosphorylation of the parasite's eukaryotic initiation factor-2α (eIF2α). In an attempt to reveal which one of three *Plasmodium* kinases, eIK1, eIK2, or PK4 is involved we generated knockouts of the enzymes. Following artesunate treatment, the eIK1(-) and eIK2(-) parasites phosphorylated their eIF2α and entered dormancy like wild type. We could not obtain knockouts of PK4 because it is essential for blood stage development where the gene targeting takes place. Nevertheless minutes after drug treatment PK4 dimerized, autophosphorylated and phosphorylate eIF2α indicating that it is the enzyme that controls dormancy. Artesunate-induced dormancy is extended by salubrinal, a selective inhibitor of the eIF2α-P phosphatases, which provides evidence for the mechanism that *Plasmodium* phophorylates eIF2α causing dormancy in response to artemisinin treatment.

852

EFFECTIVE SCALING-UP OF SEASONAL MALARIA CHEMOPREVENTION IN BURKINA FASO

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Burkina Faso scaled-up SMC implementation in 2015, covering 17 districts with a population of about 900,000 children under 5 years of age. Delivery, primarily door-to-door, was for four months starting late July. Each month the first dose of the 3-dose regimen was administered by a health worker and the remaining doses left with the caregiver. To evaluate the effectiveness of SMC delivery at scale, a survey was conducted at the end of the transmission season in 11 districts where SMC was delivered through the ACCESS-SMC project. 50 villages were selected with probability proportional to size, and households selected using compact segment sampling. SMC record cards were inspected and caregivers were asked about adherence, side effects, reasons for missed treatments, the time and any costs involved to obtain SMC for their child, the caregiver's level of education and socioeconomic status. Utilisation of insecticide-treated bednets by household members was also recorded. Children up to 7 years old were included in order to determine if children above the recommended age limit were being treated. Data were collected using Android tablet devices. 1000 children were surveyed. 741 of these were eligible to have received 4 cycles of SMC (aged between 3months and 5years at the first cycle). Of these 94% had received an SMC card and at least one SMC treatment. 83% received at least 3 cycles. 97% of caregivers reported that they had administered both unsupervised doses of amodiaquine the month before the survey. The mean percentage of children who attended for SMC who did not receive the treatment from the health worker, obtained from health worker tally sheets, was about 1% to 2%, mostly because the child was unwell. Of 95 children aged 6 to 7 years at the survey, 80% reported having received SMC. 89% of children slept under a bednet the night before the survey. In conclusion, a high level of coverage was achieved during 2015, in the first phase of implementing SMC on a large scale in Burkina Faso. Sustaining this achievement will be challenging.

853

PREVALENCE OF MUTATIONS ASSOCIATED WITH SULPHADOXINE-PYRIMETHAMINE (SP) RESISTANCE IN *PLASMODIUM FALCIPARUM* SAMPLES FROM THE GENERAL POPULATION AND PREGNANT WOMEN IN NANORO, BURKINA FASO

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Pregnant women are at increased risk of *Plasmodium falciparum* infection, which can result in maternal anemia, low birth weight babies and other sequelae. Most Sub-Saharan African countries have therefore implemented intermittent preventive treatment for pregnant women (IPTp) using sulphadoxine-pyrimethamine (SP). However, concerns are rising about its continuous efficacy because of increasing resistance against SP. Point mutations in the *Dhps* and *Dhfr* genes of *P. falciparum* are associated with resistance, especially combinations like the triple *Dhfr* mutant (51I, 59R, 108N), the double *Dhps* mutant (437G, 540E) and moreover the quintuple mutant. The aim of our study was to estimate the current levels of SP resistance in Nanoro, Burkina Faso and to test whether the mutation rate increases during pregnancy. Filter paper samples from pregnant women at first antenatal care visit (ANC1) and at delivery were collected from March 2014 till September 2015 as part of an intervention trial (COSMIC). Furthermore, samples from the general population (GP) were collected from March till May 2015. DNA was extracted and *P. falciparum* positive samples were detected by qPCR. Next, nested PCR was used to amplify the *Dhps* and *Dhfr* genes and products were sent for sequencing. We found a high prevalence of *Dhfr*-51, -59 and -108 overall, with a trend of higher levels in GP and delivery samples compared with ANC1 samples (70.7%, 74% and 61.5% triple *Dhfr* mutants respectively). Statistical analyses will follow, but this trend could possibly indicate selection of resistant parasites during pregnancy. However, the concurrent higher mutation rate in GP samples needs to be explored. *Dhps*-437 also showed high mutation rates (89.4%, 84% and 83.4% respectively) without a clear trend. The *Dhps*-540 mutation was found in one GP sample and in two delivery samples, of which one was a quintuple mutant. To our knowledge this is the first time the *Dhps*-540 mutation is found in Burkina Faso. This finding and the high prevalence of the other mutations raises concerns about efficacy of IPTp-SP in the future. Other drug combinations to tackle malaria in pregnancy should therefore be explored.

854

ON THE ADEQUACY OF A 28 DAY FOLLOW-UP PERIOD FOR ARTEMETHER LUMEFANTRINE AGAINST UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA

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Clinical trials are the gold-standard for deriving information on antimalarial efficacy from which policy decisions can be made. These estimates are vulnerable to methodological approaches such as the duration of study and loss to follow-up. The aim of this work is to explore the optimal duration of follow-up for capturing PCR confirmed recrudescences following treatment with artemether-lumefantrine (AL), and to assess the sensitivity of the recommended minimum follow-up duration of 28 days in patients from Africa and Asia. The cumulative baseline hazard, estimated from Cox regression models with shared frailty on study-sites and fractional polynomials to capture nonlinear associations, was used to estimate the probability density of recrudescences. The area under the

density curve (AUC) was calculated to determine the optimal follow-up period; accuracy of which was evaluated using simulation techniques. Data were available from 54 efficacy trials on AL (n=7,735; 2002-2014) in children less than 5 years in Africa and 10 trials in Asia in patients of all ages (n=1859, 2000-2010) with a minimum follow-up of 28 days. There were 221 (2.9%) recrudescent infections in Africa and 41 (2.2%) in Asia within 63 days. In studies with follow-up duration of 42 days or longer, 43% (47/109) of these recrudescences in Africa and 24% (10/41) in Asia were missed with a day 28 follow-up. The missed proportions were even higher when estimated using the AUC approach, which makes use of all available data. A shift to the left of the probability density function (i.e. recrudescences occur earlier) was observed for Asia compared to Africa. Effects of baseline parasitaemia and treatment dose on the shape and location of the density function were also studied. This pooled analysis confirms that the current recommended follow-up duration of 28 days remains inadequate for accurately determining AL efficacy and fails to identify an estimated 62% of the recrudescences in Africa and 49% in Asia. The feasibility and cost-effectiveness of a longer follow-up duration warrants further investigation while also considering misclassification errors associated with genotyping.

855

PLASMODIUM FALCIPARUM PARASITE CLEARANCE IN THE PERUVIAN AMAZON AS PART OF A DOD HARMONIZED CLINICAL TRIAL

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There is a global threat of *Plasmodium falciparum* resistance to artemisinin-based combination therapies observed in South-East Asia. Three DoD laboratories in Kenya, Peru and Thailand conducted a harmonized clinical trial to determine parasite clearance time after receiving artesunate. Data from Peru is presented.Participants between 5-65 years old with uncomplicated, microscopy confirmed, *P. falciparum* mono-infection, with asexual parasite density between 1,000 - 100,000 parasites/µL were enrolled. Participants were hospitalized for three days and received 4 mg/kg artesunate on Days 0, 1 and 2; 15 mg/kg mefloquine on Day 3, and 10 mg/kg mefloquine on Day 4. The parasite clearance rates were determined by microscopy every 4 h during first 12 h and then every 6 h until 72 h after first being treated with artesunate. Clinical and parasitological responses were assessed for 42 days.Between June 2014 and November 2015, 482 people with *P. falciparum* mono-infection were identified at eight health centers in Iquitos, in the Peruvian Amazon Basin, but most did not comply with the study requirements. Seventy-four subjects were consented and screened, and among them, 55 subjects with uncomplicated *P. falciparum* malaria were enrolled. Two participants were excluded due to serious adverse events and mixed infection. Participants cleared parasitemia by hour 42 (PCR uncorrected).The mean age of participants was 31.2 (95% CI 26.8 - 35.6), and 22 (41.5%) were female. The geometric mean parasite count at admission was 5514 parasites/µL (95% CI 4095-7426). The clearance rate constant (by hour) median was 0.32 (IQR: 0.29-0.39). The slope half-life median is 2.18 hours (IQR: 1.78-2.39). Finally, 50% and 99% parasite clearance median times (PC50 and PC99) were 5.84 (IQR: 3.07-7.28) and 17.02 (IQR: 15.22-19.35) respectively. All participants completed Day 42 follow-up and met the adequate clinical and parasitological response endpoint.No suggestion of resistance to artesunate was found among the participants evaluated in the Peruvian Amazon. However, surveillance using molecular markers such as K13 should be used as a complementary regional strategy.

856

DOES METHYLENE BLUE ENHANCE THE *EX VIVO* ANTIMALARIAL BLOOD SCHIZONTOCIDAL ACTIVITY OF ARTESUNATE-AMODIAQUINE?

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Reports of falciparum malaria patients in Cambodia and Vietnam failing treatment with dihydroartemisinin-piperaquine highlights the urgent need to contain and reduce the spread of artesunate based combination therapy (ACT) resistant strains. As part of this effort a triple drug strategy approach should be investigated to extend the useful life of ACTs. The objective of the present study was to determine whether methylene blue (MB) can enhance the pharmacodynamic (PD) *ex vivo* antimalarial activity of artesunate-amodiaquine (ASAQ). If ASAQ+MB can be demonstrated to be more potent than ASAQ alone, then the triple combination may provide a better option to treat ACT resistant malaria infections. In an open labelled, randomized cross-over design, a single oral dose of either ASAQ (2 tablets, with each tablet containing 100 mg AS and 270 mg AQ) or ASAQ (2 tablets)+MB (5 tablets, with each tablet containing 65 mg MB) was administered to 16 healthy Vietnamese volunteers. After an 8 week washout period the same participants received the alternative drug combination. Serial blood samples were collected up to 28 days after the last dose of either ASAQ or ASAQ+MB. The *ex vivo* antimalarial activity of ASAQ and ASAQ+MB was assessed by subjecting the participant's plasma samples collected after drug administration against an artemisinin-sensitive and an artemisinin-resistant *Plasmodium falciparum* line *in vitro*. Based on the participant's plasma inhibitory concentration profiles the preliminary *ex vivo* data revealed MB to enhance the blood schizontocidal activity of ASAQ by at least 2-fold against both *P. falciparum* lines. Additionally, using LC/MS/MS we determined the pharmacokinetic (PK) properties of the partner drugs including their principal active metabolites. The PK-PD relationship of ASAQ and ASAQ+MB will be compared and their implications for the treatment of multidrug-resistant falciparum malaria will be discussed.

857

PARASITE CLEARANCE AND DECLINES IN ARTEMETHER EXPOSURE OVER THE COURSE OF ARTEMETHER-LUMEFANTRINE TREATMENT FOR *PLASMODIUM FALCIPARUM* MALARIA IN UGANDAN CHILDREN

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We sought to assess the association between parasite clearance parameters and artemether (AR) and dihydroartemisinin (DHA) pharmacokinetic (PK) exposure in HIV-infected and HIV-uninfected children in Uganda treated with artemether-lumefantrine (AL) for malaria. Children ≤8 years underwent intensive PK sampling post-1st dose of AL followed by every 12-hour blood smears. AR and DHA exposure was compared to post-last AL dose PK in concurrently enrolled children using non-compartmental analysis. Parasite clearance parameters were calculated using the WWARN Parasite Clearance Estimator. Post-1st and last dose PK parameters were estimated in 103 children (77 HIV-uninfected and 26 HIV-infected) and 142 children (51 HIV-uninfected and 91 HIV-infected), respectively. In HIV-uninfected children, post-last dose AR area-under-

the-curve from 0 to 8 hours (AUC) was 3-fold lower as compared to the AUC post-1st dose of AL (ratiolast/first dose 0.31; p<0.0001), while DHA exposure increased (AUC ratiolast/first dose 1.75; p=0.0003). Additionally, AR exposure post-1st dose was 3 to 6-fold lower in HIV-infected children on efavirenz and nevirapine compared to HIV-uninfected children (AUC ratio 0.16; p<0.0001 and AUC ratio 0.35; p=0.001, respectively). Post 1st-dose DHA exposure was similarly lower in efavirenz-treated vs HIV-uninfected children (AUC ratio 0.45; p=0.028). Parasite clearance slope half-life was significantly longer in HIV-infected (3.51 hrs; 95% CI 2.98, 4.03) vs HIV-uninfected children (2.8 hrs; 95% CI 2.38, 3.36); p=0.003. AR exposure demonstrates a significant time-dependant decrease following the 1st dose of AL in all malaria-infected children, with those on efavirenz exhibiting dramatic reductions to AR and DHA throughout the dosing interval. Parasite clearance parameters are also prolonged in HIV-infected vs HIV-uninfected children. These findings have important implications for AL efficacy and the risk of selection for artemisinin resistance in this vulnerable population. Multivariate regression to explore the relationship between PK and parasite clearance parameters is underway and will be presented.

858

DEFINING THE DESIRED ATTRIBUTES OF NEXT GENERATION SEASONAL MALARIA CHEMOPREVENTION (SMC) DRUG

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Seasonal Malaria Chemoprevention (SMC) is implemented using sulfadoxine-pyrimethamine (SP) and amodiaquine (AQ) in areas where malaria transmission and 60% of clinical malaria cases occur during a short transmission period (~4 months), and where SPAQ remains efficacious (>90% therapeutic efficacy) -in the Sahel sub-region of Africa.Medicines for Malaria Venture (MMV) is working to identify alternative molecules with chemoprevention properties that may be suitable as next generation SMC tools. To help refine the target product profile (TPP) for potential new medicines, MMV commissioned a survey to identify the preferred attributes of next generation SMC drugs.Research focused on The Gambia and Burkina Faso, countries that have successfully implemented two SMC campaigns using a door to door approach, with >90% coverage. A varied range of international, regional and local interviewees (n=112) were selected to provide input on three TPPs in the context of a hypothetical scenario in which SPAQ would no longer be effective for chemoprevention.The TPPs tested contained fourteen attributes including indication, efficacy, dosing, administration and taste; each TPP varied in its protective efficacy and administration regimen. For each TPP, participants were asked to score the overall product and each product attribute on a scale from 1 to 5.Out of this survey, four attributes were emphasized for the development of new SMC products: (1) A well tolerated product, suitable for MDA; (2) protective efficacy at least equal to current SPAQ; (3) a child-friendly formulation to facilitate administration and adherence; (4) a monthly administration schedule to support the current effective door-to-door campaign. A fixed distribution point closer to the community, with a weekly administration could be considered if incentivized by a high protective efficacy (>80%). An alternative with a single injectable administration (before rainy season) and protective efficacy levels greater than 75% were included in the survey. Interviewees generally considered an injection to be most effective and to offer less logistical stress.

859

TARGETING ADENYLATE CYCLASE AS A NOVEL AVENUE FOR ANTIPARASITIC DRUG DESIGN

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Cyclic AMP (cAMP) is an essential, and highly conserved, secondary messenger molecule with critical signaling roles in all eukaryotes. While the function of cAMP is largely conserved, the proteins within the cAMP signaling pathway possess significant structural differences across organisms. Here, we used directed evolution and comparative chemogenomics in both *S. cerevisae* and *Plasmodium falciparum* to show that a novel class of antimalarial compounds, the phenyl-amino-methyl-quinolinols (PAMQs), specifically target the homodimeric class of adenylate cyclases (ACs), the enzyme(s) responsible for cAMP synthesis in fungi and protozoa parasites. We isolated *S. cerevisiae* resistant against two MMV malaria box compounds, MMV0570 and MMV7181, which harbored mutations in several members of the cAMP pathway, including *cyr1*, the *S. cerevisae* homolog of AC. In *P. falciparum*, we found that these compounds both possess potent (20-50 nM) activity against asexual and sexual blood stages of *P. falciparum* and strongly inhibited parasite cAMP levels. This analysis extended to 113 highly related analogs, which identified several additional compounds with strong antimalarial activity and inhibition of parasitic cAMP levels. The antiparasitic mechanism of action of these compounds was further interrogated via *in silico* molecular docking of the PAMQs with AC, recombinant expression of *P. falciparum* AC and genetic engineering to manipulate the expression level of the two parasite ACs. These compounds also show activity against several additional human pathogens that contain homodimeric ACs, including numerous fungi, *Trypanosoma cruzi* and *Leshmania infantum*. Importantly, we show that the PAMQs are highly selective, as they do not significantly affect cAMP levels in human cells. This specificity for homodimeric AC suggests that the cAMP/AC pathway is a promising pathway to target for chemotherapeutic intervention against these parasitic species. Given the increasing lack of effective antiparasitic drugs, the identification of the cAMP pathway as a candidate for small molecule intervention represents a promising new avenue for drug development.

860

KELCH PROTEIN GENE (K13) MUTATIONS IN *PLASMODIUM FALCIPARUM* POPULATIONS IN THREE MALARIA HOT SPOTS OF VIETNAM

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Malaria remains a public health challenge in Vietnam despite a substantial reduction in the incidence of disease over the last twenty years. Moreover, the spread of artemisinin resistance of *Plasmodium falciparum* which compromises the therapeutic efficacy of artemisinin combination therapies is the great threat to current global initiatives to control and eliminate malaria. An understanding of genetic factors that determine how it emerges and spreads is necessary. K13 propeller polymorphism mutations are important determinants of artemisinin resistance.In the study, propeller domain gene of K13 were successfully sequenced in 1060 isolates collected in 3 malaria hot spots of Vietnam from 2009-2016. Ten genotypes of K13 were found including 8 mutations (T474I, Y493H, R539T, I543T, P553L, C580Y, V568G and P574L) after the position of

440th amino acid. The prevalence of K13 mutations were 29%, 6% and 44% in each hot spot Binh Phuoc, Ninh Thuan, Gia Lai respectively. The most important C580Y became dominant genotype in recent year with 81% in Binh Phuoc and 67% in Gia Lai Province. There is the association between K13 mutations and prolonged parasite clearance half-life. Identification of K13 mutations and its frequency in population of *P. falciparum* in Vietnam will support surveillance efforts to contain, prevent the artemisinin resistance and facilitate the development of effective strategy to combate drug resistance.

861

INTERMITTENT PREVENTIVE TREATMENT CONTINUES TO PROVIDE BENEFIT TO MALAWIAN PREGNANT WOMEN

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Drug resistance, particularly the *Pfdhps*-581G mutation, when present with the quintuple *dhps/dhfr* mutant haplotype, may undermine the efficacy of intermittent preventive treatment in pregnancy with sulphadoxine-pyrimethamine (IPTp-SP). We conducted a cross-sectional study at delivery in an area with high prevalence of SP resistance in order to assess whether IPTp-SP remains efficacious. HIV-uninfected women with singleton pregnancies were enrolled at delivery from June to October, 2015 at two sites in southern Malawi. Demographics, clinical data, peripheral blood, and placental samples were collected, and infants were examined. Samples were tested for malaria using rapid diagnostic tests (RDT), microscopy, and polymerase chain reaction (PCR); PCR positive samples will be genotyped for mutations at *dhps*-540 and *dhps*-581. We enrolled 506 women: mean age was 24.2 years (range 15-45); mean gestational age at delivery was 39.4 weeks (range 28-43); 35% were primigravid, 17% were secundigravid, and 48% were multigravid; 81% owned an insecticide treated net, 65% of whom slept under it on the previous night. 11% received no IPTp, while 6%, 24%, and 58% received 1, 2, and 3 or more doses, respectively. Overall, 18% had evidence of malaria: 10.5% by RDT, 7.7% by peripheral smear, 5.6% by placental smear, and 15% by PCR. Malaria was more common among women who received <3 vs ≥3 doses of IPTp-SP: 15% vs 7%, p=0.003 by RDT; 10% vs 5%, p=0.04 by maternal peripheral smear; 7% vs 5%, p=0.33 by placental smear; and 14% vs 16%, p=0.69 by PCR; 20% vs 17%, p=0.42 for any malaria. Birthweight was significantly higher among women who had received ≥3doses of IPTp-SP (3121gm) compared to those who received <3 doses (3032gm, p=0.03). IPTp-SP continues to provide benefit to Malawian pregnant women, with a significantly higher mean birthweight and less maternal malaria among women who received 3 doses; the effect on patent parasitemia was greater than on malaria detected by PCR. Additional data on the prevalence of *Pfdhps*-581G and the relationship of this mutant to birth outcomes will also be presented.

862

UNCOMPLICATED MALARIA TREATMENT FAILURES AFTER ARTESUNATE-AMODIAQUINE COMBINATION THERAPY IN TWO ECOLOGICAL ZONES IN GHANA

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Continuous monitoring of the therapeutic efficacy of artemisinin-based combination therapy for the treatment of uncomplicated malaria has become critical, within the context of malaria control, in an era of the development and spread of artemisinin resistance. The therapeutic efficacy of artesunate-amodiaquine (AS-AQ) was studied from June to September 2015 among children, aged 6months to 14 years, reporting with uncomplicated malaria at two sentinel health facilities in the forest and coastal zones of Ghana. A total of 237 children were recruited for the study: 110 in the forest zone and 127 in the coastal zone. These children were followed up for 28-days using the 2009 WHO protocol for monitoring antimalarial drug efficacy. Preliminary results show no early treatment failure in the 2 ecological zones. Overall pcr-uncorrected late clinical and parasitological failure rates were 6.7% (95% CI: 3.0-13.7) in the forest zone and 11% (95% CI: 6.4-18.1) in the coastal zone (p=0.357). There were no significant differences in treatment failure rates between children aged less than 5 years and children aged 5-14 years in both ecological zones. The main adverse event reported in the 2 ecological zones was vomiting. Prevalence of vomiting in the forest zone was 6.4% (95% CI: 2.8-13.2) on day-0; 6.5% (95% CI: 2.9-13.4) on day-1, and 10.3% (95% CI: 5.5-18.0) on day-2 (p=0.474). Prevalence of vomiting in the coastal zone was 5.5% (95% CI: 2.4-11.4) on day-0; 0.8% (95% CI: 0.1-5.0) on day-1; and 2.4% (95% CI: 0.6-7.3) on day-2 (p=0.076). We conclude that AS-AQ remains efficacious and safe for the treatment of uncomplicated malaria in the forest and coastal zones of Ghana.

863

DOSE-RESPONSE EFFECT OF SULFADOXINE-PYRIMETHAMINE ADMINISTERED AS INTERMITTENT PREVENTIVE TREATMENT OF MALARIA IN PREGNANCY REDUCES ADVERSE BIRTH OUTCOMES RELATED TO SEXUALLY TRANSMITTED AND REPRODUCTIVE TRACT INFECTIONS

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The World Health Organization recommends intermittent preventive treatment with sulphadoxine-pyrimethamine for pregnant women resident in areas of moderate (stable) or high malaria transmission at every scheduled antenatal care visit from the second trimester until delivery to prevent the adverse consequences of malaria infection during pregnancy. A prospective cohort study was conducted between November 2013 and April 2014 among 1086 pregnant women attending antenatal care facilities in the Nchelenge District of Zambia. Recipients of ≥ 2 doses of intermittent preventive treatment were one-half as likely to have any adverse birth outcome - a composite measure of (1) stillbirth, (2) low birthweight, (3) preterm delivery, or (4) intrauterine growth retardation - compared to recipients of 0-1 dose (adjusted odds ratio [OR] 0.49; 95% confidence intervals [CI] 0.33, 0.75). In the sub-population of women who had an adverse birth outcome, odds ratios for mono- and co-infection with malaria and/or curable sexually transmitted and reproductive tract infections (STI/RTI) were also lower among women who received ≥ 2 doses versus 0-1 dose: malaria mono-infection (OR 0.25; 95% CI 0.09, 0.68); malaria plus trichomoniasis or bacterial vaginosis (OR 0.89; 95% CI 0.44, 1.83); trichomoniasis or bacterial vaginosis (OR 0.85; 0.38,

1.91); gonorrhoea or chlamydia (OR 0.08; 95% 0.01, 0.80); syphilis and malaria or other curable STI/RTI (OR 0.34; 0.14, 0.85). This dose-response protective effect was observed consistently across the four individual adverse birth outcomes, and in 25 of 28 categories of malaria and curable STI/RTI mono- and co-infection. Interestingly, ≥ 2 doses compared to 0-1 dose reduced the odds of an adverse birth outcome even among pregnant women who had neither malaria nor curable STIs/RTIs (OR 0.34; 0.14, 0.85), suggesting that sulfadoxine, a broad-spectrum antimicrobial drug, is protective against pathogens beyond malaria and curable STIs/RTIs.

864

POPULATION GENETICS OF THE CHLOROQUINE-RESISTANT GENE PFCRT IN CAMEROONIAN FIELD *PLASMODIUM FALCIPARUM* ISOLATES

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Understanding the population genetics of genes which shape resistance to antimalarial drugs can help in devising novel control strategies. One of the major hurdles in malaria control lies on the evolution and dispersal of the drug-resistant malaria parasite, *Plasmodium falciparum*. Specific mutations in the *P. falciparum* chloroquine resistant transporter gene "Pfcrt" have been associated with resistance to not only chloroquine, but also to amodiaquine, one of the artemisinin partners used in Cameroon for the treatment of uncomplicated malaria. We here present data on genetic variation at the single nucleotide polymorphisms (SNPs) level in the Pfcrt gene in five distinct geographical settings of the Southern-Cameroon (the most malaria endemic part), i.e. Ebolowa, Yaounde, Bertoua, Douala and Kye-ossi (a city bordering Cameroon and two others African countries). Two novel mutations, hitherto unreported (in Cameroon) were found in the Pfcrt gene and variable genetic diversity was observed across the populations. High linkage disequilibrium was found between few SNPs in all the populations traducing a synergic work for conferring/maintaining a higher level of resistance. The inference of evolutionary pattern of this gene in Cameroon based on genetic diversity data depicts a signature of Darwinian positive natural selection on these loci. While observation of novel mutations might traduce new varieties in chloroquine/or amodiaquine resistance (proposal awaiting an experimental verification), signal of positive selection can be the result of drug pressure exerted by misuse of chloroquine (though officially banned from the country) and/or amodiaquine. Our findings thus, provide a baseline understanding of the evolution of a malaria drug resistant gene in Cameroon and suggest a successful establishment of chloroquine-resistant strains which requires urgent attention of malaria control programme in Cameroon.

865

THE PROTECT STUDY: MATERNAL AND CHILD MALARIA CHEMOPREVENTION TO ENHANCE CHILD DEVELOPMENT

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More than 30 million pregnancies occur annually in *Plasmodium falciparum* malaria endemic areas. The systemic and placental changes that occur with malaria in pregnancy could adversely affect the developing fetal brain, which could in turn affect long-term childhood neurodevelopment (ND). Asymptomatic malaria, uncomplicated malaria and severe malaria in childhood have also been associated with ND deficits. However, the effects of maternal and child malaria chemoprevention on child ND have not been assessed to date. We assessed child ND at ~1 year of age in Ugandan children enrolled in a randomized, double-blinded, clinical trial of maternal and child malaria chemoprevention. In this trial, 300 pregnant HIV-uninfected women were randomized at 12-20 weeks of gestation to malaria chemoprevention with 3 doses of sulfadoxine-pyrimethamine, 3 doses of dihydroartemisinin-piperaquine (DP), or monthly DP, and their children were then randomized to receive DP chemoprevention monthly or every 3 months from 2 to 24 months age. Of the 272 children still in the study and eligible for testing, 193 have been assessed for ND outcome at ~1 year of age (mean (SD) 12.6 (0.6) months) using the Bayley Scales of Infant and Toddler Development (Third Edition). To date, the mean (standard deviation) composite scores for Cognition, Language and Motor scales are 103.2 (12.55), 99.47 (9.38) and 105.4 (12.51) respectively. There is no difference in test performance by sex. Treatment arm allocation remains concealed until June 2016, when the one year assessments will be completed. We hypothesize that monthly DP in both mothers and children will be associated with better ND outcomes than the other arms. The final one year results of the PROTECT study, the first prospective study of child ND outcomes after maternal and child malaria chemoprevention, will be presented at the national meeting of the American Society of Tropical Medicine and Hygiene.

866

THE EFFECT OF ARTEMISININ-BASED COMBINATION THERAPY (ACT) OPTIONS ON HEMATOLOGICAL RESPONSE IN *PLASMODIUM FALCIPARUM* MALARIA: A SYSTEMATIC REVIEW AND POOLED ANALYSIS OF INDIVIDUAL PATIENT DATA

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Malaria-associated anemia has a complex etiology related to increased red cell destruction and hemopoeitic suppression, compounded by malnutrition and helminth carriage. Recent reports describe variable reductions in hemoglobin after treatment of *P. falciparum* (Pf) with different ACTs, but precise quantification of the hemoglobin fall attributable to ACTs has not been evaluated widely. Understanding the normal hematological response and recovery following treatment of uncomplicated Pf malaria is crucial to quantify the risks and benefits of different ACTs and other antimalarials such as primaquine, a drug with a potentially important role in malaria elimination.A systematic search of literature databases was conducted to identify studies published from 1990 to June 2015 in which hematological data were recorded in Pf malaria patients before and after treatment with artemether-lumefantrine, dihydroartemisinin-piperaquine, artesunate (AS)-amodiaquine or AS-mefloquine. The WorldWide Antimalarial Resistance Network (WWARN), in collaboration with relevant investigators, organized an individual patient data pooled analysis standardizing and collating nearly 200 studies, with over 72,000 patients of which 70% from African countries. An a priori data analysis plan was developed to identify factors associated with anemia prevalence and hemoglobin changes following treatment with an ACT.The full analysis will be presented, including the contributions of asexual parasitemia, age, transmission intensity and drug concentration. The effect of different ACTs on hemoglobin changes (absolute and fractional) in the 7 days following treatment will be examined in relation to ACT regimen, parasite clearance time, transmission intensity and human host factors. The results of this study will be critical for better assessing safety issues and guiding the optimal therapeutic strategies for regional malaria elimination efforts.

867

ANTIMALARIAL DRUG-RESISTANCE: WHAT DO HIV AND IMMUNITY HAVE TO DO WITH IT?

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The rise and spread of drug resistant malaria parasites is one of the major challenges for malaria control, and indeed will be a huge obstacle for malaria eradication. Successful drug treatment is dependent on both the killing effect of the drug and the killing effect of the immune system. In addition, the immune system is known to play an important role in within-host competition between parasites, which in turn has been shown to be a key part of resistance evolution. Moreover, there are the historical observations that resistance initially occurs in area of low transmission intensity and hence low level of antimalarial immunity. It is thus hypothesized that the immune system is a critical factor in the emergence and spread of drug resistant mutants. If immunity indeed plays a role, this has significant implications for malaria elimination where reduced immunity is a natural consequence yet this is achieved by using a high amount of drug pressure. Using data of clinical trial on IPTp use in pregnant women in Benin, Gabon, Kenya and Mozambique, we present the occurrence of resistant mutants in a variety of immune contexts: (i) HIV-co-infections, (ii) women with different levels of antibody titers, (iii) placental and peripheral infections, and (iv) primigravidae and multigravidae women.

868

EFFICACY OF ARTEMETHER-LUMEFANTRINE FOR TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN CRUZEIRO DO SUL, ACRE, BRAZIL

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Plasmodium falciparum malaria has high morbidity and mortality; there were 143,551 cases in the Brazilian Amazon Region in 2014, of which 15.9% were *P. falciparum* monoinfection. Artemether-lumefantrine (AL) is the first-line treatment. Following the World Health Organization recommendation to routinely evaluate antimalarial treatment policies, we are conducting a therapeutic efficacy *in vivo* trial of AL for treatment of uncomplicated *P. falciparum* malaria in Cruzeiro do Sul, Acre, Brazil from December 2015 to May 2016. The objectives of this study are to evaluate the efficacy of the first-line antimalarial regimen for treatment of uncomplicated *P. falciparum* malaria and ensure effective case management practices are maintained in Brazil. Febrile participants ≥5 years old with microscopically confirmed *P. falciparum* monoinfection with parasitemia between 250 and 200,000 asexual parasites/microliter were enrolled and treated with a supervised 3-day course of AL, dosed according to Brazilian guidelines for malaria control. Clinical and parasitological parameters are monitored for 28 days. Recrudescence is differentiated from reinfection by comparing parasite genotypes from Day 0 and the day of failure. Genetic markers associated with artemisinin resistance, including the K13 gene mutation will be assessed. A total of 127 patients have been screened, and all 85 participants have been enrolled. Four participants were excluded after enrollment; two due to parasitemia below inclusion range, one due to *P. vivax* infection upon slide review, and one due to absence of fever in the previous 48 hours. Among enrolled patients, 61 have completed follow-up, 17 are still being followed and three have been lost to follow-up. No patient has met criteria for treatment failure, although one participant was diagnosed with *P. vivax* on day 28. Patient follow-up will be completed by May, 2016. Final results will be presented. Preliminary results suggest that AL remains highly efficacious for treatment of uncomplicated *P. falciparum* in the Brazilian Amazon Region. These results will be corroborated by molecular testing for the K13 resistance gene.

869

SHOULD WE STILL USE QUININE IV FOR SEVERE MALARIA?

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Malaria represents a significant health problem in patients coming back from endemic areas. Severe malaria is life threatening and the acute respiratory distress syndrome (ARDS) is among the more serious complications that invariably leads to death. We have noted the occurrence of ARDS in a few patients with severe malaria being treated with quinine IV, the only available recommended drug at the time. The question of whether quinine IV is a triggering or contributing factor of ARDS in those cases was raised. A retrospective analysis of several cases of malaria was initiated and a literature search was done. Both outcomes were not definitive in corroborating evidence against quinine IV, but the suspicion remains. In the USA, quinine IV has not been available or used in severe malaria for over 20 years! Quinidine is available in the USA. In the era of better antimalarial therapy, especially with the recommended relatively recent and effective artemisinin derivatives, or the fairly safer quinidine, should quinine IV still be listed among the treatments or used for severe malaria?

870

SAFETY AND TOLERABILITY OF DIHYDROARTEMISININ-PIPERAQUINE AS INTERMITTENT PREVENTIVE TREATMENT FOR MALARIA IN A REFUGEE CAMP, ADJUMANI, UGANDA

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The use of dihydroartemisinin-piperaquine (DP) is increasing in sub-Saharan Africa, though safety data in Africa is sparse. DP was used by Médecins Sans Frontières in an Intermittent Preventive Treatment (IPT) program in a refugee camp in northern Uganda in 2015. All children aged 6 months to 14 years resident in the camp were eligible for participation in the program, which consisted of three mass distributions of DP at 8 week intervals. Weight-based dosing following 2014 guidelines was used and a total of 40 611 doses of DP were administered during 3 distributions. A health-center based pharmacovigilance system was implemented during the program, and an existing community-based mortality surveillance system was continued. Signs and symptoms of both common and severe side effects due to DP were part of key sensitization messages during the campaign. Participants experiencing any symptoms were encouraged to present to the health centers in the camp, where free health care was provided. A total of 56 adverse events (AE) were reported during the 24 week follow-up period. All AEs were reported in the 10 days following DP administration. Of the 56 AEs, 28 were judged to be probably or definitely related to DP; the most common symptoms were rash or itching (12) and vomiting (6). One case of urticaria was notified. Symptom severity was noted for all AEs regardless of causality: 75% were mild and 25% were moderate. One SAE was reported: an unexplained death in the community which occurred in a 12-year old girl who was diagnosed with varicella 12 days after taking the first distribution of DP. On the 8th day of her

varicella, she developed swelling of her face and limbs over several hours, lost consciousness at home, and was pronounced dead on arrival to the health center. Her mother reported her taking only paracetamol and using a zinc oxide cream in the days prior to her death. The SAE was considered possibly related to DP, but the concomitant varicella provides an alternative explanation. These data show that DP is well-tolerated and safe when given in repeated doses in an IPT setting. Its use in similar contexts in sub-Saharan Africa should be considered.

871

FALSE SECURITY FROM OBSOLETE MALARIA DRUG-RESISTANCE MARKERS

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Malaria kills 500,000 people every year and this toll may increase if *Plasmodium* parasites evolve resistance to the artemisinin combination therapies that are the current mainstay of treatment. For the last two decades, artemisinin derivatives have been the frontline treatment for malaria-infected individuals and have prevented the public health catastrophes that followed the previous failure of chloroquine. To protect them as potent antimalarials for as long as possible, Artemisinin Combination Therapy (ACT) pairs artemisinins with other classes of antimalarials with the presumption that simultaneous development of resistance to all ACT components is improbable. Despite this approach, artemisinin resistance has recently been established in Southeast Asia. It is now important to understand how parasite populations may acquire resistance to ACTs. Here we report an ominous finding: *P. falciparum* isolates collected in Southwest India in 2012 displayed antifolate resistance in cell-based assays that was as high as the most resistant parasites in the world, but these parasites did not contain the full set of classic DNA sequence markers for the highest antifolate resistance. Parasites from regions using artemisinin-antifolate combinations for decades display novel mechanisms of antifolate resistance that escape routine surveillance methods. The unwitting use of ineffective partner drugs threatens to undermine the last remaining effective antimalarial.

872

ASSESSMENT OF THE USE OF MALARIA RAPID DIAGNOSTIC TESTS IN HEALTH FACILITIES IN GHANA

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Malaria rapid diagnostic tests (RDTs) have become the mainstay diagnostic tool for acute malaria infection in most health facilities in Ghana. However, in spite of the generally good performance of these tests, their use by health care providers at health facilities can be challenging. Data on the performance, reading and interpretation of RDTs under routine program conditions are limited. Since 2012, the US President's Malaria Initiative (PMI) has supported the MalariaCare partnership to collaborate with the National Malaria Control Program (NMCP) to improve quality of malaria diagnosis using RDTs in health facilities. As part of MalariaCare's quality assurance program for case management, trained clinicians and laboratory staff act as supervisors who conduct outreach training and supportive supervision (OTSS) visits to health facilities in five of Ghana's ten regions. These visits focus on skills observation and on-the- job mentoring and problem solving, with the primary aim of improving clinical assessment and evaluation skills, preparation and accuracy of malaria diagnostic tests, and adherence by clinicians to test results. A key component of the OTSS system is observing facility staff perform RDTs and providing coaching to staff on weaknesses and errors observed. Following four rounds of OTSS data tracking in focus regions, 7,502 RDT tests observed gathered information on each step in the preparation and reading of an RDT performance during 5,088 health facility visits. MalariaCare will present the findings on common errors made in conducting RDT tests, but also track progress over time in addressing these weaknesses.

873

STRUCTURAL AND FUNCTIONAL EFFECTS OF HEME BINDING TO RCPFHRP2: IMPLICATIONS FOR MALARIA DIAGNOSIS

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Early diagnosis of malaria is a key element of elimination strategies because individuals with low parasite loads can serve as transmission reservoirs. Rapid diagnostic tests (RDTs) are often used in widespread malaria screening efforts to quickly and easily detect the malaria biomarker PfHRP2, a histidine-rich protein produced by the malaria parasite *Plasmodium falciparum*, in a small drop of blood. However, PfHRP2 protein sequence variation coupled with manufacturing issues can make these tests unreliable. This work is focused on investigating the structure of PfHRP2 and its relationship to the effectiveness of current RDTs. Past studies have indicated that PfHRP2 may play a role in the parasite's heme detoxification process by binding free heme and promoting its crystallization into hemozoin. We hypothesize that heme-bound protein adopts a different conformation from free protein, and that this conformational change may affect protein binding to antibodies on an RDT. This is especially a concern since native protein will be exposed to heme in blood, but purified recombinant protein used for industrial antibody production will not. This work investigates the conformational changes of rcPfHRP2 in the presence of heme using circular dichroism (CD), and the effects of heme on antibody-based PfHRP2 detection using ELISA and RDT formats.

874

IMPROVING QUALITY OF MALARIA RAPID DIAGNOSTIC TESTING AND TEST ADHERENCE THROUGH QUALITY ASSURANCE

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As malaria prevalence in Tanzania declines, universal diagnostic testing for malaria becomes increasingly important to identify and manage other causes of febrile illness and reduce the threat of antimalarial resistance. In 2013, the Tanzania National Malaria Control Programme (NMCP) revised the National Guidelines for Diagnosis and Treatment of Malaria in line with the World Health Organization's universal diagnostic testing recommendations. MalariaCare, a partnership that provides technical assistance to rapidly scale high quality malaria diagnosis and treatment, is supporting the NMCP to design and implement a malaria case management quality assurance (QA) system with a focus on malaria rapid diagnostic tests (mRDTs). After finalizing an mRDT QA training package, the NMCP and MalariaCare trained regional and district trainers from the Lake Zone, who then trained 1,539 health care workers from 883 public health facilities in May and-June 2015. Following training, MalariaCare and

the NMCP conducted outreach, training and supportive supervision (OTSS) at a subset of facilities in July and September 2015. OTSS aims to reinforce skills developed during the mRDT training and improve adherence to test results through skills observation, mentoring and on-the-spot problem solving. At the first OTSS visit, 197 health workers from 193 facilities scored an average of 93% during mRDT observations, showing a high level of competence post-training. This visit also revealed high average adherence to positive (96%) and negative (91%) test results. Adherence to negative test results varied by facility level, with dispensaries having higher adherence (93%) than health centers (86%) and hospitals (85%). The NMCP and MalariaCare will also present results from the continuation of OTSS, expansion of training and OTSS in the Eastern Zone, and malaria testing rates among these facilities based on HMIS data.

875

ALL-IN-ONE, MULTIPLEXED ON-BEAD ELISA FOR MALARIAL BIOMARKERS PLDH AND PFHRPII

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As malaria transmission declines, accurate diagnosis becomes increasingly important for defining disease prevalence and distribution, as well as monitoring impact of interventions. Further, in low-transmission settings, identification and treatment of asymptomatic carriers are critical for eliminating the disease. Current antigen-detecting rapid diagnostic tests (RDTs) for malaria are unreliable in the asymptomatic regime (< 200 parasites/µl), and laboratory protein-based detection strategies, such as well-plate ELISAs, can require 5 - 8 hours of incubation time and are limited to one analyte. To address this, we have developed a multiplexed, magnetic bead-based ELISA for *Plasmodium* lactate dehydrogenase (*p*LDH) and *Plasmodium falciparum* histidine-rich protein II (*Pf*HRPII) with incubation times totaling less than 1 hour and detection limits rivaling those of well-plate ELISAs. In this assay, magnetic particles functionalized with antibodies specific for *p*LDH and *Pf*HRPII are added to parasitized lysed blood samples along with detection antibodies with distinct enzymes for each biomarker. Sandwich complexes for *p*LDH and *Pf*HRPII form on the surface of the magnetic beads, which are washed and sequentially re-suspended in detection enzyme substrate for each antigen. Assay detection limits are 2.7 and 1.2 parasites/µl for *p*LDH and *Pf*HRPII, respectively. Detection of both biomarkers is advantageous because it avoids false-positives due to slow *Pf*HRPII clearance and allows for differentiation between *falciparum* and non-*falciparum* infections, ultimately informing treatment. With these advantages, as well as high sensitivity and detection limits in the single parasite/µl regime, the developed multiplexed assay for *p*LDH and *Pf*HRPII is an attractive alternative to well-plate ELISAs and a promising detection strategy for an elimination setting. Further, the modularity of the multiplexed on-bead ELISA makes it applicable to any series of infectious disease biomarkers for which there are antibody pairs available.

876

MALARIA MICROSCOPY COMPETENCY IN LIBERIA POST EBOLA DISEASE OUTBREAK

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Since 2010 Liberia has progressively moved toward parasitological diagnosis of malaria. However in the last two years - since the first confirmed case of Ebola virus disease (EVD) on March 17, 2014, through January 14, 2016, when the World Health Organization (WHO) declared an end to the most recent outbreaks - the public health system has been overwhelmed managing this new disease. Due to risk from blood exposure, national policy mandated returning to clinical diagnosis of malaria instead of drawing blood. Now the Ministry of Health and Social Welfare is in the process of restoring essential and quality-assured health services in governmental and non-governmental health facilities. As a first step, County Health Team Diagnostic Focal Persons (CHT-DFPs) supporting decentralized training, and supervision activities were prioritized for retraining and competency assessment in malaria diagnostics. In February 2016, the National Malaria Control Program, with support from MalariaCare, conducted the first refresher training and microscopy competency assessment for CHT-DFPs from 13 of 15 counties post-the EVD outbreak. Trainees were assessed on parasite detection and parasite quantitation and scored against WHO minimum grades for expert level microscopists. Twelve (12) of 13 participants scored greater than 80% (M 93%; Mdn 94%) on parasite detection and all (100%) participants scored above 50% (M 66%; Mdn 67%) on parasite quantitation, resulting in equivalent designations of WHO Levels 1 (n=12) and 2 (n=1) for these 13 microscopists. There was an 11% improvement between pre- and post-test scores for parasite detection and a 47% improvement was observed for parasite quantitation. Despite an almost two-year interruption in malaria diagnostic services, microscopy capacity within the County Health Teams appears to remain strong. Continued training and monitoring of this cadre using proficiency test panels can be achieved using a recently-procured slide bank, putting Liberia on track to move forward with plans to decentralize training and supervision activities to the county level.

877

FIELD EVALUATION OF A REAL TIME LOOP-MEDIATED ISOTHERMAL AMPLIFICATION ASSAY (REALAMP) FOR MALARIA DIAGNOSIS IN CRUZEIRO DO SUL, ACRE, BRAZIL

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Traditional molecular methods, such as nested-polymerase chain reaction (PCR), are very sensitive to detect malaria parasites, but require advanced laboratory equipment and trained personnel. Real-time loop-mediated isothermal amplification (RealAmp), a LAMP-based molecular tool, facilitates rapid target amplification at a single temperature setting, reducing the need for sophisticated equipment. There is limited information on the performance of this method for the malaria diagnosis in clinical settings and field conditions. We evaluated the performance of RealAmp for malaria diagnosis in Cruzeiro do Sul, Acre state, Brazil. We enrolled 1,000 patients with fever (axillary temperature >= 37.5 C) or history of fever in last 24h presenting for malaria diagnosis from February through August 2015. DNA was extracted from dried blood spots using a crude method (heat treatment) at the sample collection site (field site), and using commercial kits at a Brazilian national reference laboratory. Genus-specific RealAmp was performed at both the reference laboratory and field site after appropriate training. In addition, Giemsa-stained blood smears were prepared and examined by two independent well-trained study microscopists. A combination of real-time PCR and nested PCR was used as reference test. The sensitivity and specificity of RealAmp from heat treatment DNA in the field laboratory were 94.1% (95% confidence interval [CI]: 90.1-96.8) and 83.9% (95% CI: 81.1-86.4), respectively, while the sensitivity and specificity of RealAmp at the reference laboratory were 83.2% (95% CI: 77.6-87.9) and 97.0% (95% CI: 95.5-98.0), respectively. Microscopy showed sensitivity of 96.4% (95% CI: 93.0-98.4) and specificity of 98.2% (95% CI: 97.0-99.0). Our findings highlight that it is possible to implement simple molecular tests at point of care in remote areas of countries such as Brazil. However, RealAmp performance was

inferior to that of microscopy performed by skilled professionals. Attempts to develop and evaluate molecular tools should continue, especially in countries targeting pre-elimination.

878

CAN PHARMACY PROVIDERS PROVIDE QUALITY MALARIA DIAGNOSTIC IN KENYA: RESULTS FROM EXIT INTERVIEW AND MYSTERY CLIENT STUDIES FROM THE KENYAN COAST

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In Kenya, 32% of people seek treatment in the private sector where availability of diagnostics testing has been low. Pharmacies often serve as the first or only point of accessing care but have not been allowed to perform blood tests, and evidence is required to show that tests can be conducted safely by this cadre. Between 2014 and 2015 PS Kenya implemented a project seeking to increase malaria testing using RDTs among private clinics and pharmacies in Kwale, Mombasa and Kilifi counties.Studies were conducted at a random sample of project facilities in Q4 2014 and Q4 2015 to track provider performance. Exit interviews were held at 130 sites with 526 clients in 2014, and 534 clients in 2015. Eligible cases were adults seeking treatment for fever for themselves or on behalf of someone else. Confirmed RDT-negative volunteers conducted 260 mystery client visits at 155 sites in 2014 and 262 visits at 113 sites in 2015. Data were analyzed using Stata v13. At endline, exit interview clients were more likely to be tested for malaria at clinics than at pharmacies (86.6% vs 59.4%, p<0.001). Between 2014 and 2015 testing by RDT increased by 22.4 points in clinics (30.1% to 52.0%); prior to the intervention RDTs were not formally available at pharmacies. Overall 83% of 238 malaria test-positive clients received an ACT in 2015, with no difference between facility types (p=0.5). However, positive clients at clinics remained twice as likely to receive an antibiotic (46.7% vs 17.1%, p<0.001). Over time untested pharmacy clients were less likely to receive an antimalarial (2014: 40.7%, 2015: 29.4%, p=0.006) while the level at clinics remained unchanged (average: 16%). Mystery clients at pharmacies were more likely to receive the correct diagnosis(negative) (2014: 72.8%, 2015: 79.6%, p<0.001), than in clinics (2014: 58.9%, 2015: 75.7%, p<0.001) At endline, 0% of test-negative clinic clients and 2% of pharmacy clients received any antimalarial, both down from over 7% in 2014.Results from this project show comparable fever management at pharmacies and clinics on the Kenyan coast. To scale up testing the MOH should consider allowing RDT testing at pharmacy-level in Kenya.

879

PFHRP2 DETECTING MALARIA RDTS: ALARMING FALSE NEGATIVE RESULTS IN ERITREA

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In Eritrea over 75% of suspected malaria cases are diagnosed using rapid diagnostic tests. Despite following WHO recommended procurement and quality assurance practices, frequent complaints of false negative RDT results were reported from most geographical settings in Eritrea in 2015. Initial investigations involved cross checking RDT results with quality assured microscopy. This exercise confirmed that SD Bioline Malaria Ag Pf/Pv (05FK80) targeting histidine rich protein 2 (HRP2) and *Plasmodium vivax*-plasmodium lactate dehydrogenase (Pv-pLDH), failed to diagnose microscopically confirmed *P. falciparum* malaria, across a range of parasite densities. RDTs were retrieved from the field and found to react against WHO-FIND quality control samples. A product recall was implemented and investigations carried out to delineate causal parasite factors. Specifically, some Pf microscopy positive samples were assessed against other brands of good performing HRP2-detecting RDTs and found to be negative. In February 2016, 50 consecutive microscopically confirmed *P. falciparum* malaria patients presenting at two regional hospitals were screened with non-HRP2 detecting RDTs. Patient specimens returning positive on pf-pLDH test line and negative on HRP2 test line provided blood samples for *Plasmodium* species identification PCR and hrp2/hrp3 PCR. The mean patient age was 29 yrs; parasite density range was 32-89,120 parasites/µl; mean of 15,347). The overall prevalence of falciparum-infected blood specimens with positive RDT results for PfpLDH band but negative to PfHRP2 band was 58% [95% C.I: 44-71]. PCR results for pfhrp2 and/or pfhrp3 gene expression and ELISA for hrp2 are pending but preliminary findings support HRP2/3 gene deletion or variation amongst *P. falciparum* parasites. Furthermore, these findings should be an early warning to neighbouring African countries to follow up reports of false negative RDT results and to consider adding investigations for hrp2/hrp3 gene deletions into surveys and surveillance activities.

880

DIRECT, HIGH-THROUGHPUT QUANTIFICATION OF PARASITIC DNA IN MULTIPLE SAMPLE TYPES WITHOUT DNA EXTRACTION

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Accurate diagnosis is essential for successful management of infectious diseases. While most molecular diagnostics are sensitive enough for clinical application, new diagnostic tools with simplified procedure and improved throughput are still needed. Capture and Ligation Probe-PCR (CLIP-PCR) has been demonstrated as a high throughput RNA quantification technology for extreme sensitive identification of malaria, but its role in DNA quantification remains to be demonstrated. In this study, we adopted CLIP-PCR for identification of DNA directly in saliva, buccal swab and whole blood samples. Target DNA from saliva, buccal swab or blood was released by lysis, heat-denatured and captured directly to 96-well plate by sandwich hybridization using multiple oligo probes with universal tail sequences. After enzymatic ligation of the probes, the single-stranded template is quantified with universal primers targeting the tail sequences by qPCR with SYBR green chemistry. To avoid false negatives caused by target polymorphism and reduce turnaround time, we adopted a multi-section strategy: multiple sets of probes that target at a continuous/semi continuous region of targeted DNA were used simultaneously, each of which being sufficient for DNA quantification by CLIP-PCR. Our DNA quantification CLIP-PCR assay was tested for direct quantification of plasmodium 18S rDNA, human 18S rDNA and schistosoma DNA. Without the need for DNA purification, CLIP-PCR quantifies DNA in multiple samples types within 4 hours. *Plasmodium* can be detected at a concentration as low as 0.19 parasites/ul in blood samples. Human 18S rDNA can be detected directly in both saliva and buccal swab samples, while Schistosoma DNA can also also be identified from serum samples of infected mice. These data indicate that CLIP-PCR has the same sensitivity as regular qPCR, but much reduced complexity and cost, making it suitable for large scale molecular surveillance studies.In conclusion, our study showed CLIP-PCR provides a highly sensitive, simple and low-cost means for direct identification of parasitic DNA in multiple sample types in a high throughput fashion.

881

HIGH-THROUGHPUT, MULTIPLEX GENOTYPING DIRECTLY FROM SALIVA AND BUCCAL SWABS WITHOUT DNA PURIFICATION

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SNPs have been found associated with disease susceptibility, drug response and complex phenotypes. Clinical population screening of significant SNP markers calls for multiplexed genotyping of SNPs on a large scale. Current SNP genotyping tools, despite many advantages, invariably require DNA extraction, which remains a key throughput-limiting step for population screening. In addition, multiplex-PCR amplification employed by these genotyping methods suffers from complex primer design and/or amplification bias. Here, we describe a novel high-throughput genotyping approach, MELPA , which has multiplex SNP genotyping capability, eliminates DNA extraction, achieves uniform PCR amplification using a single pair of universal primers, and is suitable for saliva and buccal swab samples. In brief, instead of nucleic acid extraction, MELPA lysed saliva/buccal swabs and captured the target DNA directly to 96-well plate by sandwich hybridization using multiple oligo probes with universal tail sequences. After enzymatic extension and ligation of the probes, a single-stranded template for each target SNP site was formed, and all templates were PCR-amplied using universal primers targeting the tail sequences. Multiplexed genotyping by single-base primer extensions were analyzed with a MALDI-TOF mass spectrometry platform. We tested the feasibility of the new assay for saliva and buccal swabs, and evaluated the accuracy by comparing MELPA with commercial multiplex SNP assay (iPLEX), for the detection of 20 G6PD gene variants known to be at risk for primaquine-induced hemolysis in antimalarial therapy. We successfully developed a 20-plex panel for G6PD genotyping. A typical 50 ul saliva or one buccal swab sample is sufficient for running 2 assays. Six 384-samples can be processed from sample to result in a 24-hour workflow, with a hands-on time of 2 hours. Results were consistent with iPLEX, and 100% concordant with sequencing. Saliva and swab samples can be stored at room temperature for at least 24h without affecting the performance. MELPA represents an efficient and cost-effective approach to multiplex SNP genotyping at population level.

882

UPTAKE OF MALARIA DIAGNOSTIC TESTS AND ADHERENCE TO NEGATIVE TEST RESULTS AMONG FEVER CARE SEEKERS AT INFORMAL DRUG SHOPS IN UGANDA

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There has been a steep decline in malaria prevalence in Uganda from 43% in 2009 to 19% in 2014. In light of this, the Ministry of Health changed the policy in 2010 to diagnosis prior to treatment for suspected cases to ensure patients are provided appropriate treatment for their illness and to prevent antimalarials being wasted on uninfected patients. Although a third of fever care seeking occurs in the informal private sector, there is scant reliable data on testing rates and appropriate dispensing of antimalarials in that sector. The objective of this study is to provide national estimates for malaria testing rates and to quantify antimalarial misuse. A cross-sectional survey was conducted in March 2016 using two-stage cluster sampling where up to three informal sector drug shops were sampled per cluster. Data were collected using a structured questionnaire on demographics, fever care seeking, and malaria case management. Clients were included if they exited any informal drug shop within the observation period and provided consent. Primary outcomes were proportion of clients seeking care for fever, proportion of fever care seekers testing for malaria by blood slide or malaria rapid diagnostic test (mRDT), and proportion of fever care seekers who tested negative and received an antimalarial. A total of 711 clients from 324 informal drug shops in 124 enumeration areas were interviewed. Seventy percent of all clients sought care at a shop where mRDTs were available. The proportion of clients seeking care for fever was 38%. Among fever care seekers (n=270), 33% were tested at the shop and 11% were tested elsewhere. Ten percent of clients testing negative still received an antimalarial. Among fever care seekers not taking a test (n=153), 60% received an antimalarial. Given the declining trend in prevalence, a sizable proportion of clients with fever are receiving malaria treatment without having malaria, leading to wastage and untreated illness. Educating providers on the decline of malaria prevalence as well as increasing testing availability is necessary to ensure that antimalarials are not presumptively dispensed.

883

A MINIATURIZED FLOW CYTOMETRY PLATFORM FOR MALARIA DIAGNOSTICS

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The diagnosis and treatment of malaria remains a challenging global problem due to limitations of highly sensitive and specific tests that identify and type the malarial parasites and due to the long turn-around time and expertise needed for microscopy based tests for determining the degree of red blood cell (RBC) infection. While flow cytometry has been shown to be capable of providing answers in diagnosis of malaria, the traditional use of expensive and sophisticated platforms and highly skilled operators, has restricted the speed and ease with which such diagnostic information can be provided. In this study, we evaluate the potential of a small low-cost, touch screen based miniaturized cytometer, the Muse Cell Analyzer for providing solutions related to malaria diagnostics. The platform is based on microcapillary cytometry, generates low biohazardous waste, uses small sample volume and provides the potential to add multiple diagnostic assays related to global health. A CE/IVD assay for CD4 T cell monitoring was recently released on the platform demonstrating capability of the system for use in resource-constrained settings. We have developed novel multiplexed bead based immunoassay for detection of plasmodium released antigens in blood/serum to enable parasite typing. Initial results demonstrate superior sensitivity for detection of plasmodium antigens to current RDT measurement range. In addition the platform demonstrates capability to provide percentage of infected red blood cells which is also critical to diagnostic decisions. The availability of a simple, easy to use and affordable platform like the Muse Cell Analyzer, that can provide results on plasmodium typing and percentage of infected RBC's can greatly increase capability to provide affordable and timely malaria diagnostics.

884

EVALUATION OF THE PARASIGHT PLATFORM FOR RAPID AND HIGHLY ACCURATE MALARIA DIAGNOSIS

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The WHO estimates that nearly 500 million malaria tests are performed annually. While several diagnostic assays are available, the need for an inexpensive, quick and highly sensitive malaria test remains a priority for world-wide malaria treatment. Two recent studies from our group have demonstrated a computer vision platform capable of meeting these needs. Here we present the commercially available version of this technology, the SightDx Parasight platform which provides malaria diagnosis, speciation

and parasite quantification. We conducted studies at Apollo Hospital in Chennai, India where 205 samples were tested, and at Aga Khan University Hospital Nairobi Kenya where 263 samples were evaluated. At both centers the device diagnoses were compared to microscopy, RDT and PCR results. For identification of malaria, the device demonstrated a sensitivity of 99% and a specificity of 100% at Apollo Hospital India, and a sensitivity of 100% and a specificity of 98.9% at Aga Khan University Hospital Kenya. For speciation, the device correctly identified 100% for *Plasmodium vivax* and 100% for *P. falciparum* at Apollo Hospital and 100% *P. vivax* and 99.3% *P. falciparum* at Aga Khan University Hospital. Lastly, comparing the device parasite count with that of a trained microscopist produced an average pearsons correlation of 0.83 at Apollo Hospital and 0.88 at Aga Khan University Hospital.

885

NO MORE HIDING: PICOGRAM DETECTION OF HISTIDINE-RICH PROTEIN 2 FROM *PLASMODIUM FALCIPARUM*

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Histidine-rich protein 2 (HRP2) is produced by one of the human malarias, *Plasmodium falciparum*, and is widely used for diagnostic purposes. Detection of HRP2 provides evidence for active or recent infection, but current HRP2 immunoassays are hindered by high detection limits. Here we present a novel HRP2 immunoassay for antigen capture through a bead-based system which is capable of reliable HRP2 detection at low picogram levels in a highly-specific and cost-effective manner. We compared this assay with HRP2-based rapid diagnostic test (RDT) results from community surveys in different *P. falciparum* transmission settings to assess RDT reliability in the general population. In the holoendemic setting of northern Mozambique (RDT: SD Bioline Pf), agreement between the two tests was good, with a kappa coefficient of 0.77 (95% CI: 0.74-0.79). Of 2,280 persons tested, 199 (8.7%) of were found to be positive for the HRP2 protein, but RDT negative, giving a receiver operating characteristic area under the curve (ROC AUC) of 0.95 (0.94-0.96) for the novel test. Additionally, 57 (4.3%) of all RDT positives were found to have no detectable HRP2 by the bead assay, suggesting the RDT false positive rate for this community survey. There was a clear trend for higher HRP2 concentrations in younger age groups and consequent reliability of true-positive RDT results, but this reliability diminished in older individuals. Sampling from the low-endemic nation of Haiti (RDT: First Response HRP2) revealed poor agreement between the tests (kappa: 0.30, 0.14-0.45). Of 4,350 persons tested, 62 (1.4%) were positive by the bead assay, but 53 of these persons were RDT negative (ROC AUC: 0.75, 0.62-0.89), illustrating the low HRP2 concentrations of persons in the population harboring the protein. Of the 24 RDT positive persons from this community survey, 15 (63%) had no detectable HRP2 by the bead assay, showing a high degree of false positive tests in asymptomatic individuals. The low detection limit and high specificity of the bead assay can potentially provide a national malaria control program with an objective indication of the performance of HRP2-based RDTs in an area.

886

APTAMER-BASED LOW RESOURCE DIAGNOSTICS FOR DETECTION OF MALARIAL BIOMARKER *PLASMODIUM* LACTATE DEHYDROGENASE

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Early diagnosis of malaria is critical to disease intervention, as asymptomatic individuals with malaria can serve as a transmission reservoir for the disease. Diagnostic testing has doubled since 2010 due to the rise of rapid diagnostic tests, most of which are designed using a lateral flow assay (LFA) format. LFAs overcome many shortcomings of more resource-dependent techniques (i.e. microscopy and PCR) but perform poorly at low parasitemias, thus preventing the diagnosis of asymptomatic individuals. LFAs for malaria often employ monoclonal antibodies (mAbs) for biomarker capture and detection. However, over the past twenty years aptamers have emerged as a potential alternative to mAbs in diagnostic applications. Aptamers are synthetic nucleic acid sequences that bind to target molecules with high (~nM) affinity, and offer several advantages over antibodies, namely a non-immunological origin, increased thermal stability, and affordable automated chemical synthesis. Our lab has characterized the kinetics of binding for a multitude of mAbs and aptamers specific for *P. falciparum* LDH, *P. vivax* LDH, or both. Moreover, we have developed a strategy for capturing native *P. falciparum* LDH from large volume (50-100 µL) whole blood samples using commercial magnetic beads functionalized with an X-aptamer, which is a next generation aptamer that incorporates druglike moieties into various nucleotides of the aptamer sequence to increase target affinity. The captured *p*LDH is subsequently concentrated and eluted in a small volume (10 µL) of single-stranded DNA that is complementary to the X-aptamer, and the eluent is then spotted on a lateral flow assay. This initial processing step overcomes the sample volume constraints (only 5-10 µL) of commercial LFAs, allowing more biomarker to be delivered to the LFA test line for signal enhancement in samples with low parasitemia.

887

GUIDING THE DEVELOPMENT OF IMPROVED DIAGNOSTICS FOR MALARIA: LIMIT-OF-DETECTION OF CURRENT RAPID DIAGNOSTIC TESTS

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The simplicity, rapidity and affordability of lateral flow immunochromatographic assays, referred to as rapid diagnostic tests (RDTs), have transformed our ability to diagnose malaria. The performance of quality-assured RDTs is typically equal or superior to routine microscopy and these tests can be used by community health workers effectively and with minimal training requirements. Since 2010, the World Health Organization recommends that all suspected cases of malaria should be diagnosed by either technique to prevent presumptive treatment. As a result, the WHO African Region has seen the proportion of diagnosed cases increasing from 36% of all suspected cases in 2005 to 65% in 2014, with almost three quarters of those being tested by RDTs. This and other improvements in antimalarial interventions led to a global decrease in malaria prevalence with a number of countries shifting from control to elimination strategies.Current RDTs are considered appropriate for the diagnosis of febrile patients, however more sensitive RDTs would be needed to support detection of asymptomatic infections in a context of malaria elimination. In order to guide the development of improved tests, the exact analytical limit-of-detection (LOD) of a set of current RDTs has been determined. The best-in-class RDTs detecting

the histidine-rich protein II (HRP2) or *Plasmodium* lactate dehydrogenase (pLDH) antigens were selected and then tested with serial dilutions of various reference materials, including recombinant HRP2 and pLDH proteins, *Plasmodium falciparum* culture samples as well as field isolates of both *P. falciparum* and *P. vivax*.With this study, we report for the first time the lowest concentrations of target analytes that can be detected by the best RDTs currently in the market. The determination of this key performance parameter allows for a better understanding of the advantages and limitations of current good-quality RDTs as well as of the potential improvement that could be achieved for the detection of parasite populations able to sustain malaria transmission.

888

LAMP VERSUS MICROSCOPY AND RDT TO DETECT MALARIA IN PREGNANT WOMEN: A CROSS SECTIONAL STUDY IN NW ETHIOPIA

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Timely diagnosis followed by effective treatment is the best strategy for prevention, control and elimination of malaria in pregnancy. Detection of malaria by microscopy or rapid antigen tests in resource-limited settings is hampered by quality control, infrastructure limitations, training, and poor analytical sensitivity. Low sensitivity of current testing modalities is of concern in pregnancy where parasite levels are low in the peripheral blood due to placental sequestration. We evaluated the performance of loop-mediated isothermal amplification (LAMP) compared to microscopy for the diagnosis of malaria among pregnant mothers in NW Ethiopia. A cross sectional study was conducted from January to April 2016 at Koladiba Health Center in North Gondar. Eight-seven blood samples were collected from pregnant mothers suspected of having malaria and tested by Giemsa-stained thick and thin peripheral bloodfilm, RDT, LAMP and nested PCR. Diagnostic accuracy measures (analytical sensitivity, specificity, predictive values, and Kappa scores) of microscopy, RDT (HRP2/pLDH(Pf/PAN) Combo) and LAMP (Eiken LoopAMP) was compared to nested PCR by using Simple Interactive Statistical Analysis (SISA) software and Cohen's Kappa reliability measure. A total of 87 women were enrolled, 50.6% with a previous history of malaria, 74.7% were multigravidae, 17.2% were in the first trimester, 41.4% second trimester, and 41.4% third trimester. Ten samples were positive for malaria by microscopy, 9 by RDT, and 15 by LAMP. P.falciparum, P.vivax, and mixed infections of the two species were detected. Using nested PCR as gold standard, the sensitivity of microscopy and RDT was 90 and 70%, specificity 98.7% and 97.4%, respectively. LAMP showed a sensitivity and NPV of 100%, specificity of 93.5%, with k=0.768 with nested PCR. We conclude that LAMP is a rapid molecular method and more sensitive than both microscopy and RDT for the detection of malaria in pregnancy. Mass screen and treat strategies to reduce the burden of malaria in pregnancy and reduce infant mortality will benefit from more sensitive methods like LAMP.

889

MALARIA STRATIFICATION FOR ELIMINATION ACTIVITIES

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Eliminating malaria and preventing resurgence will require targeting appropriate packages of interventions to places with ongoing transmission while managing the risk of importation and re-establishment, which relies primarily on the capacity of the health system to detect and treat new infections. Here, we describe a process for operational stratification and decision making using three available metrics in the context of a malaria elimination program in Haiti. Maps of malaria incidence data, parasite movement, and treatment seeking rates were generated and used to stratify the country into operationally relevant units. Thresholds for categorizing units by the required intervention packages, including improved routine case detection and management, indoor residual spraying (IRS), active case detection (ACD), mass drug administration (MDA), and measures targeting imported infection, were estimated by analyzing the relationships among the interlinked metrics. This methodology was applied to evaluate interventions required for elimination in Haiti. Results suggested that 75% of the population was at low risk of malaria transmission, requiring only stronger passive case detection and management. Grand Anse in the South West and Commune Ganthier in the West, near Etang Saumatre lake were identified as remote areas with the highest malaria transmission and relatively poor access to malaria treatment, requiring aggressive measures including simultaneous ACD, IRS, and MDA. Most imported infections are found in Port-au Prince suggesting that additional measures targeting travelers would be needed. This methodology provides a potential framework to help national malaria programs to develop evidence-based operational plans for targeting aggressive interventions. In a resource-limited environment, this sort of approach will be critical to ensuring optimal intervention packages are targeted to the places where they will have greatest impact, increasing the probability of interrupting transmission and maintaining malaria elimination.

890

INCREASED PREVALENCE OF ASYMPTOMATIC *PLASMODIUM FALCIPARUM* INFECTION IN DIENGA, SOUTHEASTERN GABON

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Control strategies implemented a decade ago led to a marked reduction in the prevalence of malaria in many countries. In Dienga, southeastern Gabon, the prevalence of microscopic *Plasmodium falciparum* infection was 7% in 2003, close to the pre-elimination threshold of 5%. The aim of this work was to determine the prevalence of *P. falciparum* infection in the same community a decade later.A cohort of 370 individuals aged from 3 to 85 years living in Dienga was investigated for *P. falciparum* infection; during six passages (P) in 15-month period. Demographic data were collected, along with behaviors and attitudes towards malaria. *Plasmodium* infection was diagnosed by microscopy (ME), followed by PCR to detect submicroscopic infection.The prevalence of *P. falciparum* infection in P1, P2, P3 , P4 , P5 and P6 was respectively 43.5% (25.1% ME+, 18.4% PCR+); 40.9% (27.0% ME+, 13.9% PCR+), 52.7% (26.1% ME+, 26.6% PCR+); 34.1% (14.1% ME+, 20% PCR+), 57.7% (25.4.% ME+, 32.3% PCR+);and 46.2% (21.4% ME+, 24.8% PCR+) with an overall average of 45.9% (95%CI [37.0 - 54.7], 23.2% ME+ and 22.7% PCR+). P4 and P5 prevalences were statically different throughout the six passages. Microscopic prevalence was significantly higher than that observed ten years ago (23% [n=370] vs 7% [n=323], $p < 0.001$). Asymptomatic infections were the most frequent (96%). Gametocytes were detected in levels ranging from 5.9% to 13.9%. Insecticide-treated nets, indoor residual insecticides, and self-medication were used by respectively 33.2% (95%CI [29.0 - 37.4]), 17.7% (95%CI [15.5 - 19.9]) and 12.1% (95%CI [10.6 - 13.6]) of the study population.A near-threefold increase in *P. falciparum* infection has been observed in a rural area of southeastern Gabon during a 10-year period. Most infections were asymptomatic, but these subjects likely represent a parasite reservoir. These findings call for urgent reinforcement of preventive measures.

891

MOLECULAR EVIDENCE OF HIGH RATES OF ASYMPTOMATIC *PLASMODIUM VIVAX* INFECTION AND VERY LOW *P. FALCIPARUM* MALARIA IN BOTSWANA

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Botswana is one of eight SADC countries targeting malaria elimination by 2018. Through upscaling of control activities, significant reductions in case incidence of *Plasmodium falciparum* (0.96 - 0.01) was achieved between 2008 and 2012. As part of the elimination campaign, active detection of asymptomatic *Plasmodium* species was carried out to determine asymptomatic *Plasmodium* species carriage by nested PCR in the country, in 2012. A cross-sectional study involving 3924 apparently healthy participants were screened for *Plasmodium* species in 14 districts (5 endemic: Okavango, Ngami, Tutume, Boteti and Bobirwa; and 9 epidemic: North East, Francistown, Serowe-Palapye, Ghanzi, Kweneng West, Kweneng East, Kgatleng, South East, and Good Hope). Venous blood was taken from each participant for a nested PCR detection of *Plasmodium* species. The parasite rates of asymptomatic *Plasmodium* species detected were as follows: *P. falciparum*, 0.16%; *P. vivax*, 4.66%; *P. malariae*,(Pm) 0.16%; *P. ovale*, 0%, mixed infections (*P. falciparum* and *P. vivax*), 0.055%; and (*P. vivax* and P. *malariae*), 0.027%, (total: 5.062%). The high proportion of asymptomatic reservoir of *P. vivax* was clustered in the East, South Eastern and Central districts of the country. High rates of *P. vivax* infection correlated linearly with high rates of *P. malariae* infections with a predictive value of 27.9 *P. vivax* infections for each *P. malariae* infection (95% CI 22.6-33.2, $p<0.001$). The median age for *P. vivax* infection was 5 years (Mean 5.13 years, interquartile range 3-7 years).The odds of being infected with *P. vivax* decreased by 7% for each year increase in age (OR 0.93, 95%CI 0.87-1.00, $p=0.041$) when gender was adjusted for in a logistic regression. In conclusion, we have confirmed low parasite rate of asymptomatic *Plasmodium* species in Botswana, with the exception of *P. vivax* which was unexpectedly high. This has implication for the elimination campaign, requiring a follow up study taking this evidence into account in the elimination campaign.

892

USE OF ACTIVE AND PASSIVE SURVEILLANCE TO DETERMINE THE RISK FACTORS FOR MALARIA INFECTION IN ACEH BESAR, INDONESIA, A LOW-ENDEMIC, MULTI-SPECIES SETTING (*PLASMODIUM KNOWLESI*, *P. VIVAX*, AND *P. FALCIPARUM* INFECTION) AIMING FOR MALARIA ELIMINATION

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As the number of malaria cases declines, the risk factors for infection change and transmission becomes more geographically focal and likely due to asymptomatic and non-falciparum infections. To inform malaria elimination planning, local risk factor assessments using acquired data are necessary. To identify risk factors for malaria infection, a population-based passive and active surveillance study was conducted in Aceh Besar District, Indonesia from 2014 to 2015. Malaria infection was defined as symptomatic PCR-confirmed infection passively reported from five health facilities, or asymptomatic or symptomatic PCR-confirmed infection identified in reactive case detection (RACD). Potential risk factors were assessed through a questionnaire. Multi-level logistic regression models were used to measure the associations between potential risk factors and malaria infection. Risk factors associated with species-specific infection and secondary cases were analysed by chi-squared/Fisher-exact test and Kruskal-Wallis/Wilcoxon test. Passive surveillance identified 19 *Plasmodium knowlesi*, 11 *P. vivax*, and six *P. falciparum* infections. Of 1,495 individuals screened in RACD, six (one Pk, three Pv, and two Pf) had PCR-confirmed infection. Compared to non-infected subjects screened in RACD, infections identified through passive or active surveillance were more likely to be male (AOR 12.24, 95%CI:2.84-51.05), young adults (15-30 years) (AOR 14.11, 95%CI:2.01-98.79), and work requiring overnight stays in the forest (AOR 8.19, 95%CI:1.54-43.70). Clustering of species by sub-district of residence was determined. For secondary case detection in RACD, cases were mainly afebrile (4/6), resided within 100 meters or in the same household as the index case, and had the same risk factors as index cases. The risk factors for infection in index cases and RACD identified cases were related to forest-related work. In low transmission settings, utilization of data available through routine passive and active surveillance can support targeted efforts for individuals at high risk.

893

USE OF 'EASY ACCESS GROUPS' TO GUIDE MALARIA ELIMINATION INTERVENTIONS IN HAITI - SURVEYS AMONG PRIMARY SCHOOL, HEALTH FACILITY AND CHURCH ATTENDEES TO REFINE TRANSMISSION RISK AREAS AND TARGET INTERVENTIONS

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"Malaria Zero: the Alliance for a Malaria-Free Haiti" was formed to assist Haiti in achieving malaria elimination by 2020. *Plasmodium falciparum* prevalence is estimated to be <1% nationally. In such low transmission settings, infections are likely to be spatially clustered. Targeting elimination activities to these clusters rather than the whole population will be a more effective use of resources and is expected to have a greater impact on transmission. This study investigates the use of surveys among easy access groups (EAG) to identify areas of ongoing transmission where aggressive parasite elimination activities can then be focused. Three EAG venues were identified to enable rapid sampling of populations: primary schools, health facilities (all attendees) and churches. A concurrent large-scale household survey was conducted to validate which, if any, EAG sampling approach(es) can identify areas of ongoing transmission in the community to support decision making for targeting elimination activities. EAG surveys were conducted in 25 primary schools, eight churches and 10 health facilities across four communes of Grande Anse, with 2,100 individuals randomly sampled from each venue type. Each individual was tested for a *P. falciparum* infection using an HRP2-based rapid diagnostic test (RDT) and exposure to malaria by screening for a panel of antimalarial antibodies by Luminex. Data on demographic and socio-economic factors, treatment seeking behavior, travel patterns, and prevention practices were also collected. Information on residence was collected from all RDT positive and a random selection of RDT negative individuals using a variety of approaches (GPS loggers, interactive digital maps) compared with direct tracing of households. Residence information is used to estimate the approximate catchment area for each EAG venue, as well as locate the household of detected *P. falciparum* infections. Data collected from the household survey will be used to calibrate EAG data, and determine if EAG surveys successfully identified areas of ongoing malaria transmission.

894

PRIMAQUINE SAFETY IN G6PD-DEFICIENT MILITARY COHORT IN CAMBODIA USING THE LOWER-DOSE, EXTENDED COURSE REGIMEN AS PART OF MASS DRUG ADMINISTRATION FOR MALARIA ELIMINATION

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Primaquine (PQ) implementation in malaria-endemic areas is hindered by the fear of precipitating primaquine induced hemolytic anemia in G6PD-deficient subjects. We report on safety of a modified dosing regimen of primaquine in G6PD-deficient subjects, with G6PD activity ranging from 0.04 to 2.67 U/g Hb (median 0.59 U/g Hb) who got treated with a lower dose, extended 12 week course of primaquine as part of the Malaria Elimination Pilot Study in Cambodia. This dose of PQ was well tolerated even in subjects with severe G6PD deficiency and appears to carry a low risk of significant hemolysis. 87 of G6PD-deficient volunteers were started on a weekly PQ at a dose of 22.5 mg (approximately 0.45 mg/kg) for 12 weeks, for a total dose of 270 mg. All subjects who had >10% drop of HCT and/or Hgb on day 3 post PQ, had additional CBC performed on day 7 with safety follow up until their counts stabilized. Thirteen of 87 (15%) G6PD-deficient volunteers, treated under the modified PQ regimen, had more than 10% drop in HCT and/or Hgb on day 3, the highest drop being 16%. On day 7, 2/13 had no change, 2/13 showed recovery (rise in HCT and less than 10% down from baseline), and 9/13 showed continued HCT reductions in the range of 13-22% from baseline. 3/13 volunteers had additional small drops of HCT in week 2 but none exceeded 25% safety threshold or required PQ discontinuation. The lowest Hgb value measured post PQ was 11 g/dL. No volunteers had serious adverse event due to PQ or significant symptoms of anemia or hemolysis. This is the largest cohort of G6PD-deficient subjects from Southeast Asia treated with weekly PQ and followed prospectively. These results contrast recent

report of significant safety concerns with the CDC-recommended 45 mg weekly PQ regimen in G6PD-deficient subjects in SE Asia. The better safety profile of this lower 22.5 mg weekly dose offers a more feasible strategy for targeting hypnozoites in malaria elimination settings where *P. vivax* and G6PD deficiency are common.

895

COMPARATIVE ACCURACIES OF THREE MODELS OF HOTSPOT PREDICTION IN THE PRE-ELIMINATION SETTING OF ZAMBEZI REGION, NAMIBIA

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As malaria transmission in pre-elimination settings is geographically heterogeneous, successful programs will target hotspots efficiently. These are foci with transmission intensity greater than that of surrounding areas, inside which parasite reservoirs can persist. Programs often use historical health facility (HF) case data to predict hotspots, though these are coarse data and can miss new hotspots. We compare the predictive abilities of a model utilizing solely historical HF case data, to models incorporating prevalence and/or serologic measures. The study was conducted in the catchment areas (tot. pop.~35381) of 6 randomly selected HFs in Zambezi region, Namibia, a low transmission eliminating nation. The region's annual incidence of 26/1000 contrasts with 2/1000 nationally. Potential hotspots were identified as cases clustered in space/time using SaTScan™. The datasets were: (i) HF case data extracted from registries for Jan 2012 - May 2014; (ii) PCR-confirmed parasite prevalence in a 2015 cross-sectional survey; (iii) seropositivity to AMA-1 and MSP-1 antigens in the cross-sectional survey. Receiver operating characteristic (ROC) curves are generated for clusters identified by each method. The reference value is confirmed hotspots: villages with ≥2 laboratory confirmed, locally acquired cases reported in the 2015-16 season, with geo-location confirmation. A model based solely on HF incidence (1914 total cases) revealed 2 clusters of 58 and 36 cases with radii 3.2km and 4.4km and relative risks 2.04 and 2.33, respectively ($p<0.001$). A model using prevalence (25/2017, 1.2%) demonstrated no statistically significant clusters. Serologic assays are complete and undergoing quality control. Final analyses of dataset combinations with ROC comparison are anticipated before May 30. If a hotspot prediction model including serologic data is more accurate than those using only historical incidence and/or point parasite prevalence, serologically defined hot spots may represent a useful surveillance tool in Zambezi region. Evaluation of an even broader array of candidate antibody responses can help further refine the model.

896

ENTOMOLOGICAL MONITORING ACTIVITIES DURING A MALARIA ELIMINATION PILOT PROJECT IN SOUTHERN MOZAMBIQUE

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The National Malaria Control Programme (NMCP) of Mozambique, in collaboration with key in-country malaria control stakeholders under the umbrella of the Mozambican alliance towards the elimination of malaria in Mozambique (MALTEM) and the Mozambique, South Africa, Swaziland (MOSASWA) Cross-Border Malaria Initiative, is currently designing and piloting activities to eliminate malaria from southern Mozambique by 2020. Here we show the first data from ongoing entomological monitoring in Magude, a rural district in the southern Mozambique, where we carry out a pilot malaria elimination project. We address critical issues such as (i) the anopheline species present and their vectorial capacity, (ii) how species distribution and intensities change over space and time, and (iii) which species act as a reservoir of parasites during the dry season. In addition, changes in species distribution, densities and behavior can be observed as a result of vector control interventions.

897

TIMED AND TARGETED MALARIA TESTING DURING LOW SEASONAL MALARIA TRANSMISSION IN LUAPULA PROVINCE AS A POTENTIAL STRATEGY TOWARDS ACHIEVING MALARIA ELIMINATION IN ZAMBIA

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According to the Zambia National Malaria Control Centre (NMCC), about 98% of malaria cases are caused by *Plasmodium falciparum*. From May to August, malaria transmission is at its lowest level partly because female *Anopheles* mosquitos are in aestivation, and malaria vector population is reduced. To contribute to Zambia's health strategic plan of eliminating malaria by 2020, World Vision implemented the Stop Malaria Project in 11 districts of Luapula province (Kawambwa, Nchelenge, Mansa, Samfya, Chiyengi, Mwense, Chembe, Mwansabobwe, Chipili, Lunga and Milenge) from April 2014 to March 2016 and distributed 1,172,790 long-lasting insecticidal nets (LLINs) to over 2 million people. Reducing parasite prevalence in an intermediate host is an important step in malaria elimination. As the use of rapid diagnostic tests (RDTs) are the most recommended way of detecting malaria in high incidence areas and in low-resources settings with limited malaria microscopy services, the timed and targeted malaria testing strategy was used to identify parasite prevalence. Two approaches for detecting malaria carriers were used: a) testing household members who received a LLIN after mass distribution and b) testing household members where a malaria patient came from. Malaria parasite detection increased about three times through index case testing or active case surveillance (1,540 per 1000 people) compared to households where random testing was done (464 per 1000 people). Through both approaches, a total of 24,937 were tested for malaria (8,735 people tested through index case testing and 16,202 people randomly tested) and 8,393 were positive (4,570 and 3,823 people tested positive respectively). The malaria timed and targeted testing strategy using the index case approach during low transmission helps to increase detection of malaria parasites in human reservoirs. It should be used during vector aestivation and should be supported by proven vector control interventions that can further reduce malaria transmission by *Anopheles* mosquitoes.

898

ASSESSING ASSOCIATIONS BETWEEN RECENT TRAVEL AND MALARIA PARASITE PREVALENCE DURING A MASS DRUG ADMINISTRATION CAMPAIGN IN SOUTHERN ZAMBIA

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Southern Province, Zambia has seen substantial reductions in malaria incidence over the past few years; as malaria incidence approaches zero, challenges remain in preventing importation from neighboring areas of higher transmission. The effect of human mobility on malaria incidence is unknown in this area of Zambia and efforts to identify high-risk scenarios for importation are needed. During a mass drug administration campaign conducted in diverse malaria settings in Southern Province from late 2014 to early 2015, participants were tested for *Plasmodium falciparum* using rapid diagnostic tests (RDTs) and asked about recent travel away from their place of residence. Of those that travelled in the past 14 days, both the origin and destination of each trip were categorized as low or high transmission according to area-specific malaria incidence rates (10% IR threshold). In total 2,862 (0.9%) reported traveling at least once during the 2-week period prior to the campaign visit, of which 762 were to high transmission areas; these travelers accounted for 270 (1.4%) identified infections. After adjusting for confounding factors such as vector control, age, sex and malaria incidence around their home, logistic regression showed that those traveling were twice as likely to be infected [adjusted odds ratio (AOR) = 2.3, 95% Confidence Interval (CI) 1.9-2.6] compared to those who had not traveled recently. Each day of travel conferred an average 6.4% (95% CI: 3.3-9.4%) increase in the odds of RDT positivity. Parasite prevalence was 4.9 and 9.5 times higher among participants traveling to high transmission areas for 3 or less days and 4 or more days, respectively, compared to those traveling to areas of low transmission. The increased malaria prevalence among those traveling, especially to high incidence areas, as well as the incremental increase in the odds of infection per day of travel, suggests that importation back to low incidence areas is a potential concern for elimination efforts and, though currently only a marginal contribution to overall incidence within this region, will be import to reassess as malaria programs approach elimination goals.

899

DEMOGRAPHICS AND MALARIA PREVENTION IN MOBILE AND MIGRANT POPULATIONS IN SOUTHERN LAO PDR

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Lao PDR is committed to malaria elimination by 2030. Although transmission is endemic, 97% of confirmed cases are reported from the 5 southernmost provinces. Mobile and migrant populations (MMPs) in these provinces are considered to be at risk for malaria and may be missed by surveillance systems. Appropriately targeted interventions are constrained by a shortage of information on MMP demographic, travel and health patterns. The objective was to improve understanding of these populations by describing demographics, movement, labor practices, and malaria prevention among MMPs in southern Lao PDR. Participants were confirmed malaria cases recruited from health facilities between February 25 and March 25 2015 and were eligible if they had moved from their residence within the past 12 months to stay or work elsewhere. Family and work contacts identified by enrolled cases were also invited to participate. The resulting sample population (MMPs) included all initially enrolled cases and contacts. Information on MMPs was collected via semi-structured face-to-face or telephone interviews. Descriptive statistics and logistic regression were used to characterize MMPs and compare malaria prevention methods between occupational groups. Of 189 MMPs, 69% were male. While most MMPs (78%) reported a province within Lao PDR as their place of origin, 20% were from Vietnam and 2% from other countries. Three quarters (76%) of MMPs traveled with at least one immediate family member. While 95% reported sleeping under a net or hammock the night prior to being surveyed, 82% described the net or hammock as untreated. Twenty-six percent of construction workers slept under treated nets compared with 4% of logging workers. Odds of sleeping under treated nets increased with family members present (OR=1.3 for each additional family member; p=0.033). Demographic differences and variation in malaria prevention behaviour observed in this study may indicate sociological patterns that could inform future targeted long-lasting insecticide-treated net or hammock campaigns among MMPs in southern Lao PDR.

900

AN INVESTMENT CASE FOR MALARIA ELIMINATION IN THE PHILIPPINES

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The Philippines has made significant progress in malaria control, with an 83% reduction in cases and 89% reduction in deaths since 2005. The country has launched a subnational approach towards malaria elimination and aims to reach countrywide elimination by 2030. To attain this goal, the National Malaria Control Program (NMCP) of the national Department of Health must secure sufficient funding and political commitment amid waning focus on malaria and declining donor funding for malaria-eliminating countries, including the Philippines. To support advocacy and resource mobilization efforts of the NMCP, we developed an investment case for malaria elimination and prevention of reintroduction (POR) in the Philippines. For this, we estimated the costs of malaria elimination and POR efforts in the Philippines for year 2015 using a micro-costing approach and projected the costs of eliminating malaria in the next 5 years (2016-2020). We estimated the economic benefits of investing in malaria under a hypothetical scenario of resurgence that would likely occur if all efforts on malaria are halted. The benefit of sustained investments in malaria was estimated based on the cost and impact of a potential malaria resurgence on the health system, households, and economic productivity. Cost data were collected from the NMCP and from five sample districts that were in varying phases of elimination. Our preliminary results indicate the total cost of elimination and POR activities in the Philippines in 2015 was US$ 1.03 per capita. The key cost drivers were consumables (61% of total cost) and human resources (21% of total cost). Among malaria activities, prevention and vector control incurred the highest share of the total cost (42%), followed by diagnosis (24%) and program management (15%). The benefits of investing in malaria under the hypothetical resurgence scenario were estimated to be around US$1 billion yielding a return on investment of 8.19. This remarkable return on investment in malaria makes a compelling case for sustaining investments for malaria elimination efforts in the Philippines.

901

REACTIVE CASE DETECTION FOR MALARIA ELIMINATION

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The transmission mechanism of many infectious diseases results in spatiotemporal clustering of cases. In near-elimination scenarios we can take advantage of this structure, by using the passive detection of an index case as the basis for active searches for further cases. This strategy, known as reactive case detection, is particularly attractive for moving towards elimination in settings where effective universal surveillance is not feasible. We report on microsimulations and analytical models for *Plasmodium falciparum* malaria, based on epidemiological and programmatic data from elimination projects in the Lake Kariba region of southern Zambia, which we use to quantify thresholds for success for realistic implementations in terms of transmission context, case-enrichment ratios and health system capacities. Mass drug administration has been proposed as a useful adjunct intervention to elimination programs, and we use our models to establish the contexts where this is likely to have a significant effect on the outcome and timeline of the overall strategy. The models provide an insight into the limits and potential of such strategies, which is applicable in other malarial regions and indeed in other disease areas where the focus has moved from control towards elimination.

902

MALARIA ELIMINATION IN INDIA: LARVIVOROUS FISH PLAY AN IMPORTANT ROLE UNDER LARVAL SOURCE MANAGEMENT STRATEGY

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National Framework for Malaria Elimination in India was launched on 11 February 2016. The aim is to eliminate malaria i.e. zero indigenous malaria transmission in the entire country by 2030. Based on annual parasite incidence (<1 case per 1000 population/year), the country has been classified in to three categories. Karnataka state, south India falls under category 2. Karnataka was endemic for malaria till 2009. Currently, except two coastal cities malaria in rural areas has attained pre-elimination phase. The fish-based malaria control was initiated in the early 1990's. The main compulsion of this strategy was scientifically adopted in sericulture areas in the state from 1994. Seeing this result this method of malaria control was extended in the state. Two self-sustained larvivorous fish - mosquito fish *Gambusia affinis* in ponds and tanks, while guppies in open-dug wells formally reduced the main vector *Anopheles culicifacies* population. In most of the rural areas malaria has reached a phase which needs to prevent the residual transmission possibilities. Efforts are being made to mitigate the elimination challenge in the state and aim to eliminate malaria before by 2022.

903

EVALUATING STRATIFIED MALARIA CONTROL INTERVENTIONS IN BIOKO ISLAND: DIFFERENT APPROACHES TO FOCALIZED INTENSIFIED MALARIA CONTROL INTERVENTIONS THROUGH SPATIAL CLUSTERING AND RISK MAPS

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The Bioko Island Malaria Control Project (BIMCP) created a geo-referenced mapping system in 2012 assigning a unique identifier to all households similar to an address. In 2014 this mapping system, based on ArcGIS software and satellite imagery, was linked to the Campaign Management Information System (CIMS), an Android-based tablet application, and is currently used to plan, implement, and monitor field malaria control activities on Bioko; ultimately allowing the BIMCP to accurately track all malaria control interventions at the household and community level over time. To account for budget constrains essential for long term-sustainability of malaria control programs, a stratified control strategy can provide a possible sustainable and reproducible solution. In 2015, the BIMCP developed a framework for Indoor Residual Spraying (IRS) using a stratified methodology to target communities with higher risk of malaria prevalence. The model used: pre-existing Malaria Indicator Survey (MIS) data focusing on prevalence and risk of importation, housing characteristics for all households, spray coverage, and slope and altitude. Information is linked to the unique household identifier and a risk score is created at the community level identifying the most vulnerable communities that would be selected for IRS. Using this pre-existing model for stratification, additional analysis will be carried out focusing on two different spatial clustering techniques: 1) Kulldorff's spatial scan statistics using SaTScan v9.4.2 and 2) Anselin's Local Moran's I statistics using ArcGIS v10.4; as well as regression analysis including data on socio-economic status, bed net coverage, and malaria incidence. Due to the unique characteristics of the methodologies that will be used for stratification, the parameters will not be analogous, but various thresholds will be taken into consideration in order to achieve a higher degree of comparability. Once all models have been completed and quantitatively verified, maps will be created for each methodology, overlaying the results into one map, in order to provide evidence of visual clustering of malaria risk areas.

904

THE USE OF SCHOOL RECORDS TO MEASURE THE IMPACT OF A MALARIA CONTROL INTERVENTION ON ATTENDANCE IN RURAL WESTERN KENYA

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Malaria control in school-age children is an important priority in endemic countries due to the detrimental effect of malaria on academic performance and school attendance. Additionally, the high frequency of malaria infection and gametocytemia in this age-group may contribute substantially to transmission. We reviewed public primary school attendance records of children living in villages in Siaya County Kenya that had been in enrolled in a cluster randomized trial (CRT) of a malaria intervention in 2010. The CRT showed a 42% reduction in the incidence of malaria infection in children ages 5-11 years who lived in

the intervention villages compared with those in control villages. We assessed attendance records from 2010 at 7 schools serving 8 of the 12 villages in the CRT for evidence of spillover effects from the home-based intervention. There were no records available for 2010 in 4 additional area schools. We evaluated school records for data quality by the four domains of the Prism tool: completeness, accuracy, timeliness, and relevance. We used several methodological approaches to assess attendance: time series analysis, mixed effects, and GEE, with and without imputation to adjust for missing data. Attendance records on 914 children were included in the analysis. We noted 43.7% missing data; evidence of over writing and post-date marking of registers; sample means higher than national and local mean by head count. Data quality was not adequate in any of the four Prism domains. Attendance was higher in the treated versus control group ranging from 0.67% to 0.96% (SE 0.3%, $p<0.05$), but this finding was not retained following imputation. We were unable to show an increase in school attendance associated with an intervention that reduced malaria incidence. The lack of detectable effect from the intervention may be due to asymptomatic parasitemia in this age-group, or bias from missing and poor quality data. School attendance records are not a reliable source of observational data in developing countries. Routine records should be fully vetted using a quality framework before being used as a data source document to evaluate interventions.

905

TREATMENT ADHERENCE TO DIHYDROARTEMISININ-PIPERAQUINE DURING MASS DRUG ADMINISTRATION FOR MALARIA IN SOUTHERN PROVINCE, ZAMBIA

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The effectiveness of the mass drug administration (MDA) to prevent and control malaria transmission is, in part, dependent upon the patient's adherence to the medication regimen and achieving high population treatment coverage rates. In late 2014 in Southern Province, the National Malaria Control Center of Zambia embarked upon a community randomized controlled trial of two MDA strategies with dihydroartemisinin-piperaquine (DHAp): focal MDA (fMDA), where treatment was provided to all household members if at least one household member tested positive; and, community-wide MDA, where treatment was provided to all people irrespective of test positivity. Study participants were tested for malaria, provided three doses of DHAp if treatment eligible, and visited on the third day for follow-up monitoring. The first dose of DHAp was directly observed. Analysis from the first two rounds among 75,376 persons treated indicate that 84.6% were fully adherent by reporting taking three doses of DHAp and had blister-pack confirmation and 2.9% reported taking no doses. The proportion of full adherence was greater in fMDA (91%) than MDA (81%) ($p<0.01$). The odds of full adherence in fMDA were more than two times than that in MDA, when controlling for individual RDT status and whether first dose was observed (OR 2.39, 95% CI 1.26-4.53, n=73,703). Reasons provided for incomplete adherence were forgot doses (13.6%), felt better (13.2%) and lost medication (7.4%). These results suggest that individuals recognized that if they their household qualified for treatment, they were either at risk for malaria and/or sought to clear their infection. MDA treatment campaigns may enhance treatment adherence by assuring direct observation of the first dose and sensitization efforts in areas where non-RDT positive individuals are provided treatment. Results will be updated with additional treatment rounds and expanded with comparison to household malaria indicator survey data.

906

ACCELERATING THE REDUCTION OF MALARIA TRANSMISSION IN KANEL, RANÉROU AND LINGUÈRE DISTRICTS (SENEGAL): CASE INVESTIGATION WITH FOCAL DRUG ADMINISTRATION

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Systematic investigation of malaria cases and neighboring households is a key strategy in the path to elimination in low malaria transmission settings. A pilot quasi-experimental study to evaluate whether case investigation with focal drug administration (FDA) can decrease malaria incidence was conducted during the 2015 transmission season in six health post catchment areas in the districts of Kanel, Linguère, and Ranérou (Senegal) that had received a mass test and treat campaign in 2014. Seven adjacent health posts with similar characteristics but lower malaria incidence were selected as comparison. Malaria cases passively diagnosed at the health posts or by community health workers were visited at home and all members of the household were tested with a rapid diagnostic test (RDT) and treated with dihydroartemisinin-piperaquine (DHAP). The closest five households in a 100 meter radius were also visited, all members were tested with an RDT, and in households with at least one positive RDT, all members were treated with DHAP. From September to December 2015, 1560 malaria cases in catchment area residents were passively detected (57% were male, 9% were <5 years old and 60% were aged 5-19 years old). Among these cases, 794 (51%) were investigated and 1887 households were visited, in which >95% of the members were tested. Among the 766 non-investigated cases, 71% recently received the intervention through the investigation of another case or an FDA conducted in response to an outbreak. The RDT positivity rate in the case households was 4.4% (285/6480), whereas in neighboring households it was 3.5% (250/7053). Treated individuals received a follow-up visit a median of 3 days after the initial visit; compliance with the 3-day treatment was high (>95%) and all adverse events were mild. To evaluate the impact of the interventions, the incidence of passively detected, RDT-confirmed malaria cases at the health posts will be compared before and after the intervention and between intervention and comparison villages using a difference-in-differences analysis. Results from the full transmission season and the impact evaluation will be available mid-2016.

907

HETEROGENEOUS PREVALENCE OF SUBCLINICAL MALARIA MEASURED BY ULTRASENSITIVE PCR IN MYANMAR

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A malaria elimination campaign underway in the Greater Mekong Sub-region (GMS) aims to prevent the spread of artemisinin-resistant falciparum malaria beyond the region. Elimination may require drug treatment of all malaria infections, including low density, subclinical infections that may represent a previously unrecognized transmission reservoir. In Myanmar, targeted mass drug treatment is being evaluated using ultrasensitive PCR-based testing that is thousands-fold more sensitive than rapid diagnostic tests (RDT) and hundreds-fold more sensitive than standard PCR. In collaboration with seven governmental and non-governmental malaria elimination partners, we conducted cross-sectional surveys of malaria prevalence in 43 villages located in 13 malaria endemic rural townships of in nine State and Regions of Myanmar. Finger-prick blood was collected for rapid diagnostic testing and for standard PCR and ultrasensitive multiplex reverse transcription real-time PCR (usPCR) analyses. In preliminary analyses, *P. falciparum* prevalence (both mono-infection and mixed with *P. vivax*) ranged from 0-10% by RDT, and 0-30% by usPCR; and *P. vivax* (both mono- and mixed with *P. falciparum*) 0-3% by RDT and 0-28% by usPCR. Prevalence by standard PCR, regression analysis of the data adjusted for covariates, and comprehensive village-level geospatial mapping of malaria prevalence will be presented. Subclinical malaria at very low densities can be reliably detected by a new, DNA and RNA-based, fingerstick usPCR method. The prevalence of malaria in Myanmar is highly heterogeneous from village to village, even within the same township, highlighting the need for microstratification of malaria risk to target interventions. Prospective longitudinal studies assessing the clinical and transmission risks posed by this subclinical malaria reservoir are being planned. Results are expected to guide decisions about whether, when, where and how to implement targeted mass treatment and other interventions to eliminate this reservoir.

908

REACTIVE CASE INVESTIGATION WITH REACTIVE FOCAL TESTING AND TREATMENT FOR MALARIA IN TARGETED REGIONS IN ETHIOPIA AND SENEGAL: OPERATIONAL LEARNINGS

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In low malaria transmission areas, case investigation—of individual malaria cases, their household, and neighboring households is an important tool to contain and prevent the spread of transmission. Case investigation for malaria was implemented in six health post catchment areas in Kanel, Linguère, and Ranérou (Senegal) and ten villages in Amhara Region (Ethiopia) during the 2014 and 2015 transmission seasons. When a malaria case was passively detected at the health post or by a community health worker, it was considered an index case and a field team started an investigation targeting the case's household and the closest neighboring households (up to 5 households in Senegal and 10 in Ethiopia) within a 100-meter radius. All household members were tested with a rapid diagnostic test (RDT) and treated with an antimalarial drug if positive. Operational indicators were calculated to inform the planning and training and to understand the field teams' compliance with the standard operating procedures. The mean number of households visited per investigation by health post catchment area ranged from 1.9 to 3.1 in Senegal and 1.4 to 6.4 in Ethiopia. In Senegal, the mean distance between the index case household and the neighboring households was of 77.29 meters and that between the index case household and neighboring households with positive RDTs was of 47.5 meters. The following additional indicators will be calculated when final data are available: the average number of households existing within 100 meters of an index case household, the percentage of these that were visited by the field teams during the investigations, the percentage of visited households that were within 100 meters of the index case household, and the average distance to households that were visited beyond the 100 meters radius. The relationship between distance from index case household and RDT-positivity rate will also be evaluated. Final results using complete data from both countries will be available by mid-2016.

909

DO DASHBOARDS MATTER TO DISTRICT HEALTH MANAGERS? DOCUMENTED EXPERIENCE FROM THE DEVELOPMENT AND TESTING OF VISUALIZATIONS, DASHBOARDS AND ALERTS FOR MALARIA ELIMINATION IN SOUTHERN PROVINCE, ZAMBIA

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As more National Malaria Control Programs focus on malaria elimination, real time, accurate, and actionable data are critical to target interventions to specific geographies and populations and to optimize the allocation of resources. With decentralization of decision making in Ministries of Health across Sub-Saharan Africa, decision making power will be in the hands of district health and facility managers. Efforts to date have focused on collecting data and ensuring data quality so district managers can

manage scarce resources effectively. Questions remain as to what format and frequency should these data be available to district managers and how to use the data to facilitate stronger analysis and action especially at the district level. As the Zambian Ministry of Health is embarking on an ambitious effort to eliminate malaria with significant investment in the surveillance infrastructure and data quality, we examined different visualization, dashboard and alert mechanisms tailored for district health managers and community health worker cadres. Our user group assessments were conducted in 15 districts in Southern and Lusaka Provinces. District health manager input was used to co-design and develop dashboards to facilitate better planning and action including dashboards assessing reporting, data quality, malaria case rates, case investigation and commodity stocks. We also developed and tested the usefulness of different alert systems using SMS, email and web-based communication. This co-development approach, produced well-documented promising practices on how to create and test dashboards that can help or hinder decision making for district health managers. The process of producing dashboards provided further insights into optimizing visualizations and focusing training of country counterparts. In sum, joint development of data visualization appears to improve data use for decision making.

910

AUDIT OF MALARIA DEATHS REPORTED IN THE ROUTINE MALARIA INFORMATION SYSTEM (RMIS) IN FOUR REGIONAL DEPARTMENTS, BENIN, 2015

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Malaria is the leading cause of mortality in children <5 years in Benin. National guidelines on reporting malaria death were established in 2010. Despite recent improvements in malaria case management and case reporting, the annual number of malaria specific deaths (MSD) – 1,869 in 2014 – remains high. To investigate Benin's MSD burden, the National Malaria Control Program conducted a cross-sectional study to examine case reporting and the quality of care for severe malaria cases. Using patient health records from 24 health facilities reporting at least 10 malaria deaths in 2014, we randomly sampled 20% of malaria deaths captured by the routine malaria information system and verified diagnosis, cause of death, and treatment consistency with national guidelines. A case was confirmed an MSD if the record included time and cause of death, confirmed malaria diagnosis by microscopy or rapid diagnostic test, and documented at least one sign of severe malaria. Quality of care was assessed based on: diagnostic confirmation of malaria, injection of the recommended intravenous antimalarial at the right dosage, and correct case management according to clinical signs of severity. We identified 294 records reporting malaria deaths. Of these, 210 (71%) were correctly reported as MSD. None of the health facilities surveyed had a copy of national guidelines on defining MSD. Among the 210 MSD, we were able to assess quality of care in 204. Of these, 151 (74%) deaths occurred in the first 24 hours of care and 186 (88%) were < 5 years of age. Of 204 MSD with signs of severe malaria, 190 (93%) received intravenous antimalarials, but only 34 (17%) received treatment that followed national guidelines. Anemia and convulsions were the most reported severe signs, occurring in 125 (61%) and 97 (48%) of cases, respectively. Seventeen anemia cases (14%) received a blood transfusion. Ten convulsions cases (10%) received diazepam as indicated. Poor knowledge of national reporting guidelines may influence the high number of reported malaria-specific deaths in Benin, however poor adherence to severe malaria treatment guidelines likely contributes to high malaria mortality as well.

911

EVALUATING THE COVERAGE AND IMPACT OF A UNIVERSAL COVERAGE BED NET CAMPAIGN IN TWO DISTRICTS IN NAMPULA PROVINCE, MOZAMBIQUE: A SERIES OF CROSS-SECTIONAL HOUSEHOLD SURVEYS

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Malaria is a principal cause of morbidity and mortality among children in Mozambique, with the northern provinces among the highest-burden areas in the world. One of the chief strategies to reduce this burden is the mass distribution and promotion of long-lasting insecticidal nets (LLINs). In 2013, the Ministry of Health implemented a universal coverage distribution of LLINs in highly endemic Nampula Province. An evaluation was commissioned to measure the coverage and impact of the campaign. Two cross-sectional household surveys, two weeks after the distribution in 2013 and one year later, were performed in Nacala-a-Velha and Mecubúri Districts of Nampula. Households chosen using two-stage cluster sampling were visited by survey teams. Surveyors interviewed the head of household, directly observed nets and sleeping spaces, and performed malaria rapid diagnostic tests (RDTs) on all household members, regardless of symptoms. Indicators of LLIN ownership, access, and use were calculated, and malaria RDT positivity in children under five was compared between years. A total of 1,027 households visits were made, with 2,419 individuals tested by RDT. In Nacala-a-Velha, 80% (95%CI: 72-86) of households received at least one LLIN, corresponding to 66% (58-74) of sleeping spaces. In Mecubúri, 54% (44-65) of households received at least one LLIN and 43% (35-52) of sleeping spaces were covered. The proportion of the population that reported using LLINs more than 4 times per week during the wet season was 43% (29-58) in Nacala-a-Velha and 26% (20-33) Mecubúri, falling to 22% (15-30) and 18% (14-23), respectively, during the dry season. Malaria RDT positivity in children under five was 52% (36-67) in 2013 and 61% (44-76) in 2014 in Nacala-a-Velha and 67% (53-80) in 2013 and 87% (76-94) in 2014 in Mecubúri. In the two districts, the government-led campaign did not reach the crucial coverage and usage threshold to result in a reduction in malaria positivity. The results reinforce the need for close monitoring of universal coverage campaigns, where the true achieved net use might be significantly lower than the administrative measure of ownership would suggest.

912

HIGH LEVEL OF SUBMICROSCOPIC INFECTIONS OF FOUR *PLASMODIUM* SPECIES DURING PRE-ELIMINATION PHASE IN NORTH SUMATERA, INDONESIA

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In the effort to eliminate malaria in Indonesia by 2030, the Indonesian Ministry of Health is implementing a four-stage elimination plan comprising different strategies across the archipelago. One strategy is to support all primary health centres with the capacity of malaria diagnostic testing. However, this strategy is limited by a surveillance system

which highly relies on passive clinical cases and diagnostic test using conventional microscopy examination. In the low malaria endemic areas which predominate in Indonesia, the true burden of malaria cases remains undiscovered, and the extent that asymptomatic and submicroscopic infections contribute to transmission is unknown. Therefore, this study aimed to determine the baseline epidemiological profile of *Plasmodium* species and to investigate the proportion of submicroscopic infections among all malaria cases in North Sumatera province, Indonesia. A cross-sectional survey was conducted in Batubara regency, Langkat regency and South Nias regency between January and June 2015. A total of 3635 participants were screened for *Plasmodium* infection by microscopy, rapid diagnostic tests, and molecular analysis using nested polymerase chain reaction. Primers targeting the *Plasmodium* small subunit ribosomal RNA were used to identify *Plasmodium* species, and an additional novel assay targeting the SICAvar gene was performed for *P. knowlesi* identification. All *Plasmodium* species except *Plasmodium ovale* spp. contributed to symptomatic and asymptomatic malaria cases in North Sumatera province. Overall positivity rate by nested PCR was 34.1% (1049/3080) with 10.8%, 17.1%, 6.5% and 13.3% positive for each *P. falciparum*, *P. vivax*, *P. malariae* and *P. knowlesi* infection. We found 61.1% of all malaria infections were not detected by microscopy, with *P. knowlesi* the most common submicroscopic infection. Despite the little known of the role of submicroscopic infection in malaria transmission, these results suggest that submicroscopic infections should also be targeted in malaria control and elimination programmes as they comprise a significant proportion of malaria infections.

913

AN UPDATE ON EVIDENCE OF STRATEGIES TO PREVENT MALARIA IN PREGNANCY

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In sub-Saharan Africa, approximately 45% of the 32 million pregnancies occurring annually are estimated to be exposed to malaria infection, leading to an estimated 900,000 low birthweight deliveries. WHO recommends use of insecticide treated nets and intermittent preventive therapy with sulphadoxine-pyrimethamine (IPTp-SP) for prevention, though the latter is threatened by high levels of parasite resistance. Despite a decade's worth of intensive multicentre trials, the search for safe, effective, and well-tolerated alternative drugs to replace SP for IPTp has proven elusive. These have shown that neither amodiaquine, alone or combined with SP, mefloquine, or the fixed- dose combination of chloroquine-azithromycin are tolerated well enough to replace SP for IPTp in Africa. More recently, trials from Ghana, Malawi and Kenya, looked at intermittent screening and treatment in pregnancy (ISTp) as an alternative strategy to IPTp, consisting of intermittent rapid diagnostic testing (RDT) for malaria and treatment of RDT-positive cases with dihydroartemisinin-piperaquine (DP). The results from these trials were disappointing, showing either lack of cost-effectiveness in west Africa or up to 20% higher incidence of malaria during pregnancy in east/southern Africa, rendering this test-and-treat strategy as an unsuitable alternative to IPTp-SP in high SP resistance areas. However, two recent exploratory trials have shown promise for DP as IPTp in areas of high malaria transmission and SP resistance for reducing malaria infection (Incidence Rate Ratio [IRR]=0.32) and clinical malaria (IRR=0.16). The risk of fetal loss and early neonatal death was halved (61% to 47% lower) in the IPTp-DP arm, but the lower impact on fetal growth and preterm birth led to a more restricted overall pooled impact on "adverse pregnancy outcome" of 17% (95% CI 10-37%) using fixed effects meta-analysis. We will present results of a systematic review of evidence for prevention of malaria in pregnancy both in HIV-negative and HIV-positive women in sub-Saharan Africa, with a focus on the recent data and remaining gaps in knowledge.

914

THE EPIDEMIOLOGY OF GENDER DIFFERENCES IN MALARIA UNRELATED TO PREGNANCY

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Although malaria is declining in India and Asia, it is still important to find out what factors are impacting prevalence and incidence, to assist in the development of effective strategies to reduce the burden. Malaria in pregnancy has been recognized as a clear risk factor for malaria, but it is not clear if there are additional differences by gender, and if these differences are related to exposure, or other factors. For decades it has been observed that gender differences in the prevalence of malaria exist in some areas in Asia. We examined gender differences in our studies in India, and combined our results with additional studies from the region identified in the literature using meta-analysis. Among clinic studies in five different sites in India, the pooled risk ratio (PRR) for men to be diagnosed with malaria compared to women was 1.83, 95% CI 1.48-2.27, I2 78% (n=12) for *Plasmodium falciparum*, and 1.92, 95% CI 1.72-2.13, I2 34% (n=12) for *P. vivax*. The difference was mainly among adults (any species: PRR 1.95, 95% CI 1.67-2.27, n=12), but not among children ≤15 years (any species: 1.05, 0.87-1.27, I2 42%, n=12). Results were similar for surveys in Asia, with a PRR of 1.55, 95% CI 1.16-2.06, I2 72% (n=7) for *P. falciparum*, and 1.25, 1.06-1.48, I2 0% (n=8) for *P. vivax*. Gender differences for malaria in the same direction were also noted in studies identified in the literature in Ethiopia and Uganda. We are currently in the process of adding information from the literature from other malarious areas in the world in a database to assist in an assessment of the most likely explanation for the gender differences seen in some parts of Asia.

915

ASSOCIATIONS BETWEEN MEASURE OF MALARIA DURING PREGNANCY AND ADVERSE BIRTH OUTCOMES

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Malaria in pregnancy is associated with maternal morbidity, placental malaria, and adverse birth outcomes. However, data are limited on the relationships between longitudinal measure of malaria during pregnancy and outcomes measured at the time of delivery. Data came from a RCT of intermittent preventive therapy during pregnancy among 282 HIV uninfected women followed to delivery with histopathologic assessment for placental malaria. During pregnancy, malaria was measured using passive surveillance and asymptomatic parasitemia (AP) measured every month using a sensitive molecular assay (LAMP). Placental malaria was defined as either the presence of parasites in placental blood by LAMP or the presence of parasites or pigment detected by histopathology. Adverse birth outcomes included low birth weight (LBW, < 2500 gm) and preterm delivery (< 37 weeks). Associations were made between longitudinal measures of malaria during pregnancy and placental malaria and these same measures and adverse birth outcomes. Exposure to malaria during pregnancy was divided into 3 categories: 1) No malaria or AP (n=52, 18.4%), 2) 0-1 episodes of malaria and < 50% of samples positive for

AP (n=157, 55.7%), 3) 2-3 episodes or malaria or > 50% of samples positive for AP (n=73, 25.9%). The risk of placental malaria by LAMP was significantly higher among women in category 3 (25.0%, p=0.01) compared to categories 1 (1.9%) and 2 (3.2%). The risk of placental malaria by histopathology was significantly higher among women in categories 2 (29.9%, p=0.01) and 3 (74.0%, p<0.001) compared to category 1 (7.7%). Longitudinal measures of malaria during pregnancy were not associated with adverse birth outcomes. Placental malaria by LAMP was associated with significantly higher risk of LBW (45.8% vs. 10.2%, p<0.001) and preterm delivery (29.2% vs. 7.0%, p<0.001). Placental malaria by histopathology was not associated with adverse birth outcomes. Longitudinal measures of malaria during pregnancy were strongly associated with placental malaria but not adverse birth outcomes. Placental malaria by LAMP was associated with an increased risk of adverse birth outcomes.

916

RECENT TRENDS IN MALARIA INCIDENCE AND SURVEILLANCE IN CAMBODIA

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Cambodia has the target of eliminating malaria by 2025. There are currently intensive efforts towards achieving this aim. However, there is great concern about the potential threat to elimination from increasing ACT treatment failure rates with artemisinin and ACT partner drug resistance. It is thus important to monitor trends in incidence rates of malaria in Cambodia and to understand their possible causes. Malaria surveillance data collected from multiple sources by the Cambodia national malaria control programme (CNM) from 2006 to 2016 were analysed. Overall there have been substantial decreases in malaria burden over this period, particularly for *P. falciparum*. The burden of *P. vivax* increased with roll-out of the village malaria worker (VMW) programme and wider availability of multi-species rapid diagnostic tests, peaking in 2011. In recent years, around half of malaria cases reported to CNM in Cambodia were diagnosed by VMWs. From 2013 to 2014, both falciparum and *vivax* malaria increased in incidence. This appears to have been at least partly due to a substantial increase in testing for malaria by village malaria workers (VMW) over the same period. In 2015, the number of people tested for malaria by VMWs fell and there was a decrease in the total number of cases of similar magnitude. In Western Cambodia, there was an increase in cases in 2015 due principally to a large rise in *P. falciparum* in only two operational districts with increased testing for malaria by VMWs, and a smaller increase in testing by health facilities. The data collection and analysis are ongoing and at the time of the meeting we aim to provide updated results and potential additional explanations for the observed trends. Studies are also underway to investigate the current rates of ACT treatment failure and antimalarial drug resistance and assess their potential impact on trends in malaria incidence. These preliminary results illustrate the important role of village malaria workers in diagnosing malaria in Cambodia and highlight the importance of maintaining a stable, high quality surveillance system to underpin efforts for malaria elimination.

917

PREVALENCE OF MALARIA FROM BLOOD SMEARS EXAMINATION: A TWENTY YEAR RETROSPECTIVE STUDY FROM NATIONAL MALARIA REFERENCE LABORATORY, OUAGADOUGOU, BURKINA FASO

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Malaria is still a major public health problem in Burkina Faso. According to the Burkina Faso ministry of health statistic unit, malaria prevalence is increasing. In the WHO 2014 malaria report, the trend of malaria is decreasing in sub-Sahara. Therefore, the aim of this study was to determine real malaria morbidity based on the twenty year slide positive rate of malaria. A retrospective study was conducted at "Centre National de Recherche et de Formation sur le Paludisme" laboratory from 1990 to 2013. Twenty year malaria cases data had been collected from laboratory registration book. A total of 65464 patients were examined for suspected malaria; of these, 12922 (19.74%) had objective fever; 13159 (20.10%) study subjects were positive for malaria. A slide positive rate of *Plasmodium* within the last twenty years (1990-2013) decreased from 25.8% in 1990 to 9.3% in 2013 (p< 0,0001) with slight fluctuation during the study period. A rate of gametocytemia decrease from 3.52% in 1990 to 1.09% 1n 2013 (p< 0.0001). High slide positive rate of malaria occurred during high malaria transmission season 82.97%, followed by dry season 17.02% (p< 0.0001). The age groups of 5-14 years old were highly affected by malaria infection. During the study period, the trend was to the reduction. The predominant *Plasmodium* species detected was *P. falciparum* (97.83%) followed by *P. malariae* (1.61%).. In conclusion, slide positive rate of malaria was still high in study area. During the last twenty years, Plasmodic index & gametocytemia decreased based on the microscopy diagnosis. MoH should reinforce health system capacity to improve the data report.

918

CHARACTERIZING THE IMPACT OF DYNAMIC VECTOR ABUNDANCE ON INDIVIDUAL MALARIA PREVALENCE IN A HIGH TRANSMISSION AREA OF NORTHERN ZAMBIA

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Vector control is a key strategy in reducing community malaria burden, however, little is known about the impact of vector population dynamics on individual malaria risk in multispecies systems. Malaria transmission in Nchelenge District, Zambia is complex due to the presence of two highly competent malaria vectors with distinct spatial and seasonal distributions, resulting in year-round hyperendemic transmission. From April 2012 to December 2015, the Southern Africa ICEMR enrolled 2,296 participants in active malaria surveillance and collected over 10,000 *Anopheles funestus* and 1,000 An. gambiae from indoor CDC light traps. Average mosquito counts per household were aggregated by species to each 1x1 km sampling grid by month of data collection. Multivariate spatial and temporal smoothing analyses will be conducted to further account for variation. A mean of 18.0 An. funestus and 1.7 An. gambiae were caught per household (range: 0-226 and 0-32). In preliminary regression analyses, mean An. funestus per household was significantly higher in the dry season, in inland areas, and in grids with higher use of insecticide treated nets (ITNs), and were lower in grids with greater coverage of indoor residual spraying (IRS). An. gambiae per household were correlated with climactic and environmental factors, including temperature, rainfall,

and altitude. Both species were highly predictive of individual malaria prevalence in multivariate logistic models. The odds of having a positive RDT increased 7.7% for each increase in 10 An. funestus (P=0.02) and 5.1% for each increase in 1 An. gambiae (P=0.04), controlling for clustering by household, age, ITN use, recent malaria treatment, history of IRS, education level, and distance to roads. Inclusion of environmental variables and distance to streams attenuated the association between malaria prevalence and An. gambiae and funestus, respectively, highlighting potential ecological drivers of mosquito and malaria dynamics. These findings illustrate the complexity of vector bionomics as a predictor of malaria risk and the need to target interventions to the unique ecology of each vector species.

919

SPATIAL CHANGE IN THE RISKS OF *PLASMODIUM VIVAX* AND *P. FALCIPARUM* MALARIA IN CHINA, 2005-2014

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Despite the declining trend of malaria incidence over the last decades, *Plasmodium falciparum* malaria has increased in China both in terms of the number of case and geographical coverage. To help with the disease elimination, this study examined a spatial change in the risk of both *P. vivax* and *P. falciparum* malaria across China during 2005-2014. We applied logistic regression model to understand change in the risk of *P. vivax* and *P. falciparum* in each county across study period, and linear regression model to examine annual change in longitude and latitude of affected counties. The risk of *P. falciparum* malaria significantly increased with latitude and longitude, indicating that incidence rate of *P. falciparum* malaria increased in the northern and eastern, or decreased in south and western China. Similarly, latitude and longitude of counties with *P. falciparum* significantly associated with year. However, longitude of *P. vivax* affected counties significantly decreased, showing an annual declining number of *P. vivax* affected counties in eastern or increased in western China. The risk of *P. vivax* malaria had decreased whereas, the risk *P. falciparum* malaria had increased in the northern and eastern China. For successful elimination of malaria, underlying causes of the increased *P. falciparum* malaria risk needs further investigation.

920

EVALUATING A REACTIVE TEST-AND-TREAT PROGRAM FOR SUB-PATENT MALARIA IN MACHA, ZAMBIA: OPTIMAL STRATEGIES TO ACHIEVE ELIMINATION

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In Choma District, Southern Province, Zambia, malaria prevalence by rapid diagnostic test (RDT) declined from 8% in 2008 to 1% in 2013. As part of an effort to achieve elimination, the Zambian government implemented a reactive test-and-treat (RTAT) program in parts of Southern Province in 2013. Individuals with confirmed malaria by health workers are followed-up within two weeks of diagnosis. All individuals living in households within 140 meters of the index case are tested with an RDT and treated if positive. This study aimed to optimize the RTAT strategy by characterizing infected individuals missed by both the RDT and the current screening radius. Health workers notified the study team of individuals with RDT confirmed malaria. For each study participant, a questionnaire was administered and a blood sample collected. To evaluate the optimal RTAT radius and assess the frequency of sub-patent, RDT negative infections, the radius was expanded to 250 meters and quantitative polymerase chain reaction (qPCR) testing was introduced. Spatial-temporal cluster detection was conducted to identify clusters of index households. From January 2015 to January 2016, 101 index cases were identified at health centers and health posts and followed up by the health workers with the study team. A total of 2504 individuals residing in 394 households were screened. Parasite prevalence was 2.5% by qPCR (53 positives of 2108) and 1.2% by RDT (26 positives of 2225). Of the qPCR positive cases, 66% of 53 individuals tested negative by RDT. 24 households had at least one qPCR+/RDT- individual. Nearly half of those infected resided within the index case household. The cluster detection revealed no clustering of index case households. The low number of secondary cases indicates low efficiency of RTAT beyond the index case household and the sensitivity of the RDT was too low to be an effective screening tool. Focal drug administration in which all individuals within index case households are treated may be a more efficient approach to achieving malaria elimination in southern Zambia.

921

PREVALENCE OF ASYMPTOMATIC MALARIA INFECTIONS AND ASSOCIATED RISK FACTORS IN A HIGH TRANSMISSION REGION IN WESTERN KENYA

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Asymptomatic infections pose a major threat to malaria control programs since they act as silent reservoirs for the malaria parasites. Targeting the asymptomatic reservoir is key for the success of elimination efforts. We sought to determine the prevalence of asymptomatic malaria infections, whether they show heterogeneity and their determinants in a high transmission geographically homogenous region. This was part of a larger prospective cohort study that we set up in Bungoma East sub-County. We conducted quarterly parasitological surveys for a cohort of 400 participants from randomly selected households located in known fever 'hotspots' and "cold spots". Follow-up continued for a period of one year. Generalized estimating equations were used to model risk factors associated with asymptomatic parasitemia. A total of 321 malaria infections were detected during the five cross-sectional surveys over the course of one year. Almost half (46.3%) of these were asymptomatic. Overall, most of the asymptomatic cases (67%) were in households within known 'fever hotspots'. The proportion of infections that were asymptomatic in the coldspots were 73.1%, 31.8%, 13.3%, 55.6% and 48.2% during the first, second, third, fourth and fifth visits respectively. In the known fever hotspots, the proportion of infections without symptoms was 47.7%, 48.5%, 35%, 41.3% and 47.5% during the first, second, third, fourth and fifth visits respectively. Factors associated with asymptomatic malaria include; the village one lives: people living in Maruti village were twice likely to be asymptomatic (A.O.R: 2.141, C.I: 0.03 - 1.488), age: children aged between 6 to 15 years were more than twice likely to be asymptomatic (A.O.R: 2.67, C.I. 0.434 - 1.533) and the season: infections during the dry season (January) were less likely to be asymptomatic (A.O.R: 0.26, C.I: -2.289 - 0.400). The prevalence of asymptomatic infections in this region is very high. There is a need for active surveillance to detect the asymptomatic cases as well as treat them in-order to reduce the reservoir. Targeting interventions to the asymptomatic individuals will further reduce the transmission.

922

GEOGRAPHIC DISTRIBUTION AND SPATIAL CLUSTERING OF SUBMICROSCOPIC MALARIA IN KAYIN STATE, MYANMAR

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In the Greater Mekong Subregion (GMS) malaria is spatially heterogeneous and seasonal. Since infections are relatively rare it is thought that few people should have asymptomatic and submicroscopic infections. However, recent work has revealed communities with high prevalences (>40%) of asymptomatic and submicroscopic malaria in several areas of the GMS. These preliminary studies focused on small numbers of villages and the distribution of such populations across landscapes in the GMS isn't known. The goal of this work was to assess and analyze the frequency and geographic distribution of submicroscopic malaria across four townships of Kayin State, Myanmar as part of a *Plasmodium falciparum* malaria elimination project. We used field-based geographic surveys to map a target area consisting of over 1,200 villages (approximately 16,500 km2). Villages were then randomly selected for blood surveys. Survey teams visited selected villages, randomly selected villagers for participation in the survey and took 2cc of whole, venous blood. Blood samples were analyzed using a highly sensitive quantitative PCR approach. Villages with an overall *Plasmodium* prevalence higher than 40%, of which 20% or more was falciparum malaria, were considered "hotspot" villages (43 villages out of 204 surveyed have been thus categorized so far). Clustering also occurs at scales larger than the community. Villages with high prevalences of either falciparum or *vivax* malaria occur near other villages with high prevalences. Most "hotspot" villages occurred within 5km of at least one other "hotspot" village. Finally, over 85% (37/43) of all "hotspot" villages occurred within a single subregion (less than 3,500 km2) of the overall target area. Our results indicate that submicroscopic malaria clusters at multiple scales. Populations with high prevalences of submicroscopic malaria may act as important reservoirs of the disease and could frustrate elimination efforts. These results have implications for planning and implementing interventions such as reactive screening and treatment or targeted mass drug administration.

923

MALARIA PREVALENCE IN THE URBAN AREAS OF MANGALURU IN SOUTH INDIA

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Mangaluru city, located on the Southwestern coastal area of Karnataka State in India, is the government headquarter of Dakshina Kannada district. In late 1940s, malaria was common in the rural areas of the district with parasitemia rate of 5-12% and splenomegaly case rate of 17-46%, whereas incidences were low at urban areas of Mangaluru city. The malaria control program initiated in the district in 1948 succeeded in reducing the parasitemia and splenomegaly rates to 0.5% and 9.4% respectively by 1951. However, resurgence of transmission resulted in 440 deaths between 1956 and 1959, and with renewed control efforts, there were no deaths by 1961. From 1969 malaria started resurging again, reaching a peak of 5225 cases in 1975, and with the modified plan of National Malaria Eradication Program, it declined to 86 cases in 1988. Again, the numbers of malaria cases in the district increased to 340, 4027, 6692, 21159, and 11413, respectively, in 1991, 1993, 1995, 2005 and 2015; there were 26 deaths in 1995. While 95 (83%) of 114 cases reported in 1990 occurred in rural areas of the district, during 1993 to 2015, 86% cases have occurred in the urban areas of Mangaluru city and 14% in the rural areas. The average annual parasite incidence (API) has been 16 for Mangaluru city and 0.7 for rural areas of the district. The surges in malaria cases in Mangaluru city during 1995-97 and 2004-06 were accompanied by increased incidence in the adjoining rural areas. Industrial constructions, rapid expansion of urbanization and housing constructions, and migration of workers from malaria endemic areas are the likely causes for the resurgence of malaria in Mangaluru city, while the spread to rural areas of the district has been likely occurring through the people commuting from rural areas for work in Mangaluru city. The persistence of markedly higher API in the city compared to the rural areas suggests that Mangaluru city is a substantial malariagenic locality in the district. The increasing contribution of Mangaluru city to the total cases of malaria in Karnataka state, from 1.2% in 1994 to 67% in 2015, suggests the need for more effective control measures in Mangaluru city.

924

MALARIA SURVEILLANCE DURING THE TRANSIT FROM CONTROL TO PRE-ELIMINATION

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The 2012 WHO manual of surveillance for malaria control proposed the monitoring of suspected cases, however the same manual fostered the use of confirmed malaria cases. On other hand to ensure that lab results do not influence the condition of "suspected" in the registers, has been proven to be a challenge. Following WHO guidance, Bioko Island Malaria Control Project (BIMCP) has been reporting the number of suspected cases, composed by adding a) the number of outpatients lab tested for malaria plus b) the number of presumed malaria cases (clinical diagnosis or diagnosed without lab confirmation). After 11 years of BIMCP's activity, Bioko is in transit from control phase to the pre-elimination, and a malaria surveillance system able to provide support for both phases is required. We have assessed the trends and composition of the group "suspected cases" to assess its contribution to the malaria surveillance. Between 2013 and 2016 the reduction in the average monthly number of suspected cases was quite slower (30%) compared with the decrease in its sub-group of confirmed malaria cases (75%), or the sub-groups with diagnostic behaviors potentially deviant of WHO guidelines (1. cases diagnosed as malaria without lab test, 2. cases diagnosed as malaria with negative test results, and 3. cases with a diagnosis other than malaria with a malaria test positive) which shown a decrease in the range of 68-71%. Conversely, among the suspected cases the average monthly number of cases with a malaria test negative and reported with a diagnosis other than malaria experienced an increase of 219%. We conclude that a) the number of suspected cases is not as sensitive as of confirmed cases to monitor the burden of disease, b) the training, supervision and availability of supplies for lab testing has allowed the reduction of undesirable diagnostic behavior, and c) clinicians may have in fact adopted more inclusive criteria to classify outpatients as malaria suspected cases along with the reduction of parasitemia in Bioko. NHIS data from at least the last four years will be analyzed to identify changes in the definition of suspected cases informally adopted by clinicians.

925

THE RELATIONSHIP BETWEEN ANEMIA AND MALARIA INFECTION AMONG CHILDREN UNDER FIVE YEARS IN MALAWI

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Anemia is a public health concern in resource limited settings. Although the causes are multi-factorial, a major contributor is malaria. The World Health Organization has proposed using anemia prevalence as a surrogate measure of the burden of malaria infection. Despite the fact that several studies have demonstrated a correlation between the prevalence of anemia and the prevalence of malaria infection, none have explored the relationship between anemia and malaria prevalence over time or whether widespread rollout of malaria interventions is associated with a change in the prevalence of anemia. Six cross-sectional studies were conducted in southern Malawi during the rainy and dry seasons from 2012 to 2014. A mass distribution of insecticide-treated nets (ITNs) occurred after the first survey. Children 6 to 59 months of age for whom hemoglobin data were available were included in this analysis. Blood samples were collected by fingerprick, and hemoglobin was measured using Hemocue® photometer. Malaria infection was detected by qPCR. Zero-inflated Poisson mixed-effect regression models were used to assess the relationship between anemia prevalence and malaria prevalence or ITN use accounting for clustering at the neighborhood level. Hemoglobin data and PCR results were collected on approximately 3,100 children under 5 years of age. Malaria prevalence followed a seasonal trend with the highest prevalence in the rainy seasons. In contrast, anemia prevalence and ITN use did not show consistent patterns. Additional analyses are planned for confirmation. From our initial analysis, we found no evidence of association between changes in anemia prevalence and either changes in malaria infection prevalence or ITN use in children. Anemia may not be a useful surrogate for changes in malaria prevalence.

926

ASSOCIATION BETWEEN CARRIAGE OF ASYMPTOMATIC INFECTIONS AND TIME TO CLINICAL MALARIA IN MALAWI: DATA FROM A LONGITUDINAL COHORT STUDY

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It is unknown whether asymptomatic *Plasmodium falciparum* infections lead to clinical malaria. However, there is some evidence that asymptomatic infections may protect against development of clinical disease. This study uses longitudinal data to examine the effect of asymptomatic infections on time to clinical malaria in a high-transmission region of Malawi. Individuals attending a health center with acute uncomplicated malaria were treated with artemether/lumefantrine (AL) and followed for up to 470 days. Blood samples were collected monthly for qPCR detection of infection. Passive surveillance for clinical malaria was conducted and, if RDT positive, participants were treated with AL. Mixed effect Cox models with time-varying exposure (asymptomatic infection) and random effects for repeated measures were used to estimate the effect of carriage of asymptomatic infection on time to clinical malaria. Models were adjusted for age, season, and ITN use. Follow up time for the current analysis began 30 days after each treatment with AL. There were 62 individuals with 192 periods of observation after a treatment, 137 ending in clinical malaria episodes and 55 censored observations. Median follow up time for all observations was 95 days (IQR = 39 - 202); 122 days (IQR = 51-265) for observations with asymptomatic infections and 59 days (IQR = 26 - 111) for observations without asymptomatic infection. After adjustment, individuals with asymptomatic infections had an increased time to clinical malaria compared to individuals without asymptomatic infection (Hazard Ratio = 0.67, $p < 0.05$). Asymptomatic infections are associated with decreased risk of clinical disease compared to those without asymptomatic infection. This could be attributed to a protective effect of asymptomatic infection against symptomatic malaria. Alternatively, the ability to maintain an infection without symptoms may be an indicator of host immunity. Understanding the mechanism that underlies the relationship between asymptomatic infection and clinical disease is essential to developing interventions and may provide insight into the basis of acquired immunity.

927

COST ANALYSIS OF AN LLIN KEEP-UP CHANNEL IN TANZANIA: THE SCHOOL NET PROGRAM

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Although school distribution has been named by World Health Organization as a channel for maintaining universal coverage of long lasting insecticide treated bed nets (LLIN), little is known about the cost or cost-effectiveness of this approach. In 2011-2012, Tanzania attained universal coverage, reaching over 90% ownership of at least one LLIN. Since then, in the southern zone LLINs have been distributed to school children in primary school classes (1, 3, 5 and 7) and secondary school classes (2 and 4) once a year as a keep-up strategy. A cost analysis of the third round of the School Net Program (SNP3) was implemented in 2015. During SNP3, the government distributed 494,407 LLINs to 1,919 schools in 19 districts. The study utilized the provider perspective and estimated both economic and financial costs. Costs were collected retrospectively from financial and operational records and through stakeholder interviews at the national and regional level. A survey instrument was utilized to collect resource use and expenditure information at the district and school level. Fifteen out of the 19 districts were sampled along with two schools from each of those districts. Average costs for each activity were calculated from the sampled districts and schools and applied to those not included in the sample. Overall, SNP3 was able to deliver LLINs at an economic cost of 7.70 USD per net distributed during 2015. This translates into an estimated economic cost per person year of protection of 1.28 USD. Of the total economic cost, approximately 4.33 USD (56%) was for distribution while the remainder of costs related to the cost of the net itself. These costs, as well as the cost per treated net year and cost per person year of protection appear comparable to other LLIN continuous distribution systems.

928

DO MALARIA HOTSPOTS REALLY FUEL TRANSMISSION?

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Spatial heterogeneity in malaria transmission has been recognized for some time and the idea of using these 'hotspots' of intense transmission for targeting control and elimination interventions have received considerable attention. However, despite the biological plausibility of the hotspot idea, both in terms of these areas seeding transmission and that targeting these areas with interventions can have a disproportional impact on transmission, the evidence to support this concept has been mixed. Evidence suggests that some hotspots are consistent between years while others are not which has important implications for how to inform control practices. Similarly, malaria heterogeneity exists on all scales and transmission intensities however; identifying when and whether identifying hotspots becomes operationally feasible is unknown. To study the spatial and temporal dynamics of malaria transmission, data from a longitudinal cohort in The Gambia was analyzed. All consenting individuals residing in twelve villages across the country were sampled monthly from June (dry season) to December (wet season) 2013. A study nurse stationed within each village recorded all malaria episodes between visits. *Plasmodium falciparum* infections were determined by polymerase chain reaction. Spatial and spatio-temporal analysis was conducted using the PrevMap package in R. Results indicate that malaria is focal around high burden households suggesting that such households seed transmission. However, this pattern was only observed in low transmission villages. As transmission intensity increases, households with a consistently high burden of malaria exist, but the high burden households change over time and infections are present in the majority of houses making a targeting strategy less appropriate. This study provides the first detailed assessment of the transmission patterns at the village level and has important implications for understanding the applicability of spatially targeted control approaches in this setting.

929

HEALTH WORKER ADHERENCE TO MALARIA CASE MANAGEMENT GUIDELINES AT PUBLICLY FUNDED OUTPATIENT HEALTH FACILITIES — SOUTHERN MALAWI, 2015

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Six million episodes of malaria occur in Malawi annually. Gaps in quality malaria treatment persist. We conducted a cross-sectional outpatient health facility (HF) survey in southern Malawi in January–February 2015 to identify opportunities for promoting adherence to the 2013 national malaria case management (CM) guidelines. Using the 2013 CM guidelines, surveyors classified patients as suspect uncomplicated or suspect severe malaria. Main outcomes were appropriate testing (suspect uncomplicated malaria only) and treatment (confirmed or suspect uncomplicated and suspect severe malaria) based on the CM guidelines. Weighted descriptive and logistic regression analyses of patient, health worker (HW) and HF characteristics were performed. We evaluated 105 HFs and interviewed 150 HWs and 2342 patients. Of 1427 suspect uncomplicated malaria patients at HFs with malaria testing, 1072 (75.7%) were tested, and 547 (53.2%) tested positive. In total, 511 (92.7%) confirmed and 98 (60.3%) suspect malaria patients (at HFs without testing) were appropriately treated. Only 8 (5.7%) suspect severe malaria patients received appropriate pre-referral treatment. Patients were more likely to get tested for malaria if they reported fever (odds ratio [OR] = 2.6; 95% confidence interval [CI]: 1.7–4.0), headache (OR = 1.5; 95% CI: 1.1–2.1) or vomiting (OR = 2.0; 95% CI: 1.0–4.0) to HWs and less likely to be tested if they reported a skin problem (OR = 0.4; 95% CI: 0.2–0.6). For patients with suspect malaria, appropriate treatment was more likely with elevated temperature (OR = 1.5 per 1°C increase; 95% CI: 1.2–1.9), patient reported fever (OR = 7.2; 95% CI: 2.6–20.2), being seen by HWs with a copy of the 2013 malaria CM guidelines (OR = 11.8; 95% CI: 3.9–34.8) or HWs with additional supervision visits in the last 6 months (OR = 1.3 per additional visit; 95% CI: 1.1–1.5), but less likely for those attending rural hospitals versus health centers (OR = 0.2; 95% CI: 0.1–0.3). HW solicitation of patient symptoms may improve malaria testing practices. Appropriate treatment of malaria may be improved by extra supervision visits to HWs, plus better access to tests and guidelines.

930

THE CHANGING BURDEN OF MALARIA IN PREGNANCY AND CURRENT EFFECTIVENESS OF INTERVENTIONS

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The risk of malaria in pregnancy (MiP) has fallen considerably in the 21st century as interventions have successfully reduced prevalence within the general population. Combining an existing mathematical model of MiP with estimates of endemicity between 2000 and 2015, we estimate that the risk of MiP has fallen on average by 37% across all areas of sub-Saharan Africa at risk. The per-pregnancy risk of MiP-attributable low birthweight (LBW) faced by women not covered by interventions such as Intermittent Preventative Treatment (IPTp) with sulfadoxine-pyrimethamine (SP) has fallen by 32%. However, the risk of MiP remains substantial: in the absence of pregnancy-specific intervention we estimate 8.5 million women would have experienced MiP leading to 660,000 LBW deliveries in 2015. We combined these estimates of MiP risk with current intervention coverage from population based surveys. Across 26 countries median use of LLINs in multigravidae (1 previous child or more) was 40%, but in primigravidae was substantially lower (median 25%). Only 21.1% of pregnant women at risk are receiving any IPTp, lagging well behind antenatal clinic (ANC) attendance (64.1% of these women attended ANC at least three times). We then combined our maps of the MiP burden with maps of SP resistance mutations. IPTp efficacy in different areas was estimated based on IPTp studies in areas with different mutation frequencies. Based upon 2010 levels of resistance, 9 million women reside where IPTp-SP is still likely to be highly effective and attended ANC at least three times during pregnancy, but did not receive any IPTp. This represents 200,000 LBWs which could be averted highly cost-effectively. We found 24% (6.2 million) deliveries occurred in settings where the quintuple SP resistance mutation had saturated by 2010, with 2 million occurring in settings with appreciable levels of the sextuple haplotype. Increasing LLIN use, particularly in primigravidae, and providing IPTp to women already attending ANC should be key priorities. Increased monitoring of the effects of SP resistance, as well as research into suitable alternatives to current IPTp regimens is also crucial.

931

PREVALENCE OF ASYMPTOMATIC MALARIA AND ANAEMIA AMONG SCHOOL AGE CHILDREN IN TWO ECOLOGICAL ZONES IN GHANA

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Asymptomatic Malaria in school children is a public health challenge because of its implication in anaemia and cognitive functioning of school children. We compared the prevalence of *Plasmodium* spp. and anemia among school children in the forest and the coastal zones of Ghana over a three year period. A cross sectional survey was conducted yearly for 3 years in randomly selected schools in a coastal savanna town (Cape Coast) and a town located in the Forest zone (Begoro). A total of 2751 children aged 6 to 14 years were screened between 2013 and 2015 during the rainy season (September to November). The pupils provided their clinical and medication history two weeks prior to the screening and their heights body weights and axillary temperatures were taken. Each pupil provided a finger-prick blood for the preparation of thick and thin blood films for detection of parasitaemia by microscopy. The overall prevalence of asymptomatic malaria ranged from 17.5% to 23.1% over the 3 years period. The prevalence was higher in Forest zone (Begoro) than in coastal savanna town (Cape Coast) consecutively over the 3 years however significant deference was only observed in 2014 with a prevalence of 24.2% and 11.3% respectively (P value < 0.0001). Highest prevalence of malaria was observed in children age between 9 and 11 years (36.5% in 2013, 43.1% in 2014 and 42.1% in 2015). *P. falciparum* is the predominant species in both sites (79%, 86.2% and 94.3% for 2013 to 2015 respectively). Other species identified in Begoro (forest zone) were *P. malariae* and in Cape Coast were *P. malariae* and *P. ovale*. The prevalence anaemia were 10.2%, 14.5% and 16.5% respectively from 2013 to 2015. Anaemia was associated with asymptomatic malaria (OR=0.42, p value <0.0001 in 2013, OR=0.62, p value = 0.049 for 2014 and OR=0.46, p value <0.0001 for 2015). We conclude that malaria parasite prevalence is relatively high among school children in the coastal and forest zones of Ghana. Anaemia in school children increased from 2013 to 2015 and is associated with asymptomatic malaria. Asymptomatic malaria in basic schools can be used to monitor the effectiveness of the malaria intervention.

932

FAILURE OF AVAILABLE MALARIA CONTROL INTERVENTIONS IN DANGASSA, MALI: CONTINUOUSLY HIGH PREVALENCE OF *PLASMODIUM FALCIPARUM* INFECTION IN A COHORT OF 1,400 INDIVIDUALS FROM 2012 TO 2015

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During the past decade, major investments have been made in sub-Saharan Africa leading to improved case management, greater coverage and use of LLINs and scale up of Seasonal Malaria Chemoprevention (SMC). We have examined changes in the seasonal prevalence of *Plasmodium falciparum* infection over a 4 year period in the context of malaria control interventions such as universal coverage with LLINs, IPTp for pregnant women, free ACT treatment for malaria and have recently introduced SMC. Our hypothesis is that long-term carriage in asymptomatic subjects is essential to maintain transmission in such populations/conditions. From 2012 to 2015, seven (7) blood smear surveys were performed in both the dry and rainy seasons in a well characterized cohort of adults and children in Dangassa (n=1500). Clinical and parasitological measurements to detect both symptomatic and asymptomatic infections were performed during each survey. Asymptomatic infection was defined as a positive smear for *P. falciparum* asexual parasites with an axillary temperature ≤ 37.5o C and no history of fever in the previous 24 hours. Seven point-prevalence of asymptomatic carriage across age groups and among pregnant women was assessed. The overall prevalence of asymptomatic infection varied from 13.3% to 51.3% in June 2015 and October 2015. Symptomatic carriage was most frequent at the end of the rainy season with 12.2% in 2012 and 11.9% in 2014. High frequencies of asymptomatic infection were observed in the dry season for children 5 to 14 years of age: 57.2% in 2013 and 64.5% in 2014. Prevalence of asymptomatic infection was also higher in pregnant women during the low transmission varying from 24.3% in 2013 to 28.6% in 2014. In conclusion, these results suggest that in Dangassa, despite multiple interventions against malaria, the prevalence of asymptomatic *P. falciparum* infection remains high, emphasizing the need for additional control approaches targeting the dry season reservoir and persistent transmission in order to reduce the continuing burden of malaria.

933

TREATMENT SEEKING BEHAVIOR, DIAGNOSIS AND TREATMENT PRACTICES IN TANZANIA: COMPARISON BETWEEN COMMUNITY SURVEYS CONDUCTED SOON AFTER THE IMPLEMENTATION OF THE AFFORDABLE MEDICINES FACILITY - MALARIA AND THE MRDTS ROLL OUT, AND THREE YEARS LATER

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Considerable efforts have been made to increase access to first-line antimalarials and mRDTs across Africa. In Tanzania mRDTs were rolled out in the public sector from 2009-12, while ACTs are subsidized in the public and private sectors. We present findings from community surveys conducted in 2012 and three years later to assess the degree to which these interventions have led to sustained changes in case management. Both surveys took place in the same regions of Tanzania (Mwanza, Mbeya and Mtwara) after the rainy season. In each region, community surveys were conducted in randomly selected wards of urban and rural districts. Health seeking behavior and drug use were inquired from individuals reporting fever in the previous two weeks. All participants were tested for malaria using mRDTs. Between 2012 and 2015, parasite prevalence increased from 16% (1428/8834) to 21% (497/2351) in Mwanza, 2% (125/5941) to 4% (82/1980) in Mbeya and 16% (837/5327) to 26% (554/2138) in Mtwara. A larger proportion of febrile cases who sought treatment were tested in 2015 (20% (210/1037)) than in 2012 (10% (169/1653)). This also translates into more antimalarials delivered. Results from both surveys show that in private as in public sectors, febrile cases were more likely to be treated than tested for malaria (14% (379/2690) tested and 35% (949/2690) received an antimalarial), especially in regions of high parasite prevalence. The majority of antimalarial therapies were obtained from private sector (25% (669/2690) and 10% (280/2690) in public sector), which is also the sector where the febrile cases were the least tested (10% (108/1096) of individuals seeking treatment in private sector tested against 55% (195/354) in public sector). Despite increasing between 2009 after roll-out of RDTs and 2012, testing still remains poorly used, especially in private sector. Increase of drug use consumption may

reflect better health seeking behavior but probably more a remaining overuse of antimalarials. These results highlight the importance of targeting the private sector for improving and encouraging the use of mRDTs in order to ensure rational and adequate use of antimalarials.

934

ACCESSING *VIVAX* MALARIA WITH OVERLOOKED GENES: THE DIVERSITY OF VIR GENES IN *PLASMODIUM VIVAX* FROM NORTHERN REPUBLIC OF KOREA

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Plasmodium vivax is the most prevalent human malaria parasite causing most of the cases outside of sub-Saharan Africa. Since 1993, *P. vivax* has been consistently arising in the northern area of Gyung-gi and Gang-won, Republic of Korea (ROK). The vir proteins, variant surface antigens (VSAs) of *P. vivax*, are considered as the key proteins to escape a host immune system through their antigenic variation, to infect erythrocyte's surface, and partially to induce the adherence of *P. vivax*-infected reticulocyte to the endothelial cell receptor. In these regards, vir genes were proposed to be the candidates for a target of vaccine development, but variant nature of VSAs have been an obstacle to develop a potent vaccine. In this study, genetic diversity of four vir genes, vir 27, vir 21, vir 12, and vir 4, was evaluated using 85 venous blood samples collected from *vivax* malaria patients in 2011-2013, ROK. The number of SNPs was distributed from low as 5 (vir 4) to high as 143 (vir 21). The average number of haplotypes of all vir genes was 8.25, and average Hd was 0.727. Vir 12 (H= 9, Hd= 0.795) showed the most genetically diverse followed by vir 21 (H= 9, Hd= 0.752), vir 27 (H= 6, Hd= 0.774), and vir 4 (3, 0.530,). Tajima`s D values of vir 27 (1.08530, $P > 0.1$), vir 12 (3.22553, $P < 0.01$), and vir 21 (0.52098, $P > 0.1$) were all positive, meaning decreased size of these genes' population and process of balancing selection. Moreover, Tajima`s D value of vir 12 was statistically significant, indicating that vir 12 was under balancing selection and/or decreasing population size. This study was the first survey about the vir gene in ROK, which provides the information of vir gene in ROK on genetic level. Among the four vir genes, it seemed that the most divergent gene was vir 12 and the vir 4 gene the most conserved. However, the sample sequences used in this study have shown a clear difference with Sal 1 reference gene sequence, while vir 4 gene was very similar to Indian isolate.

935

PACBIO TECHNOLOGIES FACILITATE GENERATION OF A HIGH-QUALITY *PLASMODIUM COATNEYI* GENOME SEQUENCE AND ASSEMBLY

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High-quality genome sequence assembly and annotation are imperative for system biology-based studies to understand the complexity of parasite-host interactions in malaria. After decades of research, the first *Plasmodium* whole genome sequence, *P. falciparum* was published in 2002 and both genome and other technologies have been advancing ever since, to the benefit of research aimed at understanding the biology of the parasite and the disease. *Plasmodium coatneyi* infection of rhesus macaques is an exceptional model to study malaria severity, given the biological and clinical features that are shared with *P. falciparum*. However, a complete genome sequence and annotation of *P. coatneyi* has not been available. As a solution, we developed a *P. coatneyi* assembly using PacBio (RS SMRT®) sequencing. This long-read technology (at least 10kb) has resulted in an assembly with high coverage and statistical confidence, with only one gap existing in the parasite's14 chromosomes. A technology comparison as well as an evaluation of the current *P. coatneyi* genome sequences (nuclear, mitochondrial, and apicoplast), gene annotation, and repetitive regions will be presented.

936

GENETIC POPULATION STRUCTURE IN HOTSPOTS OF *PLASMODIUM VIVAX* INFECTIONS IN THE PERUVIAN AMAZON: CLOSING THE GAP BETWEEN GENETICS AND EPIDEMIOLOGY

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Molecular analysis and spatial statistics provide new insights toward understanding the complexity of malaria transmission in the Peruvian Amazon as well as to better design interventions to move toward elimination. In this study, we evaluated the genetics of parasite populations in hotspots of *Plasmodium vivax* detected by microscopy in a seasonal endemic community (Lupuna, Iquitos) of the Peruvian Amazon. A population-based surveillance was conducted from March 2013 - March 2014. Every three months, we georeferenced houses and took blood samples for light microscopy examination. Samples on filter paper were collected by finger-prick for RT-PCR diagnostic and microsatellite (MS) genotyping. Of 4,090 samples (902 individuals) collected, 210 were positive by microscopy and 154 were analyzed with 9 previously reported and 7 new MS. Although four main subpopulations were identified by Bayesian analysis (STRUCTURE v2.3.4), only subpopulation 4 was stable across all surveys. Statistically significant hotspots with Satscan v9.4.2 (applying a Bernoulli model) were identified in every survey except in December (low transmission). A logistic regression showed that infections inside hotspot (n=31.2%; RR=2.62; p=0.024) during the high transmission month of June were two times more likely to be from subpopulation 4 than those located outside hotspot (OR=2.73; 95%CI=1.02-7.29). Concurrent vector biology analysis determined that in June, indoor and outdoor human biting rates by *Anopheles darlingi* decreased dramatically, supporting the hypothesis of bottleneck and clonal expansion of subpopulation 4 despite introduction of many other parasite populations. Our study findings confirmed the endogenous parasite population source of local transmission and hotspots in riverine Amazonian communities.

937

TEMPORAL DYNAMICS OF GENOME-WIDE TRANSCRIPTION IN MALARIAL CHILDREN IN BURKINA FASO

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Malaria is a complex infectious disease with many genetic and environmental determinants influencing host immune response to infection, the progression of the disease and its severity. To date, surveys of host transcriptional responses used largely on cross-sectional study designs. Here we investigate the temporal dynamics of peripheral blood

transcription response to *Plasmodium falciparum* infection using RNA sequencing of a pediatric cohort in Burkina Faso. Children from 2-10 years living in two malaria-endemic villages of Banfora health district in Burkina Faso were sampled between May and November 2015 before and during the course of infection. Hundred bp Paired-End RNASeq profiles were generated and sequenced on an Illumina HiSeq instrument. Supervised statistical analysis of host genome-wide gene expression profiles revealed the nature, magnitude and significance of transcriptional changes taking place during the course of malaria infection. Gene set and pathway enrichment analysis identified key signature pathways, molecular and biological processes of host immune system modulated in response to infection. Integrated genotype and gene expression analysis allowed quantification of the contribution of host genotype to response to infection. The study provides a high-resolution picture of temporal transcriptional changes in a highly malaria-endemic region.

938

GENOMIC SIGNALS OF CHANGING MALARIA TRANSMISSION

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Levels of genetic variation in the malaria parasite *Plasmodium falciparum* are known to vary by transmission intensity. The chance of the exact same multi-locus pattern of variation occurring several times in the population tends to be very low when transmission is intense, due to the increased opportunity for sexual reproduction and recombination. In contrast, in regions approaching elimination the same haplotypes tend to be found repeatedly due to clonal propagation. The tipping point at which this change in the population occurs is a crucial consideration for surveillance programmes, where genetic technologies are likely to play an increasingly important role in future. Here we use a fully integrated model of malaria genetics and epidemiology to explore the bottlenecks in the *P. falciparum* lifecycle that are capable of generating the observed patterns. Our results are able to capture the major trends in genomic variation over a range of transmission intensities, while also providing insight into the relative strengths of the different processes that lead to a loss of variation in decreasing transmission. We apply this model to a range of elimination and pre-elimination scenarios to explore the ways in which malaria epidemiology can influence and shape parasite population genetics. These results add to an expanding body of evidence that highlight the importance of genetic data as part of future control and elimination schemes.

939

DEVELOPMENT OF A MULTIPLEX QPCR ASSAY FOR THE QUANTITATION OF *PLASMODIUM FALCIPARUM* GAMETOCYTOGENESIS IN A COHORT OF ASYMPTOMATICALLY INFECTED ADULTS IN WESTERN KENYA

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Plasmodium falciparum gametocytogenesis and gametocyte carriage remain poorly understood in areas of endemic malaria transmission, in spite of the important correlation between malaria transmission and gametocyte carriage. A sensitive and efficient method for quantitation of gametocytes in field samples is needed to determine transmission potential and evaluate mechanisms leading to increased gametocyte production. A multiplex quantitative PCR (qPCR) assay is well suited for this purpose because it uses multiple different fluorescent probes to simultaneously quantify the expression of several markers, reducing the amount of time and sample needed as compared to running multiple individual assays, and it is capable of detecting even very low levels of gene expression. Using the TaqMan qPCR chemistry, we are developing and validating a multiplex assay for simultaneous quantification of the expression of *Pfs25*, *Pfs230p*, and *PfAP2-G*. *Pfs25* and *Pfs230p* are markers of female and male gametocytes, respectively, and are being quantified because the ratio of female to male gametocytes can have implications on transmission, and because together, they represent total gametocytemia. *PfAP2-G* is an important master regulator of gametocytogenesis, and its expression correlates strongly with gametocyte commitment in cultured parasites. Additionally, we are further validating the utility of the multiplex assay by quantifying the expression of our markers of interest in a large cohort of adults with asymptomatic *P. falciparum* infections in western Kenya. Multivariate analysis of this expression data could revel trends in gametocyte development in this population. In turn, a better understanding of gametocyte development and prevalence could inform vector control programs to focus resources on the areas with the highest transmission potential, and educate clinical decision making to benefit patients while also reducing malaria transmission.

940

TURNING BACK THE CLOCK: A HISTORY OF APICOMPLEXAN SPECIES DIVERGENCE

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An accurate timescale of the evolutionary history of apicomplexan parasites is critical for understanding rates of speciation, interactions between parasites and their hosts, the acquisition of new virulence traits, and the emergence of new diseases. Despite the tremendous public heath, socio-economic and agricultural impact of diseases caused by apicomplexan parasites such as *Plasmodium*, *Cryptosporidium* and *Theileria*, key phylogenetic aspects remain controversial or unknown, and an extensive estimate of the age of speciation of many lineages has never been attempted. Some studies have calibrated divergence times with substitution rate estimates for rRNA or organellar genes, which are not adequate for all levels of divergence, and may not be representative of the entire genome. Recently, we developed and validated a novel statistical approach to estimate the age of speciation events, using genome-wide protein sequence divergence. We used this approach to date the relative age of evolutionary lineages of several mammal-infecting *Plasmodium* species. Here, we re-apply this method to updated and expanded *Plasmodium* genomic data, including avian parasites, and further widen the taxonomic breadth of the study to include other genera, such as *Cryptosporidium, Theileria* and *Eimeria*. We find that protein divergence within genera, but not between genera, can be aptly described by a molecular clock model, and provide a comprehensive timeframe for the evolution apicomplexan parasites that yield novel insights into the co-evolution of these species and their hosts.

941

DETERMINATION OF THE *PLASMODIUM VIVAX* RECURRENCE PATTERN IN INDIVIDUALS OF THE COMMUNITIES OF CAHUIDE AND LUPUNA OF THE PERUVIAN AMAZON

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Plasmodium vivax is a major cause of malaria in Perú, and most cases are concentrated in the region of Loreto. The presence of latent hypnozoites makes it difficult to classify recurrent *P. vivax* infections as relapses, recrudescences or re-infections. The characteristic of the different

recurrences has not been defined adequately in this region despite obvious relevance to malaria control and elimination. This study determined the *P. vivax* recurrence pattern in individuals of the communities of Cahuide (CAH) and Lupuna (LUP) in the region of Loreto. A total of 50 individuals of both communities with *P. vivax* recurrence that were followed up monthly from August 2012 to March 2014 were screened for parasitemia determination by qPCR 18S rRNA and subsequently all *P. vivax* infections were genotyped using 17 microsatellites. STRUCTURE analysis were performed and the recurrences pattern were determined. After the analysis only 44 individuals where evaluated for recurrence pattern, 24 from CAH and 20 from LUP; of 24 individuals of CAH 6 individuals had one homologous recurrence, 15 had one heterologous recurrence, 1 had two homologous recurrences, 1 had two heterologous recurrences and 1 had three heterologous recurrences. In LUP of 20 individuals 6 had one homologous recurrence, 13 had one heterologous recurrence and 1 had one homologous and one heterologous recurrence. In both communities, the presence of individuals with recurrent *P. vivax* infections were observed, the majority of these cases were heterologous recurrences and most of them were detected in the seasons of intense malaria transmission of this region. Finally the parasitemia in recurrences were lower than first episode despite the greater amount of heterologous recurrences which could indicate that although they can be reinfections these would generate an immune response that controls the parasitemia.

942

A PARASITE GENETICS APPROACH TO EVALUATE MALARIA TRANSMISSION DYNAMICS IN ZAMBIA

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Malaria endemicity is highly heterogeneous in Zambia with *Plasmodium falciparum* prevalence by rapid diagnostic test ranging from 50% in Nchelenge District, located in northern Zambia, to 1% in Choma District in the south. Committed to malaria elimination by the year 2020, Zambia is faced with developing control strategies which will be effective in diverse epidemiological settings. Moving forward, it will be critical to understand the mechanisms by which transmission is sustained in these various settings. In particular, we are interested in evaluating the extent of cross-border transmission between Nchelenge District and the Democratic Republic of Congo (DRC). Parasite genetics offers a means through which we can assess this threat to local control efforts and better understand transmission dynamics in Zambia. We performed amplicon deep sequencing at the var2CSA locus of *P. falciparum* samples collected from Nchelenge District, Zambia and Haut-Katanga District, DRC, on the opposite side of Lake Mweru. Deep sequencing allows us to enumerate the haplotypes present in a given population. Subsequent comparisons of genetic relatedness and diversity between samples, including Principal Component Analysis will enable us to evaluate genetic diversity with the aim of determining the extent to which cross-border transmission occurs between Nchelenge District and the DRC.

943

ORIGIN AND SPREAD OF *PLASMODIUM VIVAX* AND *P. FALCIPARUM* IN THE AMERICAS

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Plasmodium falciparum is thought to have been introduced in the continent from Africa, over the past 400 years, as a result of to the transatlantic slave trade, although evidence to support this hypothesis is lacking. The date(s) and routes of spread of *P. vivax* to the Americas is unknown. Here we examine patterns of genetic divergence between malaria parasite populations from the Americas and other continents with the purpose of inferring their origins and time of divergence from putative founding populations. We sequenced the mitochondrial genome of 114 isolates of *P. vivax* (including 9 isolates from howler monkeys from southeast Brazil that have been identified as *P. simium*) and 223 isolates of *P. falciparum* from different continents and compared these data with publicly available sequence data. Most *P. vivax* parasites from the Americas clustered together, in phylogenetic analysis, with samples from Africa and South Asia, consistent with a recent spread of this species throughout these regions. However, one highly differentiated cluster (including mostly samples from Eastern Brazilian Amazon and Venezuela) was observed, which may suggest an independent introduction (but geographically restricted) of this parasite in the Americas. *Plasmodium* I samples clustered together, being clearly differentiated from the rest of American *P. vivax* samples. There greatest *P. falciparum* diversity was found in South Asia and Africa, while South American parasites were the least diverse. Interestingly, nearly all *P. falciparum* samples from the Americas clustered together and were clearly differentiated from those from other continents, consistent with a major population bottleneck during the (recent) introduction of this parasite into the Americas.

944

EVOLUTION OF THE TRANSMISSION-BLOCKING VACCINE CANDIDATES PVS28 AND PVS25 IN *PLASMODIUM VIVAX*: GEOGRAPHIC DIFFERENTIATION AND EVIDENCE OF POSITIVE SELECTION

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Transmission-blocking (TB) vaccines are considered an important tool for malaria control and elimination. Among all the antigens characterized as TB vaccines against *Plasmodium vivax*, the ookinete surface proteins Pvs28 and Pvs25 are leading candidates. These proteins likely originated by a gene duplication event that took place before the radiation of the known *Plasmodium* species to primates. We report an evolutionary genetic

analysis of a worldwide sample of pvs28 and pvs25 alleles. Our results show that both genes display low levels of genetic polymorphism when compared to the merozoite surface antigens AMA-1 and MSP-1; however, both ookinete antigens can be as polymorphic as other merozoite antigens such as MSP-8 and MSP-10. We found that parasite populations in Asia and the Americas are geographically differentiated with comparable levels of genetic diversity and specific amino acid replacements found only in the Americas. Furthermore, the observed variation was mainly accumulated in the EGF2- and EGF3-like domains for *P. vivax* in both proteins. This pattern was shared by other closely related non-human primate parasites such as *Plasmodium* cynomolgi, suggesting that it could be functionally important. In addition, examination with a suite of evolutionary genetic analyses indicated that the observed patterns are consistent with positive natural selection acting on pvs28 and pvs25 polymorphisms. The geographic pattern of genetic differentiation and the evidence for positive selection strongly suggest that the functional consequences of the observed polymorphism should be evaluated during development of TBVs that include Pvs25 and Pvs28.

945

ASSOCIATION BETWEEN THE ALPHA THALASSEMIA TRAIT AND *PLASMODIUM FALCIPARUM* GAMETOCYTEMIA IN A MALARIA ENDEMIC AREA IN GHANA

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The gametocyte stage of *Plasmodium falciparum* represents an important stage in human/vector malaria transmission and a highly relevant target for disrupting transmission. The alpha thalassemia trait has been associated with protection against severe malaria, however its influence of parasitemia carriage remains unclear. This study aimed to determine the prevalence of *P. falciparum* asexual stage parasitemia and gametocytaemia in association with alpha thalassaemia. The study involved three cohorts of children, non-pregnant male and female adults, and pregnant women from Asutsuare, a malaria endemic community in Ghana. The prevalence of sub-microscopic gametocytaemia was detected by Pfs25 real time PCR. The prevalence of α-thalassaemia was determined by genotyping the African α -globin deletion, α 3.7, by PCR. Children with wild type or heterozygous α-thalassemia were two times less likely to harbour parasites, compared with those having the mutant allele (OR; 0.52; 95% CI, 0.28-0.97; P=0.037), however, this association was not present in adults and pregnant women. Sub-microscopic gametocytaemia prevalence was 39.5% in children, 29.7% in pregnant women and 8.7% in adults. There was no association between any α-thalassemia allele and gametocyte carriage in any of the cohorts. In conclusion, children and pregnant women had higher asymptomatic parasitemia and may potentially serve as reservoirs of transmission. Wild type and heterozygous α-thalassemia alleles were protective against asymptomatic parasitemia in children but not in adults and pregnant women. This study found no association of alpha-thalassaemia with increased risk of gametocyte carriage, perhaps due to the limited data on the gametocyte prevalence in the study community.

946

PLASMODIUM VIVAX ISOPRENOIDS BIOSYNTHESIS PATHWAY ENZYMES AS PROBABLE DRUG TARGETS

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Malaria remains one of the world's major infectious disease imposing significant economic burden on various developing countries. Among the major *Plasmodium* species causing human malaria, *Plasmodium vivax* is now known to cause severity similar to that caused by *P. falciparum*, which has attracted a lot of attention towards the parasite. There are reports on parasitic strains showing drug resistance with the current therapeutic regimen and vaccine trials are still underway. Thus, there is a constant demand for a stable and effective antimalarial. In this context, apicoplast, a relict plastid in the *Plasmodium* is looked upon as a putative drug target due to its prokaryotic origin. It acts as a site for four major metabolic pathways which includes a prokaryotic type (Non-mevalonate) Isoprenoids biosynthesis or MEP pathway. All four pathways are hypothesized to be essential for the survival of the parasite. Majority of the enzymes involved in all these pathways are encoded by the parasite nuclear genome and are targeted to the apicoplast via a bipartite leader sequence. In the present study we have characterized two major enzymes IspD and IspG from *P. vivax* isoprenoid biosynthesis pathway. This pathway is responsible for the synthesis of isoprene units and is indispensible for the erythrocytic stages of the parasite. We have cloned and expressed PvIspD and PvIspG proteins from Indian field isolates. The proteins were purified and tested for their biochemical activity. The purified proteins were used to raise antibodies which were further used to co-localize the protein in the apicoplast of the parasite, indicating to the transcriptional activity of these proteins in the parasite. In-silico studies of proteins indicate the presence of conserved domains across all apicomplexans and prokaryotes. We have tested binding of different drugs with these enzymes and hypothesize a strong probability of using these enzymes as targets to inhibit erythrocytic stages of parasite.

947

NOVEL CLUSTERING ALGORITHM IMPROVES HAPLOTYPE DETERMINATION FROM PACBIO TARGETED AMPLICON SEQUENCING DATA

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Next-generation sequencing of targeted PCR amplicons provides improved understanding of microbial population structures such as those found in *Plasmodium* infections. While Ion Torrent and Illumina are regularly used for amplicon sequencing, these technologies are limited to amplicons less than 500 bp. Longer amplicons and read lengths can capture more complete haplotypes and transverse highly variable or repetitive regions. Technologies such as PacBio circular consensus sequencing can generate sequences kilobases in length, but suffer from higher error rates, which make analysis difficult when attempting fine resolution in determining haplotypes (e.g. detecting one base pair difference). Several approaches have been used to help correct for these errors including using Illumina in conjunction with PacBio to help correct the error prone long reads as well as several clustering and data curation methods. However, de novo assembly and clustering of long haplotypes remains a challenge. We have developed a novel clustering algorithm to mitigate the error rates of PacBio without the aid of Illumina. This algorithm outperforms current clustering methods and will be integrated into our SeekDeep software suite (baileylab.umassmed.edu/SeekDeep). The algorithm is based on rapidly creating clusters using kmer distance between sequences. Once

sequences have been clustered, a consensus sequence for each cluster is generated creating a high-fidelity haplotype. We have tested the algorithm on mixtures of *P. falciparum* lab strains from 1.6kb and 3kb fragments of the VAR2CSA gene and detected all known haplotypes perfectly down to 1% abundance. The algorithm has performed well on clinical samples including the 1.6kb VAR2CSA *P. falciparum* fragment and the protein sequences of *P. vivax* CRT and *P. vivax* CSP, both of which contain repetitive regions. Thus, our new algorithm provides researchers with the ability to further detail and define the diversity within microbial and parasitic populations.

948

EVIDENCE OF SELECTION AND GENE COPY NUMBER VARIATIONS IN VIRULENCE FACTORS AND RESISTANCE GENES IN *PLASMODIUM FALCIPARUM* FROM LORETO - PERU

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Malaria is affects more than 300 million people causing more than 2 million deaths per year. Of the four *Plasmodium* species known to infect humans, *P. falciparum* stands out as the most severe. There is increasing evidence that the emerge of resistance to anti-malarial drugs, migration and limited access to adequate health care contribute to the spread of malaria in endemic regions. This data underscores the need of constant surveillance in these settings. In this study, we analyzed the genomes of 16 *P. falciparum* strains isolated from the endemic Amazonian region of Loreto in Peru during a high incidence period between 2004 to 2008. For this purpose, we conducted a genomic population analysis focused on the detection of SNPs, gene copy number variations and selection. Our results identified the presence of four subpopulations with a very recent origin in the region with evidence of gene flow between them and presence of polyclonal infections. Read depth analysis detected five clusters with increased gene copy numbers in chromosomes 4,5,10,11 and 12 that affected genes with roles in invasion and immune evasion like reticulocyte binding proteins, MSPs, duffy binding-like merozoite surface protein and others. Additionally, we found increased gene copy number in GCH1 in five isolates and MSP1, MSP2 and VP2 in one single isolate. The genomic analysis of neutrality employing TajimasD and FuFst identified two genomic regions under balancing selection. Genes located in these regions include reticulocyte binding proteins, erythrocyte membrane protein and SURFIN. The finding of increased copy number in genes mediating host-parasite interaction can result in different expression profiles potentially influencing parasite development and severity of certain strains. Increase in copy number in genes associated with resistance pose a potential threat to anti-malarial drugs employed in this setting and underscore the need to track the origin of the VP2 expansion. Finally, the evidence of selection in genes mediating invasion point towards the effect of immune pressure in positive selection on diversity.

949

INFLUENCE OF SEASONAL MALARIA CHEMOPREVENTION ON MARKERS OF T CELL EXHAUSTION AND IMMUNOREGULATION

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In addition to causing acute and sometimes fatal illnesses, *Plasmodium falciparum* infections may also compromise immune responses to some vaccines, and thus antimalarial treatments have been considered in conjunction with vaccination. Here, in the context of Seasonal Malaria Chemoprevention (SMC), we examined whether malaria prevention decreases the frequency of exhausted T cells and regulatory T cells. The study was conducted in two villages in the district of Ouelessebougou in Mali, an area of intense seasonal transmission. Children either received SMC (Beneko village) or not (Ferekoroba village), and were followed for 6 months with monthly assessment of T cell markers in *ex vivo* assays. In the group that received SMC, 42% of children (21/50) remained free of *P. falciparum* (as assessed by blood smears) during the follow-up period compared to only 16% (8/50) in children who did not receive SMC. Despite the impressive effect of SMC treatment on blood stage parasitemia rates, there was no discernible effect on the level of exhausted T cells as measured by the expression of PD-1, LAG3 and CD160. The levels of T regulatory cells (CD4+FOXP3+ T cells) were increased in the non-SMC group and in a subset of the SMC group that were infected. We speculate that under intense malaria transmission, frequent antimalarial treatment (45/50 children in the group that did not receive SMC and 28/50 in the group that received SMC) complicates the ability to observe the effect of SMC on the immune system. We are currently evaluating the effect of malaria prevention during the low transmission season when malaria incidence and treatment is low, and we will present these data for comparison.

950

SEASONAL MALARIA CHEMOPREVENTION IS ASSOCIATED WITH A REDUCTION IN SEROPOSITIVITY TO BLOOD-STAGE ANTIGENS

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Seasonal Malaria Chemoprevention (SMC) entails 3-4 therapeutic doses of antimalarials given to children at monthly intervals. SMC was approved by WHO in March 2012 for malaria control in countries with seasonal malaria transmission, and implementation is being scaled up in Mali. While SMC has substantial benefits against malaria infection and disease, the long-term impact on immunity to malaria is not well understood. We conducted cross sectional surveys at the beginning (N=579) and at the end (N=559) of the 2014 transmission season. Antibody levels to *Plasmodium falciparum* antigens MSP-142, AMA-1 and CSP were measured by ELISA.

Seropositivity was defined as OD above the mean plus three standard deviations of naïve donors. *P. falciparum* infection was diagnosed by blood smear. In August 2014, 53.4% of the children were infected prior to first SMC dose, compared to 22.2% in November one month after last dose. Seropositivity rates prior to and after dosing among uninfected children were 85.9% vs 73.6% to MSP-142, p<0.001; 42.2% vs 30.3% to AMA-1, p=0.001; and 19.4% vs 15.2% to CSP, p=0.2. Among *P. falciparum*-infected children, the rates prior to and after SMCwere 95.1% vs 91.1% to MSP-142, p=0.1; 54.0% vs 43.3% to AMA-1, p=0.05; and 30.4% vs 25.0% to CSP, p=0.3. The lower seropositivity rates at the end of the malaria season among children who received 3 monthly doses of SMC suggest that the reduced exposure to blood stage parasites is reducing immune recognition of blood stage antigens like AMA-1 and MSP-1. We are currently evaluating the effect of SMC given over 2 or 3 years on seroreactivity rates to blood stage and preerythrocytic malaria antigens.

951

IDENTIFICATION OF IMMUNE SIGNATURES UNDERLYING CLINICAL IMMUNITY TO *PLASMODIUM FALCIPARUM* MALARIA

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Naturally acquired immunity to malaria is a complex and poorly understood process, with increasing evidence pointing towards an orchestration of multiple immune mechanisms triggered by a highly diverse set of antigens. Various studies have shown how the acquisition of antibodies against individual or subsets of surface antigens correlate with increased protection against clinical malaria in an exposure and/or age-dependent manner. Nevertheless, inconsistent and sometimes contradictory results raise the question if the observed correlates are indeed the functional components of a protective immune response or to what degree they proxy previous exposure and are indicative of other underlying immune mechanisms. Furthermore, limited efforts have so far been made in relating these immune correlates to a particular degree of individual-level protection. Here we analysed cohort-based, *P. falciparum*-specific antibody profiles using a machine learning approach to identify immune signatures predictive of an individual's protective status against clinical infection. We demonstrate that due to small effect sizes, antibody responses against commonly assumed immune correlates and potential vaccine targets are poor predictors of clinical immunity. On the other hand, highly predictive immune signatures can be found in data incorporating a much broader set of internal and surface-expressed parasite proteins. Models build on these signatures show a high degree of accuracy in predicting individual-level protection against symptomatic infections during the next transmission season. Our results suggest that naturally acquired protection is the consequence of a focused accumulation of responses to a variety of both conserved and polymorphic antigens but with no single antigen offering high-level protection on its own.

952

EFFECT OF ALLELIC POLYMORPHISM ON MALARIA PARASITE SPECIFIC *EX VIVO* IFN-GAMMA (IFN-γ) RESPONSES TO APICAL MEMBRANE ANTIGEN 1 (AMA1) IN A MALARIA EXPOSED POPULATION

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One of the leading causes of morbidity and mortality, especially in children in Africa, has been malaria caused by *Plasmodium falciparum* species. However, the delivery of core malaria interventions is responsible for the percentage decrease in malaria death rate since the year 2000. But the global technical strategy and targets for malaria 2016-2030 cannot be attained with these interventions because, malaria has not been controlled by widespread deployment of existing control tools in many endemic areas. An effective vaccine would complement these efforts. Effective anti-malaria vaccine will likely require vaccine constructs designed to induce protective CD8+ T cells against malaria liver stages. The leading malaria vaccine candidate antigen expressed by sporozoite, liver and blood stage parasites is the *P. falciparum* Apical Membrane Antigen-1(AMA1), but its allelic polymorphism is a stumbling block for vaccine development. Limited data exists on the effect of AMA1 polymorphisms on T cell responses. This study is therefore designed to investigate the effect of allelic polymorphism on malaria parasite specific *ex vivo* IFN-γ response to AMA1 in a malaria exposed population. Five study volunteers with the following HLA A and/or B super types (A01, B27, B07, B58, A02, A01A03, A03, B44, and A03) were selected from a previous study. Bioinformatically selected MHC class I-specific AMA1 epitopes from 3D7 strain, were aligned with sequences from 7G8, FVO, tm284, FC27 and AAN35928 AMA1 strains for selection and synthesis of peptides with variability. A total of 133 peptides were selected and synthesized, for stimulation of volunteers' peripheral blood mononuclear cells in IFN-γ Enzyme Linked Immunospot Assay (ELISpot). The Fisher's exact test will be used to compare proportions of IFN- γ responders between the different allelic forms of AMA1. Pairwise post hoc test for differences in proportions will be performed if statistically significant differences are observed in proportions of positive responders to the various antigens.

953

CONTRIBUTION OF PARASITE AND HOST DIVERSITY TO MALARIA TRANSMISSION IN GHANA

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The transmission of malaria parasites depends on the presence of the sexual stages (gametocytes) of *Plasmodium falciparum* in the blood. The probability of establishing an infection in the mosquito depends on several factors including the densities of male and female gametocytes as well as their sex ratio. This then makes it interesting to determine how gametocytes are rendered infectious as well as understand the dynamics of the development of transmission blocking antibodies which are developed against these gametocytes and also better understand how these work together to cause malaria trasnmission. We intend to i)- compare the prevalence of gametocytes and transmission blocking antibodies against Pfs48/45 and Pfs230 and ii)- determine the diversity of factors such as cytokines which also influence malaria transmission in two areas with different malaria transmission intensities. Blood samples were collected from *P. falciparum* infected and uninfected patients living in two different malaria transmission intensity. Total IgG (and subclasses IgG1 and IgG3), IgM, IgE levels were determined by ELISA. The levels of cytokines in the plasma (TNF, INF-γ and IL-10) were estimated by Multiplex. Gametocyte prevalence was measured by microscopy and qRT-PCR. Highly variable seroprevalence of antibody responses against sexual stage parasite antigens was found. A marked prevalence and significantly higher level of antibodies was found in volunteers from the high malaria transmission intensity. However the cytokines profiling is negatively correlated to antibody level. But these results are preliminary we expect to have more before the meeting and better interpreted.

954

PURIFICATION OF *PLASMODIUM* SPOROZOITES ENHANCES PARASITE-SPECIFIC CD8+ T CELL RESPONSES

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Malaria infection caused by *Plasmodium* parasites continues to cause enormous morbidity and mortality in endemic populations, and there is no licensed vaccine capable of inducing sterile protection. Hyperimmunization with attenuated whole sporozoites can induce sterily protective immune responses targeting pre-erythrocytic antigens. Most animal models of hyperimmunization rely on sporozoites dissected from

mosquito salivary glands and injected without further purification. In BALB/c mice, repeated small doses of P. yoelii sporozoites progressively expand the population of sporozoite-specific CD8+ T cells. In this study, large secondary doses of unpurified sporozoites unexpectedly led to contraction of sporozoite-specific CD8+ T cell responses in sporozoite-primed mice. While sporozoite-primed CD8+ T cells can alternatively be expanded by secondary exposure to Listeria monocytogenes expressing recombinant *Plasmodium* antigens, such expansion was potently inhibited by co-injection of large doses of unpurified sporozoites and by uninfected salivary glands alone. Purification of sporozoites away from mosquito salivary gland debris by density gradient centrifugation eliminated salivary gland-associated inhibition. Thus, the inhibitory effect appears to be due to exposure to uninfected mosquito salivary glands rather than sporozoites. To further assess the effect of salivary gland exposure on later sporozoite vaccinations, mice were immunized with uninfected salivary glands from a single mosquito. Compared to naïve mice, salivary gland pre-sensitization reduced subsequent liver burdens by 71%. These data show that component(s) in mosquito salivary glands reduce liver infection, thereby limiting antigen dose and contributing to lower magnitude T cell responses. These findings suggest that sporozoite immunogenicity studies be performed using purified sporozoites whenever feasible.

955

T-CELL DYNAMICS REVEAL A POTENTIAL ROLE FOR CD8+ T-CELLS DURING BLOOD-STAGE *PLASMODIUM CYNOMOLGI* INFECTION OF RHESUS MACAQUES

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T-cells play a critical role in malaria immunology. They are important for the development of protective immunity against the parasite but can cause immunopathology when over-exuberant or dysregulated. The roles of CD4+ T-cells are well-appreciated in these processes, but the role of CD8+ T-cells during blood-stage malaria has received less attention. Most studies to-date have focused on studying T-cell responses against *Plasmodium falciparum* or mouse models of malaria. Contrastingly, T-cell responses during *P. vivax* infection are less understood, in part due to the lack of appropriate and tractable animal models for this malaria parasite. In this study, the *P. cynomolgi* –rhesus macaque model of *P. vivax* infection was used to monitor the T-cell dynamics during primary and relapse blood-stage infections. Immunophenotyping of T-cells during acute infection revealed a decrease in the total number of T-cells compared to pre-infection values as expected based on previous experiments. Although both CD4+ and CD8+ T-cell numbers decreased at this point, the CD8+ compartment was the most affected. Interestingly, the loss of CD8+ T-cells in the periphery appeared to correlate with clinical presentation. In agreement with the flow cytometry data, whole-blood transcriptomic analysis also indicated that the peripheral T-cell compartment, in particular CD8+ T-cells, was altered during acute infection. These results corroborate previous findings showing decrease in CD8+ T-cell subsets in *P. vivax*-infected patients. Interestingly, the transcription of IL-15 and Granzyme B was decreased in peripheral cells but this did not correlate with protein levels that were increased in the plasma. Collectively, these data suggest that activated Granzyme B-secreting CD8+T cells, although reduced in number in the circulation, may be homing to other organs and releasing inflammatory mediators during the infection. Overall, these data indicate that CD8+ T-cells are involved in blood-stage *P. cynomolgi* infection and may be related to disease severity.

956

DECREASED MALARIA TRANSMISSION IN KENYA LED TO DELAYED ACQUISITION OF ANTI-MALARIAL ANTIBODIES IN CHILDREN AND ADULTS

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Malaria transmission is declining worldwide due to interventions such as insecticidal treated bed nets and indoor residual spraying. In our study population in western Kenya, we have observed a decline in the prevalence of asymptomatic *Plasmodium falciparum* (Pf) infection in children from 81% in 2003 to 14% in 2013. It has long been known that antibodies play an important role in the development of immunity to malaria. We sought to analyze changes in acquisition of anti-malarial antibodies over a 10-year period of declining transmission. Bioplex was used to measure immunoglobulin (Ig) G antibodies specific for 34 malarial proteins in healthy Kenyan children and adults from two cross-sectional cohorts (n=82 children, n=95 adults in 2003, and n=97 children, n=50 adults in 2013). The antibody magnitudes, prevalence, and seroconversion rates were directly compared between the two cohorts (2003 and 2013). Compared to the 2013 cohort, children and adults in 2003 had higher antibody magnitudes and prevalence, and faster seroconversion rates for the majority of the antibodies measured. For example, in 2003 the age groups 1-3, 4-6, 7-10, and 18+ years had prevalence values for merozoite surface protein (MSP) 6 of 50.0%, 58.3%, 83.3%, and 88.4%; in 2013 prevalence values for the same age groups were 17.2%, 27.0%, 32.3%, and 68.0%, respectively. Antibody magnitudes and prevalence in both cohorts tended to increase with age, as expected. An exception to this pattern was seen in antibodies to 4 out of 5 domains of Pf Erythrocyte Membrane Protein 1 (PfEMP1), in which children in 2003 and had higher antibody magnitudes than adults in 2003, while in 2013 antibody magnitudes increased with age. In summary, decreasing Pf transmission is associated with a decline in several anti-Pf antibodies across age groups. Lower transmission rates may lead to a slower acquisition of anti-malarial immunity and longer duration of susceptibility to symptomatic Pf infections.

957

ANTIBODY RESPONSES IN MALARIA-NAÏVE ADULTS AFTER IMMUNIZATION VIA MOSQUITO BITE WITH RADIATION-ATTENUATED *PLASMODIUM FALCIPARUM* SPOROZOITES (IMRAS)

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Immunization with radiation-attenuated *Plasmodium falciparum* sporozoites (PfRAS) via mosquito bite serves as the gold standard for protective immunity against malaria and provides a relevant model for studying protective immune effector mechanisms. We recently completed a phase 1 clinical trial (Immunization via Mosquito bite with Radiation-Attenuated Sporozoites, or IMRAS), a comprehensive systems biology-based effort to identify biomarkers of protection, including host response and antigenic targets, by comparing protected, non-protected, and mock-immunized subjects. There is evidence that humoral immunity

contributes to RAS-induced protection in that immune serum from RAS-immunized humans can block the invasion of Pf sporozoites into hepatocytes. In addition, high titers of CSP-specific antibodies in humans correlate with protection against infectious challenge. In the present study, we investigated the generation of malaria-specific antibody responses in PfRAS-immunized subjects, and assessed their associations with sterile protection from controlled human malaria infection (CHMI). Serum IgG antibodies specific for pre-erythrocytic antigens (CSP, AMA-1, and CelTOS) were quantified by the International Reference Center for Malaria Serology Laboratory (WRAIR) using validated ELISA assays at the following time points: pre-immunization, day 14 post 3rd immunization, day of CHMI and day 28 post CHMI. In addition, sporozoite-specific antibodies were measured by an immunofluorescence antibody (IFA) assay using imaging analysis software at the pre-immunization and day of CHMI time points. Results from this study will be integrated with those from current and future studies with IMRAS samples using advanced immunobiology systems analysis to identify immune signatures of protection elicited by whole sporozoite vaccination.

958

IDENTIFICATION OF PFEMP1 EPITOPES USING A DIVERSITY-COVERING ULTRADENSE PEPTIDE MICROARRAY

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Plasmodium falciparum erythrocyte membrane protein-1 (PfEMP1) antigens play an important role in parasite sequestration and host immune system evasion. PfEMP1s are encoded by the extraordinarily diverse *var* gene family, present in 40 to 60 copies per genome. 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. While we have previously identified PfEMP1 fragments whose seroreactivity is associated with increased malaria exposure and thus potential immunity, the critical PfEMP1 epitopes that drive such immune-mediated protection have not yet been identified. A peptide microarray is a tool that, with carefully chosen serum samples, allows for recognition of peptides that may harbor epitopes critical for natural protection against malaria. We designed a pilot PfEMP1 peptide array to identify such PfEMP1 epitopes. Eighty-three thousand 16-amino acid (aa) peptides were spotted onto an array, spanning the complete *var* repertoires of the reference genome 3D7, the Indochina strain DD2, and an Indian isolate, RAJ116, with 12-aa overlap. This included a total of 151 PfEMP1s, providing coverage of both extracellular and intracellular PfEMP1s and all previously described PfEMP1 domain cassettes. We probed this array with sera from children aged 1-6 years old and adults from rural Mali. Among extracellular and intracellular PfEMP1 fragments recognized more intensely by adults than children, potentially representing an effective immune response, we identified key peptides associated with this seroreactivity. We also identify such peptides in domain cassettes associated with severe malaria. We anticipate that this approach will allow targeted identification of specific regions in PfEMP1 constitutive domains that harbor epitopes critical to natural immunity. We present plans for future work with this powerful new tool.

959

AN ULTRA-DENSE PEPTIDE ARRAY FOR IDENTIFYING HUMAN ANTIBODY BINDING SITES ON MALARIA PARASITE ANTIGENS

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A high-throughput means of identifying epitopes involved in antibody recognition of malaria antigens would heighten our understanding of natural immunity to malaria. Previous epitope mapping approaches do not enable simultaneous study of multiple antigens or antigenic variants. We designed an ultra-dense peptide array containing multiple *P. falciparum* antigens and field sample-derived diverse variants. Arrays were fabricated *in situ* using light-directed maskless array synthesis. We included vaccine candidate antigens, gametocyte antigens, variant surface antigens, and mosquito salivary antigens to inform vaccine design, development of serosurveillance assays, transmission capacity, naturally acquired immunity, and species-specific vector exposure. 172,396 peptides representing 209 antigens were included. Sequences were derived from Sanger and genome sequencing of samples from Mali, including those from a phase II pediatric vaccine study, and Southeast Asian samples from an artemisinin resistance study. PfEMP1s (n=151) were included as 16-amino acid (aa) peptides overlapping by 12-aa (16/12 overlap). Other antigens were included in triplicate with 16/12 overlap; a subset was additionally included with 16/15 overlap. The most diverse antigen, AMA1, had 331 variants (14,153 non-redundant peptides). PfEMP1s represented the greatest proportion of the array with 83,760 peptides. Reactivity of sera extracted from dried blood spots (DBS) on Whatman® 903 cards and from whole blood was highly correlated, albeit with some loss of dynamic range for DBS samples, thus justifying future field sample collection without a cold chain. Sera collected from Mali and Southeast Asia recognized previously described and novel epitopes in pre-erythrocytic and blood stage antigens including CSP, AMA1, and variant surface antigens. We identify antigens and peptides underlying the higher reactivity of adult sera as compared with pediatric sera. With this approach, an ultra-dense peptide array illuminates acquisition of natural immunity to malaria and can provide insights applicable to vaccine design and serosurveillance.

960

THE TRANSCRIPTION FACTOR T-BET COMPROMISES HUMORAL IMMUNITY TO BLOOD-STAGE MALARIA BY INHIBITING THE EFFICIENT DEVELOPMENT OF GERMINAL CENTRE RESPONSES

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Naturally acquired immunity to malaria develops only after many years of repeated exposure to *Plasmodium* parasites. Despite the key role that antibodies play in protection against malaria, the cellular processes underlying the slow acquisition of immunity are unclear. 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. We have recently addressed this hypothesis using a pre-clinical model of severe malaria and found that the same inflammatory pathways mediating disease syndromes impair T follicular helper cell differentiation and the development of germinal centre (GC) responses required for antibody-mediated control of parasitemia. To further define the impact of inflammation in the induction of protective immunity, the development of GC responses to *P. berghei* ANKA was examined in mice deficient in the pro-inflammatory transcription factor T-bet. Genetic deletion of T-bet significantly improved T follicular helper cell differentiation rates, which translated in enhanced GC and parasite-specific antibody responses to infection. Infection of T-bet$^{fl/fl}$CD23^{Cre} mice, with specific deletion of T-bet in their B cell compartment revealed that antibody production and isotype-switching was also regulated by B-cell-intrinsic expression of T-bet. Moreover, the induction of GC B cells and total plasma cell responses to infection were significantly improved in the absence of T-bet expression specifically in B cells. Thus these data suggest that inflammatory pathways elicited in response to clinical malaria negatively impact the development of long-term humoral immunity not only by inhibiting T cell help for antibody formation but also by directly modulating B cell responses to infection.

961

REDUCED *PLASMODIUM* BURDEN IN HUMANS ASSOCIATES WITH CD38+ CD4+ T CELLS DISPLAYING CYTOLYTIC POTENTIAL

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Malaria is associated with complex multi-factorial immune responses, and the exact molecular mechanisms required to control parasite burden remain largely unknown. The recent discipline of systems immunology integrates immunology with molecular and computational sciences to enable comprehensive and quantitative evaluation of human immune responses at a level of detail previously restricted to murine models. We are applying systems immunology approaches to characterize the host response to the *Plasmodium spp* parasite in humans. Using a unique resource of samples from a controlled human malaria infection study, we identified a novel population of CD4$^+$ T cells whose frequency in peripheral blood was inversely correlated with parasite burden following *P. falciparum* infection. These CD4$^+$ T cells expressed the multifunctional ectoenzyme CD38 and had unique features distinguishing them from other CD4$^+$ T cells. Specifically, their phenotype was associated with proliferation, activation and cytotoxic potential as well as significantly impaired production of IFN-γ and other cytokines and reduced basal levels of activated STAT1. A CD38$^+$ CD4$^+$ T cell population with similar features was identified in healthy uninfected individuals, at lower frequency. This is the first report of a population of CD4$^+$ T cells with a cytotoxic phenotype and markedly impaired IFN-γ capacity. The expansion of this population following parasite infection and their ubiquitous presence in humans suggests that they may have a broad role in host-pathogen defense.

962

HOST IMMUNITY TO MALARIA INFECTION, ANAEMIA AND SOCIO-ECONOMIC IMPACT AMONG CHILDREN LESS THAN 10 YEARS IN NORTHERN CAMEROON

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Malaria and anaemia are key public-health challenges in Cameroon. However, little has been reported on the interaction between these interconnected health determinants. The present study was designed to investigate the relationship between malaria occurrence, immunity, anaemia and socio-economic impact among under - ten children living in an area of intense seasonal malaria transmission in Northern Cameroon. A Cross- sectional survey was conducted in November 2013, in Pitoa and Mayo-Oulo Health Districts, Northern Cameroon. Total, 368 children aged 6months - 10 years were recruited. Finger-prick blood samples collected were used for haematocrit; immunoglobulin gamma level determination using ELISA; malaria parasite prevalence, specie and density by microscopy; *Plasmodium* DNA extraction from filter paper for PCR. A structured questionnaire was used to assess Socio-economic status.Data analysis was by SPSS 20.Overall prevalence of malaria and anaemia were 32.9% and 20.6% respectively. Globally, 46.4% of the children (95% CI: 41.1 - 51.8) were low anti-malarial Total IgG producers, 36.2% (95% CI: 31.2 - 41.5) low IgG1 producers and 19.8% (95% CI: 15.7- 24.3) were low IgG3 producers. There was no statistically significant (p>0.05) association between immunity and malaria status for all the categories of IgG. The Socio-economic status of the population was poor. Malaria was not the cause of anaemia in the children. Therefore, other factors may have accounted for anaemia. Since no effect of malaria and immunity was observed in the low production of IgGs, the IgG levels observed could not be an indicator of any protection against malaria but may be due to humoral response to malaria infection.Malaria programmes should rapidly scale up on improving the health and immunity status of the anaemic in poor communities.Future studies should focus on finding out the causes of anaemia in malaria - infected children.

963

PROFILES OF PFEMP1-SPECIFIC IGG ANTIBODIES FROM BIRTH TO 12 MONTHS OF AGE IN BENINESE INFANTS

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The cytoadherence properties of *Plasmodium falciparum* infected erythrocytes (IE) represent a major contributor to the pathogenesis of malaria through interactions with various endothelial cell surface receptors. These interactions are mediated by members of the highly variable *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) expressed on the IE surface. One particular component of PfEMP1 proteins, the cysteine-rich interdomain region (CIDR), is known to play a very important role in the adhesive interactions between IE and endothelial receptors, making this region a potential vaccine target of interest. Here, we investigated the dynamics of maternally-transferred IgG antibodies targeting the CIDR of a panel of different PfEMP1 proteins, as well as infants' own acquisition of antibodies with the same specificities during the first year of life. We used plasma samples collected longitudinally from the offspring of a cohort of pregnant women who had themselves been followed closely through pregnancy. We show that the levels of all anti-CIDR antibodies quantified declined to the point of disappearing over the 6 first months of life. Antibodies with specificity for the CIDR predicted to adhere to selected receptors (CD36, EPCR) or for the CIDR associated with the unknown phenomen were subsequently acquired by infants between 7-12 months of age, their levels being a function of *P. falciparum* history during infancy. Infected infants developed stronger antibody responses to the CIDR associated with either EPCR binding or unknown compared to uninfected infants. The transcriptional profile of var genes showed no obvious difference between parasites infecting the children before and after 6 months except for some genes of group B var.

964

PLASMODIUM FALCIPARUM EPIDEMIOLOGY IS GOVERNED BY MULTI-SCALE IMMUNE SELECTION AND A DIVERSITY-TRANSMISSION FEEDBACK

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The human malaria parasite *Plasmodium falciparum* displays an extensive degree of antigenic diversity that facilitates long periods of infections and high re-infection rates. Of particular interest are the variant surface antigens (VSA) encoded by several multi-gene families, including the *var* genes whose protein products PfEMP1 are implied in malaria infection pathology and immune evasion. Within-host clonal antigenic variation of PfEMP1 limits host exposure to the parasite's antigen repertoire by the predominant and cyclic expression of single variants during infection. At the population level, high levels of antigenic diversity between parasites result in hosts acquiring protection against severe and symptomatic infections in a piecemeal process over years of repeated exposure. As antigenic diversity is predominantly generated through recombination it is expected to respond dynamically to changes in transmission and immune selection. On the other hand, population-level prevalence and incidence can be seen as a direct consequence of antigenic diversity, thus forming a non-linear feedback between transmission intensity and diversity. To explore this feedback and its consequences on malaria epidemiology in more detail we developed an individual-based, multi-scale modelling framework in which antigen diversity emerges as a dynamic property from the underlying transmission dynamics. New antigenic variants are generated through recombination and mutation during infection and the mosquito-stage and are subjected to within and between-host selection pressures. Our results show that immune selection severely limits the level of antigenic diversity that can be stably maintained at any given time and that the transmission-diversity feedback can significantly affect how population-level prevalence responds to changes in disease transmission due to natural fluctuation or intervention measures, which has important implications for our understanding of malaria epidemiology and disease control efforts.

965

A PROSPECTIVE STUDY OF B CELL DYNAMICS IN PATIENTS WITH MALARIA USING MASS CYTOMETRY

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Clinical immunity to malaria is largely mediated by humoral immune responses. Protective immunity, however, appears to require repeated exposure and wane in the absence of continuous infection. It has been proposed that malaria impairs the function of B cells and contributes to the slow development and incomplete immunity observed in malaria. The mechanisms behind this are as of yet unclear. In the current study we have investigated the dynamics of B cell phenotypes at six time points over the course of one year, in patients treated for *Plasmodium falciparum* malaria at the Karolinska University Hospital in Stockholm. Study participants were stratified according to previous malaria exposure in order to compare immune responses of malaria-naïve individuals (n=3), with individuals from malaria-endemic areas (n=3). We present an extensive mass cytometry (CyTOF™) characterisation of B cell phenotypes. Our results demonstrate the existence of specific B cell subpopulations that arise at different time points and differently in individuals with previous malaria exposure.

966

LONGITUDINAL ASSESSMENT OF PFSPZ-SPECIFIC T CELL RESPONSES IN MALARIA-NAÏVE ADULTS VACCINATED WITH PFSPZ VACCINE

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An attenuated *Plasmodium falciparum* (Pf) sporozoite vaccine (PfSPZ Vaccine) is being evaluated in humans for efficacy. Here we present T cell responses after vaccination with 9.0x10^5 PfSPZ administered 3 times at 8 week intervals to malaria-naïve adults. The phenotype and effector functions of Pf-specific memory CD4, CD8, and γδ T cells were assessed by 14-16-color flow cytometry throughout the course of vaccination. Pf-specificity was determined by incubating PBMCs with aseptic PfSPZ or the vaccine diluent (HSA). PfSPZ Vaccine induced high magnitude multi-functional IFNγ, IL-2, and/or TNFα cytokine-producing memory CD4 T cell responses. Surprisingly, these responses peaked at 1.5% (mean) after the 1st immunization, and declined to 0.57% and 0.51% after the 2nd and 3rd immunizations, respectively. PfSPZ-specific cytokine-producing memory CD8 T cell responses also peaked after the 1st immunization (0.39% mean) and declined to pre-vaccine levels after the 3rd immunization. The activation markers HLA-DR and CD38 on T cells were used to provide a sensitive measure of T cell activation *in vivo*. The frequency of HLA-DR+CD38+ memory CD4 T cells peaked two weeks after the 1st immunization, but returned to baseline (pre-vaccine) levels two weeks after the 2nd and 3rd immunizations. Last, the Vδ2+ sub-family of γδ T cells expanded 2.8-fold above pre-vaccine levels, and this increase persisted after all immunizations. γδ T cells showed a very high level of HLA-DR+CD38+ co-expression, peaking at a mean of 34% two weeks after the 2nd immunization, and declining after the 3rd immunization. In conclusion, PfSPZ Vaccine induced high frequency Pf-specific CD8, CD4, and γδ T cells after the first vaccination with 9.0x10^5 PfSPZ. The magnitude of Pf-specific CD8 and CD4 T cells was dramatically reduced after the 2nd and 3rd immunizations, suggesting that PfSPZ Vaccine induced rapid anti-PfSPZ immunity that limited subsequent boosting with PfSPZ Vaccine at the dose and interval tested here. Based on these data, it may be possible to achieve equivalent protective efficacy with fewer immunizations.

967

ASSESSING THE IMPACT OF NON-ADHERENCE TO ANTIMALARIALS USING WITHIN-HOST MODELING OF FALCIPARUM MALARIA

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Artemisinin combination therapy regimens typically contain 3 or 6 doses spread over 3 days. This ensures that the blood concentration of the drugs remains high enough for a sustained period of time, reducing the probability that parasites survive and recrudesce. Taking the correct doses of antimalarials at the right time will maximise their clinical impact. The efficacy of ACTs is usually compared in clinical trials where there is a high level of patient adherence to the drug regimen, but adherence can be much lower in routine health care settings, and is likely to vary according to the complexity and length of the regimen, as well as side effects of the

drugs amongst other factors. Much information on patient adherence has been collected, but it is difficult to assess the clinical impact of incomplete adherence in research settings. In this work, we have developed a stochastic within-host model of asexual parasitaemia in individuals with no previous exposure to malaria, which we calibrated against data from malariatherapy patients. Combining this model with a pharmacokinetic-pharmacodynamic (PKPD) framework enables us to estimate potential levels of treatment failure following poor adherence to malaria treatment. We include variability between individuals both in terms of parasite dynamics and pharmacokinetics. We simulate treatment with artemether-lumefantrine (AL) and estimate the probability of treatment failure after taking different numbers of doses, or taking the doses at the wrong time, using values based on adherence studies. Our modeling structure also allows other drug combinations to be scrutinized in this way.

968

FORECASTING MALARIA ADMISSIONS AT A RURAL DISTRICT HOSPITAL IN WESTERN KENYA USING REMOTE SENSING DATA

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In sub-Saharan Africa, malaria kills a child every two minutes. In response to still high incidence of malaria, WHO developed the new global technical strategy for malaria 2016-2030. One of the three pillars of this strategy is to use surveillance as a key intervention in malaria control and elimination. Malaria surveillance data provides opportunity to develop a malaria early warning system and tracking of progress towards elimination. Therefore, in this study we use malaria admission data of children under five years of age at Siaya district hospital in Western Kenya to develop models to forecast malaria admissions incorporating remotely sensed environmental data. Three models were developed assessing forecast accuracy at 1-month, 2-month and 3-month lead times. We used monthly totals of malaria admissions and precipitation. For Normalized Difference Vegetation Index and Land Surface Temperature we computed monthly means. The data covered the period from 2002 to 2013. Malaria admissions were modelled using a general additive model with a smooth function of time to capture trend; a cyclic cubic spline of month to capture seasonality; and splines of the environmental variables for each lead time. To adjust for residual autocorrelation, we incorporated autoregressive term of order 1 as random effect in the models. Malaria admissions were assumed to follow quasi-Poisson distribution adjusting for over dispersion. The period from 2002 to 2012 was used for model training and the year 2013 for model validation. The 1-month lead time model had the least Root Mean Squared Error for both the training and the test periods with values of 19.30 and 6.02 respectively. The 3-month model had the least mean absolute percentage error (MAPE) of 39.31% for the 2013 forecast values while 1-month lead time model had the least MAPE for the training period. The 3-month lead time model provided better forecast accuracy in terms of absolute differences between forecast and observed values for the 2013 malaria admissions. The 3-month lead time model can potentially be utilized as an early warning system allowing sufficient lead time for control activities.

969

A QUANTITATIVE ANALYSIS OF THE IMPROBABILITY OF FERTILIZATION AT LOW GAMETOCYTEMIAS IN THE ABSENCE OF TRANSMISSION-ENHANCING MECHANISMS

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Successful infection of an *Anopheles* mosquito by *Plasmodium falciparum* gametocytes can occur over many orders of magnitude of gametocyte density in the human host, from levels that are not detectable with light microscopy to thousands of gametocytes per microliter of blood. The empirical curves for the success of infecting a mosquito as a function of gametocyte density are difficult to fit, as the success of infection at low gametocyte densities can be higher than expected, while the success of infection at high gametocyte densities rises slower than expected. Indeed, even at high densities, there is often incomplete infection success. We are particularly interested in the low-end of gametocyte densities because people carrying undetectable gametocytes contribute heavily to the infectious reservoir. At the low-end of densities, overdispersion of gametocytes in a bloodmeal partially helps to explain the observed success rate, but it is not the full story. Within the mosquito midgut, a male gamete searches a crowded environment in which there can be more than one million uninfected red blood cells per female gametocyte. We reexamine the sexual phase of malaria transmission from gametocytes in the human host up to gametes finding each other and fertilization occurring in the mosquito midgut. We construct a detailed analysis of the within-midgut dynamics and study the effects of male gamete swimming rate, gametocyte density, overdispersion of gametocytes per feed, clustering, and diuresis of the bloodmeal on the probability of successful infection at different human host gametocyte densities. Finally, we show residual under-prediction of success probability at low gametocyte density that could be explained by possible alternative mechanisms such as clustering or chemotaxis in the mosquito midgut and then provide estimates of the magnitude of these possible mechanisms based on observed success probability.

970

A SIMULATION STUDY OF WHEN MALARIA CONTROL AND ELIMINATION PROGRAMS CAN SAFELY REDUCE VECTOR CONTROL EFFORTS

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Scale up of the coverage of malaria vector control interventions has led to significant reductions in malaria disease burden globally. Due to the considerable resources currently devoted to vector control, there is a need to determine if this expenditure should be sustained after significant reductions in transmission. We use a simulation model of malaria epidemiology and immunology (OpenMalaria) to predict malaria transmission and disease outcomes after stopping the deployment of vector control interventions under various settings. We conduct regression analysis of simulation results to derive predicted probabilities of resurgence, severity of resurgence and time to resurgence under scenarios defined by the pre-intervention entomological inoculation rate (EIR), case management coverage, and vector control coverage, amongst other parameters. Results indicate that, in the absence of secular changes in the underlying determinants of transmission (historically called receptivity), there are few scenarios under which vector control can be removed without a strong expectation of resurgence. These, potentially safe, scenarios are characterized by low historic EIR, successful vector control programs that achieve elimination or near elimination, and effective surveillance systems with high coverage and effective treatment of malaria cases. Programs and funding agencies considering scaling back or

withdrawing vector control from previously malaria endemic areas need to first carefully consider current receptivity and other available interventions in a risk assessment. Surveillance for resurgence needs to be continuously conducted over a long period of time in order to ensure a rapid response should vector control be withdrawn.

971

EPIDEMIA: AN ONLINE PLATFORM FOR DATA ACQUISITION, INTEGRATION, AND ANALYSIS TO SUPPORT ECOLOGICAL FORECASTING OF MALARIA OUTBREAKS

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Malaria early detection and early warning systems are important tools for public health decision makers. Here we present technical information on the design and implementation of the EPIDEMIA online platform which serves as an automated tool for forecasting and early detection of malaria outbreaks in the Amhara Region of Ethiopia. The platform uses freely available, community-driven open source software including the R language for statistical computing, MySQL and PostgreSQL for relational data storage, and PHP, HTML, and CSS for web front-end. The R language was chosen because it is widely accessible and allows for the use of advanced statistical modeling techniques. As a result of these choices, the EPIDEMIA data platform can easily be modified to incorporate potential future statistical models, applied to other geographic areas, or applied to other diseases linked to environmental conditions. The EPIDEMIA platform's epidemiological data acquisition subsystem consists of a securely hosted and encrypted website where public health collaborators in Amhara upload malaria morbidity data which is then parsed and stored in a MySQL database. The environmental data acquisition subsystem uses our previously-developed EASTWeb software to continuously monitor, download, and summarize earth-observation data derived from satellite remote-sensing products that are freely-available on the internet. When new environmental data has been processed in EASTWeb's PostgreSQL database, a listener triggers a PHP script to import the new data into EPIDEMIA's MySQL database. The data integration subsystem, which is a series of scripted actions written in PHP, R, and MySQL stored procedures, integrates the various datasets. After each weekly upload of epidemiological data, the forecasting subsystem uses dynamic linear models implemented in R to analyze the integrated dataset for early signs of malaria outbreaks and to generate malaria forecasts. The reporting subsystem uses R to generate a weekly malaria forecast in PDF format and allows users to generate custom reports, visualizations, and data summaries using a query interface on the website.

972

MATHEMATICAL MODELLING OF TAFENOQUINE FOR *PLASMODIUM FALCIPARUM* MALARIA ELIMINATION

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The 8-aminoquinolines are the only known class of antimalarials with action against *Plasmodium vivax* hypnozoites and *P. falciparum* gametocytes. Primaquine is widely used as part of treatment of *P. vivax* malaria to clear hypnozoites from the liver and thus prevent relapses. This requires a 14-day course to be taken. Primaquine has also been recommended by WHO since 2012 as a single dose for *P. falciparum* infection to kill gametocytes and thus interrupt transmission. A recent mathematical modelling consensus exercise for WHO to predict the efficacy of mass drug administration (MDA) for *P. falciparum* elimination found the additional impact of single dose primaquine to MDA with artemisinin combination therapy (ACT) to be very small. Tafenoquine is an investigational 8-aminoquinoline antimalarial currently being trialled as a hypnozoiticidal for treatment of *P. vivax* infection. Its mode of action and side effect profile are very similar to primaquine however it has the advantage of a much longer half-life. It has thus been proposed as a single dose alternative to 14 days of primaquine for *P. vivax*. However, tafenoquine is not currently being considered as a gametocytocidal for *P. falciparum* infection. In this role, its long half-life may confer greater transmission blocking activity than primaquine which could make it a valuable tool for falciparum elimination. In the absence of trial evidence, a mathematical model was developed to predict the efficacy of single dose tafenoquine alone and when given with ACT for *P. falciparum* malaria elimination. Using this model, a range of scenarios is being explored including its use for treatment of clinical cases, in mass drug administration, and as part of a combined strategy with vector control measures. In addition, its impact in low, medium and high transmission settings in Asia and Africa and for the elimination of artemisinin and ACT partner drug-resistant falciparum malaria. The results of this mathematical modelling exercise will be presented with recommendations for the possible role of tafenoquine in *P. falciparum* elimination and for potential clinical study designs to assess its efficacy in the field.

973

IDENTIFYING MALARIA RISK FACTORS IN A HYPERENDEMIC SETTING USING BAYESIAN MODEL SELECTION

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The epidemiological dynamics of malaria, as with many other vector-borne diseases, have been linked to a wide variety of environmental, socio-economic, and demographic factors. Traditional statistical approaches for evaluating the contribution of each of these potential disease drivers present critical tradeoffs. Modeling all possible combinations can be computationally intensive and make it difficult to draw definitive conclusions when numerous disease factors are considered. Conversely, selecting a subset of potential drivers can fail to describe the relative importance of a particular covariate and can exclude important risk factors. To address these issues we propose a Bayesian probit regression model that contains a model selection procedure which proposes new candidate models through the random addition, subtraction, or swapping of covariates. A new model is proposed and evaluated at each step of the iterative Markov Chain Monte Carlo algorithm, generating parameter estimates and inclusion frequencies for each potential disease driver. We used this approach to simultaneously evaluate the relative importance of a wide range of environmental, socio-economic, and medical risk factors for malaria in the Bunkpurugu-Yunyoo district of northern Ghana, using existing data from six malaria surveys conducted in 2010-13. Our analysis identifies substantial protective socio-economic and medical factors related to the two modest "urban" centers in this small geographic area, indicating that the small towns in this hyperendemic setting may buffer nearby rural areas from environmental conditions that are traditionally linked to high malaria transmission. This Bayesian model selection technique offers a promising solution for dealing with the practical and computational constraints of evaluating numerous diverse risk factors for malaria and other diseases.

974

ATTACKING THE MOSQUITO ON MULTIPLE FRONTS: INSIGHTS ON OPTIMAL COMBINATIONS OF VECTOR CONTROL INTERVENTIONS FOR MALARIA ELIMINATION FROM A MATHEMATICAL MODEL

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Despite great achievements by long-lasting insecticide-treated nets (LLINs) and indoor residual spraying (IRS), research demonstrates that these tools are insufficient to eliminate malaria transmission in many settings today. Protective coverage from these interventions is attenuated where mosquitoes can access blood resources from non-human hosts or from humans when they are outdoors. Fortunately, field experiments indicate that there are many promising vector control interventions that can be used to complement LLINs and/or IRS by targeting a wide range of biological and environmental mosquito resources. The majority of these experiments were performed to test a single vector control intervention in isolation; however, there is growing evidence and consensus that effective vector control will require a combination of interventions tailored to the target ecological and epidemiological setting. We present a mathematical modeling framework designed to examine combination interventions prior to empirical field trials. The model framework incorporates all stages of the mosquito life cycle from egg, larva, pupa, adult, and, crucially, the female gonotrophic cycle whereby females blood feed and lay eggs. We describe how the framework may be used to evaluate the impact of combining existing and novel interventions in synergistic ways in areas where LLINs and/or IRS are widely used but where malaria transmission persists. We consider the following vector control interventions in addition to LLINs and IRS: larvaciding (conventional and aerial), attractive toxic sugar baits (ATSBs), insecticide spraying of male mating swarms, mosquito-proofed housing, spatial and topical mosquito repellents, systemic and topical insecticide-treatment of cattle, odor-baited traps and space spraying. We describe optimal combinations of these interventions needed to significantly reduce entomological inoculation rate (EIR), a widely accepted measure of malaria transmission, in a range of ecological and epidemiological settings.

975

IMPACT OF SEASONAL MALARIA CHEMOPROPHYLAXIS IN A HIGH AND SEASONAL MALARIA TRANSMISSION SETTING IN BURKINA FASO

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Seasonal malaria chemoprophylaxis (SMC) is a strategy endorsed by the WHO to prevent malaria in children aged 3-59 months living in seasonal malaria transmission areas. In Burkina Faso where malaria transmission is highly endemic and seasonal, more than 60% of malaria cases (clinical malaria) occur during the rainy season from June to October with the majority falling on younger children. In 2015, the SMC campaign in Burkina aimed at giving sulphadoxine-pyrimethamine and amodiaquine anti-malarial treatments (SP+AQ) to children below 5 years at one month interval during the rainy season. At each time point, children were treated with one dosage regimen (three unit doses over three days). About 8,000 community health workers were recruited to administer the SMC preventive treatment to about 650,000 children across 17 health districts. Here we aim at modeling the long term impact of SMC on *Plasmodium falciparum* clinical incidence and prevalence in the targeted population as well as in the general population. We used baseline and post intervention data on clinical and parasite rate detected by RDT and accounted for the selection of parasites with decreased drug sensitivity to calibrate the EMOD agent-based mechanistic model. Our 5 years simulations with a scenario of 80% SMC coverage were proven to substantially drop the prevalence of clinical malaria and parasite rate with a community based effect on *P. falciparum* prevalence in the general population.

976

SPRAYING OF MALE MATING SWARMS AS A NOVEL VECTOR CONTROL INTERVENTION: INSIGHTS FROM A MATHEMATICAL MODEL

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Despite the success of long-lasting insecticide treated nets (LLINs) and indoor residual spraying (IRS) in controlling malaria transmission, challenges posed by elimination in residual transmission settings and waning effectiveness of current interventions prompt the investigation of novel vector control tools. Interventions that target hitherto unaddressed elements of vector biology may be of particular interest. Targeted insecticidal spraying of male mating swarms is a novel intervention that reduces the mating rate of female mosquitoes, thereby decreasing population density and biting rates. The only field trial of this intervention to date was undertaken in 2013 in Burkina Faso where it proved highly effective at reducing densities of *Anopheles gambiae*, one of the most dangerous African malaria vectors. Trial results showed that over a single month, mosquito densities were reduced by 80% in an ecological setting characterized by exceptionally high vector density. To understand the mechanisms through which spraying of male mating swarms impacted vector populations, a differential equation based model was developed and fitted to data. Parameters estimated through the model fitting procedure can give insight into mosquito mating dynamics in the natural setting as well as under an extreme external stressor. These results can give insight into how other interventions targeting mating dynamics may function. Results also allow an understanding of how female mating rate changes as males become scarce, indicating the importance of Allee effect driven population dynamics. The model-based analysis of these data suggest further research into targeting mosquito mating dynamics for vector control should be explored across a variety of ecological settings, both to better understand mosquito mating dynamics, as well as to evaluate the potential impact of swarm spraying across a variety of settings.

977

DEVELOPING AN EARLY WARNING SYSTEM FOR MALARIA IN THE AMAZON: PROGRESS IN PERU

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Reported cases of malaria in the Peruvian Amazon have increased 5-fold since 2011, from 11,779 to 60,254 in 2015. Given the rate of detection up to April of this year, the number of cases are likely to exceed 70,000 in the region of Loreto. Responding to this epidemic has been challenging given the reduced financial support from the Peruvian government combined with large-scale environmental impacts that have affected Peru, including massive flooding in 2012 and the current "mega" El Nino. An

underappreciated driver of malaria transmission is the human-environment interface resulting in increased human contact with high-risk transmission environments. With support from NASA, a team of investigators from the US, Peru and Ecuador are developing a 2-component system that will improve detection of high-risk environments using large-scale prediction models, followed by application of focused agent-based models (ABMs) to predict local transmission dynamics and simulate potential intervention strategies. This presentation will provide an update of the system after one year of development. Data include: weekly case detection reports for 2000-2015 from all health posts in the region of Loreto in the northern Peruvian Amazon; satellite-derived estimates for meteorology, land cover, hydrology, and eco-region; and estimates of population density. The large-scale model identifies districts with high spatial and temporal clustering using polynomial distributed lag models. Initial formulations of these models have been presented previously. The small-scale ABMs, described in Pizzitutti et.al. 2015, have been improved to incorporate human mobility. This long-term project has both scientific and political (implementation) challenges that will be discussed.

978

REQUIREMENTS FOR EFFECTIVE CRISPR-CAS9-BASED GENE DRIVE FOR THE CONTROL OF MALARIA AND OTHER VECTOR-BORNE DISEASES

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Recent demonstrations of CRISPR-Cas9-based gene drive by Gantz et al. and Hammond et al. highlight the huge potential of this technology for the control of mosquito-borne diseases such as dengue, Zika and malaria through genetic modification of their vectors. Two promising strategies have been proposed - one in which the gene drive system is used to spread a disease-refractory gene into the mosquito population, and another in which the gene drive system is used to spread a fitness load or male gender bias, thus suppressing the mosquito population. While encouraging as a proof-of-concept, these constructs are highly error-prone and induce large fitness costs, leading to certain failure in the wild. Using a population modeling framework, we determine minimal properties that these systems must have in order to be successfully implemented. Required improvements address the following issues: a) disruption of the CRISPR-Cas9 target site through non-homologous end-joining (NHEJ); b) fitness costs induced by the transgene and/or gene drive system; and c) evolution of pathogen resistance to the disease-refractory gene. We use our modeling framework to explore potential solutions to these issues. For instance: a) the use of second or third-generation CRISPR-Cas9 systems to enable sustained population replacement or suppression; and b) the use of CRISPR-Cas9 systems that prevent the accumulation of NHEJ-generated mutant alleles by cleaving a target sequence in an essential gene while also containing a recorded copy of the essential gene. Finally, we project the dynamics of the constructs of Gantz et al. and Hammond et al. beyond the experimental data, and explore tailored improvements. High homing rates were observed for both constructs, with over 90% transmission of the CRISPR-Cas9 system to offspring of heterozygous parents (c.f. 50% expected for purely Mendelian inheritance); however, high rates of NHEJ were also recorded, and females heterozygous for the construct of Hammond et al. had their fertility reduced by 90%. We recommend specific improvements for each system and thus suggest research priorities for the CRISPR-Cas9 gene drive field.

979

MODELING THE USE OF A 20-HYDROXYECDYSONE STEROID AGONIST FOR MALARIA CONTROL

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Mathematical models have played an important role in designing malaria control policies by relating aspects of mosquito biology and behavior to malaria transmission. We developed a discrete-time compartmental model of the mosquito lifecycle to investigate the use of a novel compound for impregnated bed nets. This compound, a dibenzoylhydrazine, is a 20-hydroxyecdysone (20E) steroid hormone agonist with multiple effects on *Anopheles gambiae*, an important vector of the malaria parasite *Plasmodium falciparum*. The application of this 20E agonist to lab-reared mosquitoes reduced their mating success and egg development, shortened their lifespan, and blocked parasite development - effects that interfere with their ability to transmit malaria. We incorporated these varied effects into our model to predict the impact of this 20E agonist on the mosquito population and malaria transmission when applied via bed nets at varying levels of coverage. We find a non-linear relationship between bed net coverage and the mosquito population size, with a slightly increased population size at low coverage due to density-dependent larval mortality. However we predict, at all coverages, a shift in the mosquito age distribution toward younger mosquitoes, which are unable to transmit malaria, and reduced malaria prevalence in the human population, with the possibility of elimination at high coverage. These results show the potential of 20E agonists as new compounds for malaria control and highlight the utility of mathematical models when studying multiple aspects of mosquito biology that are simultaneously affected.

980

WITHIN-HOST MATHEMATICAL MODELS OF MALARIA BUILT FROM MULTI-OMIC DATASETS

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The characterization of a malaria infection via a mathematical model that takes into account essential molecular interactions has been elusive. In the recent past, mathematicians trying to characterize the behavior of molecular and/or cellular quantities relied on simple diagrams (i.e. involving a reduced set of quantities) of processes described by biologists. Now instruments produce up to billions of measures in a single run from samples isolated from a particular biological domain (genomics, transcriptomics, metabolomics, proteomics, etc). The -omics revolution has changed the overall situation from data-poor to data-rich and thus has put many modelers in a situation of data overload. In this talk we address the integration of multiple -omic technologies to characterize the pathogenesis of malaria. Particularly, we will see how metabolic, transcriptional, lipidomic, and clinical data collected from a non-human primate infection with *Plasmodium* Cynomolgy can be used in unison to produce a mathematical model capable of differentiating between susceptible and resilient individuals. Such model is based on transport partial differential equations involving red blood cells, the immune system, and parameters that are functions of molecular quantities. This model makes use of a novel approach to cluster time series. The outcome of this model is a quantitative prediction of the course of a malaria infection based on known and anticipated molecular interactions that take place within a single individual. The lessons learned and methods developed during the course of this research are of ample applicability, thus the benefit of this aggregated knowledge expands beyond the field of malaria and outside the realm of research.

981

ORDE WINGATE'S SUICIDE ATTEMPT, CAIRO, 1942: A CASE STUDY IN ACUTE ATABRINE PSYCHOSIS

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One of the best known field commanders of the Second World War was Orde Wingate. His daring guerilla tactics in British-mandated Palestine, Ethiopia, and Burma have become legendary among military historians. Indeed, Wingate's military career in the field has rightly attracted the bulk of attention of biographers and military historians. Such well-deserved attention to Wingate's military career, however, has tended to divert attention from a 1942 suicide attempt. Suffering from malaria—possibly cerebral malaria--Wingate received atabrine therapy from his physician. According to his biographers, he abruptly increased its recommended doses. In a botched suicide attempt that followed, Wingate thrust a knife into his neck. This proposal will examine the probable cause of Wingate's suicide attempt. Was it, as some observers suggest, the severity of Wingate's malarial condition? Was it the high dose of atabrine with which Wingate recklessly self-medicated that caused his acute psychosis. Or was it, in fact, a combination of the two, exacerbated by an undiagnosed psychiatric condition? The authors conclude that pre-existing psychiatric issues (which the overdose of atabrine exacerbated)—not cerebral malaria—precipitated his 1942 suicide attempt.

982

JOINT EFFORTS, A KEY TO SUCCESS FOR THE MALARIA IN PREGNANCY PROGRAM IN LUANDA, ANGOLA

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Angola, in response to WHO's 2012 updated guidance on Intermittent Preventive Treatment in pregnancy (IPTp), revised its national malaria protocol to better address the fact that 25% of maternal mortality is caused by the disease. The new protocol was a collaborative effort of a national technical working group assisting the National Malaria Control Program (NMCP) including the National Reproductive Health Program, the national AIDS Institute, WHO, UNICEF, UNFPA and implementing partners of the U.S. Presidents Malaria Initiative (PMI). The updated Prevention and Treatment Manual for Malaria in Pregnancy, based on the revised protocol, was approved in 2014, and efforts continued with reviewing and updating training modules, job aids and monitoring tools that would reflect the additional doses of IPTp. The Ministry of Health, with support from partners, then disseminated these materials in the provinces and municipalities where they worked. USAID's ForçaSaúde program, with support from PMI, worked with the Provincial Health Directorate of Luanda to build capacity of 297 health professionals to implement the new guidance in 78 health facilities of four municipalities, Belas, Cazenga, Cacuaco and Viana, with a combined population of 4.3 million. Comparing the IPTp data from the four municipalities between 2014 and 2015, one can see that the new guidance has started to take effect. In both years approximately 70,000 pregnant women received the first dose or around 60% of women registering for antenatal care (ANC). For the new third dose there was an increase of 85% (from 12,490 women to 23,046), and receipt of the fourth dose rose by 164% (3,345 to 8,839). Two major challenges remain: increasing ANC registration and addressing missed opportunities to provide ANC doses for those who do attend including ensuring regular supplies of sulfadoxine-pyrimethamine for IPTp. Future progress requires continued inter-departmental collaboration among NMCP, Reproductive Health and the AIDs Institute, on-the-job training, enhanced statistical capacity, and supervision.

983

STUDY ON PATIENT ADHERENCE TO CHLOROQUINE AND PRIMAQUINE TREATMENT FOR *PLASMODIUM VIVAX* MALARIA IN MANAUS, STATE OF AMAZONAS

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Compliance to treatment is one of the most important factors to ensure radical cure, avoid relapses, prevent the emergence of parasite resistance to anti-malarial drugs and decrease transmission. Brazil has been using the "short" treatment scheme for *Plasmodium vivax* malaria - 3 days chloroquine +7 days primaquine - for many years trying to ensure patient`s adherence. Currently the country also use, when adherence could be secured, the "long" scheme with 14 days primaquine (or primaquine adjusted by weight). This study was conducted in Manaus, Amazonas to assess patient adherence to the standard *P. vivax* malaria scheme with 3 days chloroquine+7 days primaquine in the health centers' routine care; and identifying factors that determine low treatment adherence and adverse events associated with the treatment of *P. vivax* malaria. It was a transversal study conducted with patients seen in 11 health centers and designed to quantify adherence by conducting home interviews and verifying how many pills were remaining one day after the last treatment day. Sample size was determined by using an expected 15% proportion of low adherence, a 95% confidence range, and a 5% level of significance. 165 patients were interviewed, 98 were male and 67 female, and 100 were the patients themselves and 65 their guardians.There were a total of 31 patients who failed to adhere, representing 18.8% of the total, of which 6.1% had blisters with pills and 12.72% reported non-adherence. The study demonstrated problems in adhering to primaquina related to various factors that vary from the instructions given by the dispensers but not really understood by patients to the presentation of the medications. The value found for non-adherence/probable non-adherence may still be understated due to the possible introduction of selection biases: patients who are available for a visit and interview may cooperate more, for example. The result of this study reinforces an important problem with the *P. vivax* scheme that need to be addressed. In view of the malaria elimination interest it is important to show problems that need to be solved in order to reach the elimination goal.

984

PRE-SERVICE TRAINING INSTITUTIONS: AN IMPORTANT CONTRIBUTOR TO SCALE UP OF HIGH QUALITY MALARIA CASE MANAGEMENT SERVICES IN MALAWI

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Pre-service training institutions are an important, but often neglected, component of improving the quality of malaria case management. In 2007, Malawi updated its national guidelines on first line malaria treatment (from sulfadoxine-pyrimethamine to the artemisinin-based combination therapy (ACT) artemether-lumefantrine). By 2012, the Ministry of Health began reviewing the corresponding national training curriculum to orient health providers to the new malaria case management treatment guidelines. To support rollout, MalariaCare partnered with the National Malaria Control Program to train 3,035 health care providers (across 14 districts) on the updated national case management guidelines. Between 2007 and 2014, limited efforts were made to target pre-service training institutions as well. To enhance timely scale up of high quality case management services, MalariaCare trained 22 lecturers from eight training institutions, representing half of all training institutions in Malawi. Participants' knowledge and competencies related to the updated malaria case management guidelines were evaluated through pretests and posttests. Key findings include that average scores increased

from thirty eight percent (38%) at pretest to seventy three percent (73%) at posttest - indicating that baseline knowledge of the updated malaria case management guidelines was low among this group. Next steps for ensuring that new graduates continue to be well prepared will also be shared, including partnering with training institutions and Malawi's National Malaria Control Program to orient final year students to Malawi's updated malaria case management guidelines and support for incorporating the updated national guidelines into pre-service training curricula. In addition to practicing health providers, training institutions should be engaged to ensure that new health providers entering the workforce are up to date on changes to national guidelines, allowing them to contribute to the scale up of high quality malaria case management services.

985

IMPROVING QUALITY OF MALARIA CASE MANAGEMENT IN MALAWI THROUGH TARGETED CLINICAL MENTORING

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The Malawi Ministry of Health estimates that malaria accounts for 34% of all outpatient visits and 40% of all hospital admissions, with approximately 5 million cases treated each year. MalariaCare seeks to test promising new methods for more rapidly improving the quality of malaria diagnosis and treatment. In Malawi, the project is working closely with the National Malaria Control Program, Malaria Alert Center and Queen Elizabeth's Hospital to enhance routine outreach training and supportive supervision (OTSS) efforts by supporting a core group of clinical experts to provide intensive clinical mentoring to health facilities that are not meeting the project's minimum standards for quality case management. Intensive mentoring is used to target areas of weakness using problem-solving approaches and experiences that have worked in similar settings to address challenges and improve performance. Intensive mentoring is a collaborative approach engaging the commitment of both mentor and mentee. MalariaCare's hypothesis is that intensive mentoring will improve performance in a sustainable way on gaps identified during OTSS. Given the lack of literature on the contributions of clinical mentoring towards improved quality of malaria case management, MalariaCare will share findings regarding the use of mentoring toward this purpose in select health facilities. A core group of clinical mentors has been trained to provide intensive clinical mentoring in selected high-volume low-performing health facilities, following supervision rounds and through remote support by phone and email. Data will be collected using a standardized malaria case management evaluation tool developed by MalariaCare. A brief competency-based pre-post assessment tool will be used to assess provider competencies at the beginning and end of the mentoring process. The primary outcomes, however, will be changes in performance on case management indicators after mentoring and over time compared with a baseline. Results and lessons learned are expected to generate evidence on the potential role of clinical mentorship for improving the quality of malaria case management.

986

CLINICAL, DEMOGRAPHIC AND LABORATORY DATA AND METADATA COLLECTION FOR HUMAN MALARIA BLOOD SAMPLES COLLECTED FROM INDIVIDUALS LIVING IN DIVERSE EPIDEMIOLOGICAL SETTINGS

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Malaria is a leading cause of disease and death in many developing countries and is a severe public health problem worldwide. Independent research centers are active in many malaria endemic countries in Asia, Africa, and South America. To enhance the effectiveness of global malaria research and help accelerate progress, collaboration and data sharing between and among national and international research centers and other facilities is critical. Such collaboration is possible when easy to follow standards for data and metadata collection and dissemination are available and widely adopted. The NIH/NIAID-supported Malaria Host Pathogen Interaction Center, MaHPIC, consortium involves international collaborators from Brazil, Thailand, Colombia, Malaysia and Nigeria, with more coming onboard. We have collected clinical, demographic and laboratory data and metadata from diverse research collaborations from around the world, and developed easy to use data entry templates that are compatible with the data standards developed by the International Centers for Excellence in Malaria Research (ICEMRs) and the NIH/NIAID Clinical Data Working Group. The challenges faced with respect to mapping terms to the currently available and accepted ontologies will be discussed and solutions provided.

987

USING OUTREACH TRAINING AND SUPPORTIVE SUPERVISION (OTSS) RESULTS TO MONITOR ADHERENCE TO REVISED MALARIA TREATMENT GUIDELINES IN THE EASTERN REGION OF GHANA

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Following WHO's 2010 recommendation, Ghana revised its national malaria case management guidelines in 2014 to include laboratory confirmation for all suspected malaria cases before treatment with ACT. Training was conducted for public health facility workers on the new guidelines, but training numbers were inadequate. MalariaCare supports the National Malaria Control Program to conduct regular outreach training and supportive supervision (OTSS) visits to health facilities as part a strategy to improve adherence to the new guidelines and overall provision of febrile case management in five supported regions. The first round of OTSS following the training on updated guidelines resulted in a total of 426 health facilities receiving supervision visits. These visits included observations, practical demonstrations, interviews and patient record reviews to identify weaknesses, encourage problem solving and strengthen performance through on-the-job training and mentoring. OTSS data from this round shows that 82.9% of staff interviewed had participated in case management training, and that the overall score for clinical fever evaluations observed was 92.7%. The clinical fever evaluation score is a composite of the following: in 92.2% of observations, clinicians appropriately classified fever and 95.5% requested a malaria test to confirm the clinical diagnosis. Following a diagnostic test, 81.8% adhered to a negative test result and 87.6% prescribed according to guidelines as observed by supervisors during patient encounters. In a review of facility clinical records, adherence to a negative test result was higher at 92.7% of the sampled records. Comparison with the two previous rounds of

OTSS, which occurred before training on the updated guidelines, indicated improvement in performance of all four clinical fever evaluation indicators to above 80%, and an improvement in adherence to negative test results from 57.2% to 81.2%.

988

LESSONS LEARNED: MALARIA CASE MANAGEMENT TRAINING IN MADAGASCAR

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To help scale up high-quality diagnosis and case management services for malaria and other febrile illnesses, MalariaCare is supporting Madagascar's National Malaria Control Program (NMCP) to conduct clinical case management training in the Menabe region, with training sites in Morondava, Belo, Miandrivazo and Mahabo. Training materials were aligned with NMCP guidelines, with emphasis on orienting participants to Madagascar's updated policy around first-line treatment (three days of ACT), use of artesunate injection for severe malaria, as well as clinical and diagnostic practices. Participants were assessed using pretests and posttests. Of the 55 participants, 71% were nurses and 29% were physicians. Overall, 85% of participants stated that the training met their expectations. In addition, 87% of participants in Morondava improved their knowledge and skills after training - with an average increase of 16 percentage points between pretest and posttest. Similarly, 80% of participants in Belo improved their scores by an average of 17 percentage points, 94% of participants in Miandrivazo increased their scores by an average of 18 percentage points, and 93% of the participants in Mahabo increased their scores by an average of 18 percentage points. Average pre and posttest scores (out of 30) were 20 and 25 for Morondava; 18 and 23 for Belo; 19 and 25 for Miandrivazo, and 20 and 25 for Mahabo. Participants will use the knowledge gained from this training to improve the quality of diagnostic and treatment in their communities, aimed at reducing malaria related morbidity and mortality rate in remote areas of Madagascar.

989

ASSESSING THE PERFORMANCE OF AN INTEGRATED DISEASE SURVEILLANCE AND RESPONSE SYSTEM IN THE CONTEXT OF VARYING MALARIA TRANSMISSION: A CASE STUDY FROM MADAGASCAR

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The Madagascar's ministry of health (MOH) is building an integrated health information system to streamline reporting at all levels. One component of this is an integrated disease surveillance and response system (IDSR) for notifiable diseases. Madagascar's entire population is at risk of malaria with epidemic prone areas. As malaria transmission decreases and Madagascar considers pre-elimination, a specific surveillance system is also required. In October 2015, the MOH conducted an assessment of the IDSR in 93 randomly selected health facilities (HF), 218 community workers, and 19 sentinel sites. Results showed that 70% of HF reported accurate data, 62% of expected reports sent to district, 50% of expected reports received on time. IDSR guidelines were found in 20% of HF, 37% of HF have the official list of diseases under surveillance, and 54% have the weekly reporting form. Only 50% of IDSR staff at the district level, 57% at the region level were trained in surveillance. Supervision was irregular with 50% of HF supervised in the past six months. Only 52% of districts and 3/10 regions have an epidemic management committee. Data use was limited, only 40% of districts could investigate all epidemic alerts in the past 12 months. These findings suggest the need to develop and implement a comprehensive IDSR-strengthening strategy including data quality assurance procedures, use of new technologies, staff capacity building, coordination among partners, and use of data to respond to disease control in Madagascar.

990

MONITORING AND EVALUATION WORKSHOPS: AN APPROACH TO IMPROVE MALARIA INFORMATION SYSTEMS AND DATA USE TO BETTER INFORM PROGRAM IMPLEMENTATION

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Malaria control interventions in most endemic countries have intensified in recent years and there is a need for robust monitoring and evaluation (M&E) systems to measure progress and achievements. Providing program and M&E officers with appropriate skills is a way to strengthen malaria's M&E systems and enhance information use for programs' implementation. From 2010 to 2015, capacity strengthening efforts included organizing regional in-person workshops for M&E of malaria programs for Anglophone and Francophone countries in sub-Sahara Africa in collaboration with academic institutions from Ghana and Burkina Faso. Open-sourced online courses were also available in English. A post-workshop assessment was conducted after five years to assess the effects of these regional workshops and identify gaps in capacity. The regional workshops trained two hundred and eighteen participants from 28 countries from 2010 to 2015. Trained participants were from ministries of health, national malaria control programs, non-governmental organizations, and development partners. The average score (%) for participants' knowledge tests increased from pretest to posttest for the Anglophone workshops (2011: 59 vs. 76, 2012: 41 vs. 63, 2013: 51 vs. 73; 2014: 50 vs. 74, 2015: 52 vs. 69). Similarly, Francophone workshop posttest scores increased, but were lower than Anglophone due to higher scores at pretest. (2011: 70 vs 76, 2012: 74 vs 79, 2013: 61 vs 68; 2014: 64 vs 75, 2015: 70 vs 79). Results of the post-workshop assessment revealed that participants retained practical M&E knowledge and skills for malaria programs, but there is a need for a module on malaria surveillance adapted to the pre-elimination context. The workshops were successful because of the curriculum content, the facilitation quality, and the engagement of partner institutions with training expertise. Results from the post-workshop assessment will guide the curriculum's development and restructuring for the next phase of workshops. Country-specific malaria M&E capacity needs assessments may also inform this process as countries reduce malaria burden.

991

MAPPING MALARIA RESEARCH FOCUS, CAPACITY AND INTERNATIONAL COLLABORATION IN NIGERIA

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Nigeria has the highest burden of malaria globally with the entire population (est. 182 million) at risk of infection and disease. Nigeria is scaling up its efforts to control and eliminate malaria, which requires a wide range of country-specific technical support and scientific expertise for mapping, monitoring and evaluating, operational research and documenting programmatic impact and success. To better understand the distribution of skills and institutional capabilities that may provide programmatic support within Nigeria, this study aims to identify and map malaria research focus, areas of scientific capacity and international collaborative links by analyzing data from published scientific articles. Using electronic searches in online bibliographic archives, a systematic collation of articles from the past 5 years with at least one Nigerian-based author is being conducted. From this, a comprehensive geo-referenced database is being developed by categorizing key information including authors, institutions, location (i.e. state, zone, place), research type (i.e. molecular, parasitological, immunological, epidemiological and/or entomological), journal, journal impact factor, number and country of international collaborators and main sources of funding. To date, more than 400 articles on malaria research in Nigeria have been identified and being entered into the database. Maps will be presented on the number of articles, institutional type, and research focus with key collaborative networks and funding sources highlighted. The study will provide a useful resource for the national malaria programme as well as national and international scientists. It will highlight the current technical and scientific potential that could be maximized for programmatic purposes, and also provides a model approach that can be applied in other endemic counties.

992

THE INCUBATION PERIOD OF MALARIA AMONGST TRAVELERS RETURNING TO THE UK

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The introduction of imported cases of malaria to non-endemic countries remains an on-going concern for healthcare professionals. The United Kingdom (UK) alone continues to receive and treat ~ 1,500 cases of imported malaria each year. This number remained roughly constant between 2003 - 2013. Accurate quantification of the mean and variance of this key epidemiological parameter is essential to inform public health policy and specify case definitions. To reduce the risk of severe malaria and death associated with infection, rapid diagnosis has been demonstrated to be key for prevention. However, to date, few studies have estimated the incubation period of malaria collectively from a large number of individuals. Current UK guidelines available to the public suggest that the incubation period of malaria is approximately 7 -18 days. We use data on clinically confirmed cases of *Plasmodium falciparum* and *Plasmodium vivax* imported to the UK between 1991 - 2006. Amongst cases of in travellers arriving in the UK, onset of symptoms occurred within a mean of 19 days after arrival for *P. falciparum*. For *P. vivax* the observed incubation period distribution was bimodal with the first peak of infections reported with a mean of 22 days, the data showed the classic second peak around 265 days post infection for *P. vivax*. For a subset of cases, the use of anti-malarials and less severe malaria infection lengthened the mean incubation period of *P. falciparum* from 13.4 days, by 4 days and 5.1 days respectively in a multivariate regression model. For *P. vivax* our findings demonstrated that women had shorter incubation periods. Our findings highlight that while for some cases the incubation period falls within the current recommended guidelines, on average the reported estimated incubation period duration was longer, particularly for *P. vivax*.

993

THE USE OF RESPONDENT-DRIVEN SAMPLING TO ASSESS MALARIA KNOWLEDGE, TREATMENT-SEEKING BEHAVIORS AND PREVENTIVE PRACTICES AMONG MOBILE AND MIGRANT POPULATIONS IN AN ARTEMISININ RESISTANCE SETTING IN WESTERN CAMBODIA

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The movement of populations within and between malaria-endemic regions is a major contributor to the spread of artemisinin resistance along the Cambodian borders. Mobile and migrant populations are poorly connected to public health and surveillance systems. Their high mobility makes them difficult to survey and reach with health interventions. The objective of this study was to determine knowledge of malaria, treatment-seeking behaviours and preventive practices among migrant/mobile workers in Western Cambodia. A structured survey of mobile and migrant populations was implemented in two provinces of Western Cambodia along the Thai-Cambodian border using a Respondent Driven Sampling (RDS) approach. Results: There were 764 participants in Pailin and 737 in Veal Veng. Most respondents (93.7% in Pailin and 96.1% in Veal Veng) received health messages, predominantly on malaria. Knowledge of malaria transmission, prevention, and symptoms was found to be very high (94.4% in Pailin and 98.2% in Veal Veng); however, other beliefs persist, predominantly in Veal Veng (24.4% listed a dirty or unsanitary environment and 57.6% listed contaminated food or drink in Veal Veng). Knowledge of using a mosquito net was high (95.5% in Pailin and 99.1% in Veal Veng), however specific knowledge of the use of an insecticide treated bed net (ITN) for prevention was relatively low. Stated ownership of an ITN or treated hammock net was low (25.3% in Pailin and 53.2% in Veal Veng). Of those that took an antimalarial, greater than 60% used artemisisnin-based combination therapy (ACT) or atovaquone-proguanil. In conclusion, RDS sampling methodologies were used to effectively statistically robust data to evaluate malaria risk and to assess protective behaviours of this difficult-to-survey population. There is a need for sustained public health efforts to reach these populations within an elimination context. These findings are critical for informing strategies to more effectively deliver health services and promote behaviour change among a population at high risk for contracting and spreading artemisinin-resistant malaria.

994

LACK OF MORTALITY IN CHILDREN WITH SICKLE CELL DISEASE AND SEVERE MALARIAL ANEMIA WHO RECEIVE TIMELY BLOOD TRANSFUSION

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Hemoglobin AS (sickle cell trait) has a strong protective effect against malaria, particularly severe malaria, but studies with small numbers of children with HbSS (sickle cell anemia, SCA) and severe malaria have suggested that children with SCA who develop severe malaria have a high mortality rate. To better characterize hematologic parameters and risk of mortality in children with SCA and severe malaria, we compared these factors according to HbS genotype (AA, AS, SS) in a cohort of children 18 months – 12 years of age with severe malarial anemia (SMA, n= 184), cerebral malaria (CM, n= 213) or healthy community controls (CC, n= 197). All children with SMA received blood transfusion. HbAS (41, 6.9%) was more frequent in CC (37, 18.8%) than in SMA (2, 1.1%) or CM (2, 0.9%, *P*<0.001), confirming the protective effect of HbAS against severe malaria. HbSS (18, 3.0%) was more frequent in SMA (17, 9.2%) than CM (1, 0.5%, P<0.001). Among children with SMA, children with HbSS and HbAA had similar hemoglobin levels, but children with HbSS had a higher WBC count and lower parasite density (both *P*<0.001). There was no mortality in children with SMA, even in those with HbSS. Our findings show that SCA is a major risk factor for SMA in this population, but SCA is not associated with increased mortality in SMA if timely transfusion is available.

995

AGE- AND PREVALENCE-RELATED MALARIA INFECTION RISK AND TREATMENT BEHAVIOR: EVIDENCE FROM A HOUSEHOLD SURVEY IN UGANDA

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In Sub-Saharan Africa, both under-treatment and over-treatment of malaria are common since illnesses are often diagnosed and treated on the basis of symptoms. Using data on fever treatment and malaria rapid diagnostic testing from 2,285 households in 92 villages in Uganda, we find that although households treated 40% of clinical malaria episodes with artemisinin-based combination therapies (ACTs)—the first-line, most effective, class of drugs for the disease—households also treated the same proportion of non-malarial febrile illnesses (Unadjusted Difference: -0.036, 95% CI: [-0.111 0.039], p=0.345). We show that both the age of the patient and the village prevalence rate are strongly associated with the probability that a febrile patient is infected with malaria: each additional age year is associated with a 0.6 percentage point decline in the probability of testing positive for malaria (95% CI: [-0.008 -0.005], P<0.001), and a one standard deviation increase in the village malaria prevalence rate is associated with a 13 percentage point increase in the probability that a febrile patient is infected with malaria (95% CI: [0.105 0.154], P<0.001). However, ACT treatment rates for febrile illnesses are not significantly associated with either age (Unadjusted Coefficient: -0.001, 95% CI: [-0.003 0.001], P=0.180) or with standardized village malaria prevalence rates (Unadjusted Coefficient: -0.032, 95% CI: [-0.074 0.009], P=0.121). We also present some evidence that whether a caregiver believes a febrile illness is malaria is unrelated to the febrile patient's age and to the local prevalence rate, suggesting that information gaps on malaria risk may play a large role in the under-treatment of malaria.

996

RELATIONSHIP BETWEEN THE PREVALENCE OF PARASITEMIA IN PREGNANT WOMEN AND CHILDREN: BIOKO ISLAND MALARIA INDICATOR SURVEY 2008-2015

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Pregnant women have been one of the main targets in the efforts to control malaria and in some settings they are routinely screened and treated or provided anti malaria prophylaxes and also provided ITNs. Studies have indicated that they are more likely to have detectable malaria due to higher parasite densities. Prevalence in children (2-14 years) is one of the main standard measures used to monitor the outcomes and impacts of malaria control programs in moderate to high transmission areas. Pregnant women attend ANC and blood samples are routinely taken and could be used to screen for parasitemia, allowing for an insight into the monthly variation of the incidence of malaria without need for extra sampling. Knowing the relationship of the prevalence of malaria in children and pregnant women could have far reaching implication the various control interventions in terms of timeliness of data and the implementation of the routine MIS. Using 8 years data of the annual MIS Bioko islands of Equatorial Guinea from 2008-2015, the yearly prevalence of parasitemia in children and pregnant women was calculated and Pearson's product-moment correlation was run to assess the relationship. Prevalence among children is relatively higher, there exist similar trends in the two groups; There was a very strong positive correlation between prevalence of the two groups $r(6) = 0.909$, $p < .002$. In conclusion, ANC attendees can be used as a sentinel group to monitor malaria prevalence because they are readily available and show similar trends with the children whose malaria prevalence is a standard measure to estimate malaria endemicity. More importantly, there will be timely and seasonal data for decision making.

997

ONE YEAR STABILITY ANALYSES OF PFS25-EPA AND PFS230-EPA CONJUGATES ADJUVANTED WITH ALHYDROGEL®

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Plasmodium falciparum Pfs25 and Pfs230 proteins are important malaria transmission-blocking vaccine candidates. Pfs25 is expressed as a surface protein during zygote and ookinete stages in infected mosquito, while Pfs230 is expressed in gametocytes in the human host and on the surface of gametes in the mosquito host. To increase immunogenicity, the recombinant Pfs25 and domain 1 of Pfs230 (referred to as Pfs25M and Pfs230D1M) were conjugated to the recombinant, nontoxic *Pseudomonas aeruginosa* ExoProtein A (rEPA) in conformance with current good manufacturing practices (cGMP). The resultant clinical grade conjugates (Pfs25M-EPA and Pfs230D1M-EPA) were formulated on Alhydrogel at 78 µg/mL for Pfs25M and 50 µg/mL for Pfs230D1M. To ensure the vaccines are in compliance with the regulatory specifications during the phase 1 human clinical trial period, annual evaluation of these vaccines was performed. The one year stability analyses on the clinical lots included appearance, endotoxin content, sterility, strength (protein content tested by *o*-Phthaldialdehyde assay), identity (SDS-PAGE and Western blot after antigen extraction from Alhydrogel), integrity (pH, percent protein bound to Alhydrogel, SDS-PAGE, Intrinsic Fluorescence CD, Direct Alhydrogel Formulation Immunoassay), and efficacy (the mouse potency assay). Our results showed that the Drug Products Pfs25M-EPA and Pfs230D1M-EPA formulated on Alhydrogel are biochemically, biophysically, and biologically stable after storage for one year at 4ºC. Thus, we conclude that sufficient stability of these candidates supports further studies in human clinical trials.

998

LONG TERM IMMUNE RESPONSES INDUCED AGAINST *PLASMODIUM VIVAX* CSP AND MSP-1 CHIMERIC VACCINE CANDIDATES DELIVERED BY NOVEL ADENOVIRAL VECTORS

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Protection against malaria based on pre-erythrocytic and erythrocytic antigens depends on IFN-γ production by CD4 and CD8 T cells. Adenoviral vectors have been used to induce robust cellular immune responses in recent malaria vaccine clinical trials and reports indicate that adenoviruses are capable of long periods of transgene expression, a desirable for malaria vaccines since immune responses lasting several transmission cycles are necessary to exert a substantial effect on disease incidence. To test the durability of the immune responses induced by adenoviral vectors encoding a multistage *Plasmodium vivax* chimeric protein vaccine including promiscuous T cell and B cell epitopes from the circumsporozoite protein (CSP) and merozoite surface protein-1 (MSP-1), we primed BALB/c mice with one of two different recombinant adenoviral vectors and assessed their immune responses and ability to induce memory T cells by boosting the mice one year post priming. We found that mice immunized with the recombinant proteins or one of the two protein-encoding adenoviral vectors - Simian Adenovirus 36 (SAd36) or a recombinant human adenovirus 5 modified to contain the fiber-knob region of adenovirus serotype 3 (Ad5/3) - displayed no significant decrease in antibody titers against the CSP and MSP-1 protein chimeras when antibody titers were compared between day 20 and 1 year post priming. We subsequently boosted mice with the heterologous adenoviral vector 1 year post priming, followed by two protein boosts 20 and 40 days later. We found that in comparison to mice receiving only protein immunizations, both heterologous adenoviral regimens induced CD4 and CD8 T cells capable of producing higher levels of IFN-γ, IL-2, and TNF-α upon *ex vivo* stimulation with the MSP-1 or CSP chimeric recombinant proteins or peptide pools representing T or B cell epitopes derived from these chimeric proteins. Taken together, the data demonstrate that our novel multistage malaria vaccine is able to induce long lasting humoral and cellular immune responses able to recognize two different *Plasmodium* antigens when delivered by a regimen that includes adenoviral vectors.

999

A HYBRID *PLASMODIUM VIVAX* BLOOD STAGE-TRANSMISSION BLOCKING VACCINE CANDIDATE ELICITS ROBUST CELLULAR IMMUNE RESPONSES AND LONG-LIVED FUNCTIONAL ANTIBODIES

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Malaria control efforts focused on the use of vector based interventions have resulted in a significant reduction of *Plasmodium falciparum* cases. Unfortunately, the unique biological features of *P. vivax* make vector control interventions ineffective against this parasite. Transmission Blocking Vaccines (TBVs) are considered the best alternative for achieving malaria control as antibodies induced by TBVs taken up by the mosquito during the blood meal interrupt parasite development. However, a major concern for post-fertilization TBVs is a short lived antibody response as the human host is not exposed to these mosquito stage antigens, needed to induce a natural boosting effect. A transmission blocking formulation could also result in low compliance since it would not be protective against the disease. To address these problems, we designed a multistage chimeric vaccine denominated *P. vivax* erythrocytic stage-transmission blocking chimera (PvES-TBC). This vaccine candidate includes 5 promiscuous T helper cell epitopes derived from the erythrocytic antigen MSP-1 which were genetically linked to the MSP-1 19kDa fragment and to the transmission blocking antigen Pvs25. PvES-TBC was tested in comparative experiments with Pvs25 in mice. PvES-TBC induced a strong cellular and humoral immunity able to recognize both MSP-1 and Pvs25. Additionally, when compared to Pvs25, PvES-TBC elicited a more robust antibody response as determined by a significantly higher avidity and a bias toward IgG2a production. This response was determined to last for a period longer than 2 years after the final boosting in mice immunized with PvES-TBC, an effect not observed in the Pvs25 group. The long lasting response was related to a significantly higher proportion of long lived plasma cells in mice immunized with PvES-TBC. When the transmission blocking ability of antibodies was tested by membrane feeding assays using wild *P. vivax* isolates, the antibodies produced in response to PvES-TBC were able to block 90% of mosquito infections. These characteristics make PvES-TBC a promising transmission blocking vaccine candidate that warrants clinical development.

1000

SAFETY AND IMMUNOGENICITY OF THE NOVEL *PLASMODIUM FALCIPARUM* BLOOD-STAGE VACCINE CHAD63-MVA RH5 IN A PHASE IA CLINICAL TRIAL

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The development of an effective malaria vaccine remains a vital goal if eradication is likely to be achieved. Candidate antigens for blood-stage *Plasmodium falciparum* vaccines have been hampered with issues of polymorphism and the need to induce extremely high antibody concentrations for these vaccines to be effective. The reticulocyte-binding protein homologue 5 (RH5) is the most promising blood-stage *P. falciparum* candidate antigen to date. It is essential for parasite survival and erythrocyte invasion and, unlike other blood-stage antigens (e.g. AMA1 and MSP1) RH5 does not appear to come under significant immune pressure in endemic settings, and there is limited polymorphism. Vaccination with an RH5-based vaccine confers protection against malaria challenge in non-human primates and the antibodies induced by vaccination are able to cross-inhibit *in vitro* all *P. falciparum* isolates tested to date. We report on a first-in-human Phase Ia clinical trial (NCT02181088) of an RH5-based vaccine delivered using the viral vectors chimpanzee adenovirus serotype 63 (ChAd63) and modified vaccinia virus Ankara (MVA) in a heterologous prime-boost strategy. The trial was conducted in Oxford and Southampton, United Kingdom and recruited twenty-four healthy, malaria-naïve volunteers aged 18-50. The first four volunteers (Group 1) received a lead-in dose (5×10^9 viral particles, vp) of the ChAd63 RH5 vaccine alone before dose escalation to the full dose (5×10^{10} vp) in Group 2. The first four volunteers in Group 2 received ChAd63 alone, and the final sixteen were boosted with MVA RH5 eight weeks after ChAd63 RH5 at doses of $1 – 2 \times 10^8$ plaque-forming units, pfu. Data on all adverse events were recorded for 28 days after each vaccination and serious adverse event (SAE) data were collected for the duration of the study. This is the first RH5-based vaccine to be tested in humans. The vaccines were well tolerated, with no safety concerns and there were no SAEs. T cell and serum antibody responses were assessed by *ex-vivo* interferon-γ ELISpot, ELISA and *in vitro* growth inhibition activity (GIA) assays. The vaccines were immunogenic and these data will be presented.