was administered to new TB patients, older than 15 years, who had received at least one month of TB treatment and given consent. Data was collected through informal discussions with TB coordinators and facility heads, desk top review of patient records and intervies with TB patients to assess direct costs prior to being diagnosed and direct and indirect cost of current treatment. Of the 159 patients interviewed, 64% were in the three lower socio economic quintiles with monthly income less than US$ 42.55. Health system delay was estimated at 1.4 weeks with males taking longer to seek care than females. DOTS patients paid a mean total direct cost of US$ 0.50 for each visit and spent of 58 minutes per DOTS visit. The mean number of days spent in the hospital was 22.7 days and direct cost of hospitalization was US$ 48.32. Whilst 48.3% of the patients borrowed, 37.7% sold assets to cope with paying for their ill health. Reduction of monthly household and patients income due to TB was 44.5% and 82.6% respectively. Sixty-one percent of the TB patients lost their jobs, 11% got separated from their spouse/family and stopped attending public functions. Through this study, the NTP has identified constraints faced by TB patients and their families that have an effect on case finding and treatment adherence. We recommend that TB patients and their families should benefit from social protection packages that will ease the financial burden. Employers should not hesitate to take back workers who have been diagnosed and treated with TB.

1251

CLINICAL OUTCOMES ASSOCIATED WITH ROUTINE USE OF INTERFERON-Γ RELEASE ASSAYS IN A CENTRAL LONDON TB SERVICE

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Interferon-γ release assay (IGRA) has been shown to have higher specificity than tuberculin skin testing (TST) for screening for latent TB and is recommended by the National Institute for Clinical Excellence (NICE). The aim of this study is to review the indications for and analyse the results of IGRA testing in our central London BCG vaccine positive population. We analysed routinely collected clinical and demographic data on patients referred for IGRA testing to a TB service at a large London teaching hospital from September 2007 to January 2012. Reasons for referral for screening included contacts of active TB, pre-monoclonal antibody therapy, recent migrants and occupational health. Quantiferon Gold InTube was used. We used the London TB registrar to identify patients that were diagnosed with active TB either by bacteriological or clinical evidence from November 2007 to February 2012. We determined the sensitivity and specificity of IGRA for diagnosing active TB and screening for latent TB. We used univariate linear regression to assess the incremental impact of IGRA result on having a diagnosis of active TB. 961 IGRAs were performed on 917 patients. 51% were male with a median age of 30 years (IQR 19-40). There were 46 (4.8%) indeterminate, 703 (73.2%) negative and 212 (22%) positive results. Indeterminate results were more prevalent amongst immunosuppressed than immunocompetent patients 66.7% (24/36) vs. 33.3% (12/36). 46 cases of active tuberculosis were identified from the London tuberculosis register, 15 of which had a negative IGRA result. The sensitivity and specificity of IGRA for diagnosing active TB were 67% and 79% respectively. We found a direct correlation between a positive IGRA test result and active TB diagnosis (p 0.00 coefficient 1.343). The sensitivity and specificity of IGRA for latent TB screening were 55% and 89% respectively. We found an overrepresentation of indeterminate results amongst immunosuppressed patients. IGRA was used in addition or other conventional diagnostic modalities for the diagnosis of active TB, which is outside the scope of the NICE guidelines. Although the use of IGRA for this purpose is approved by the US Food and Drug Administration, caution should be exercised due to its low sensitivity for diagnosing active TB. However the usefulness of IGRA in screening for latent TB was conferred by its high specificity in our setting.

1252

EVALUATION OF HOUSEHOLD LEVEL INTERVENTIONS DURING A LARGE, URBAN TYPHOID FEVER OUTBREAK - HARARE, ZIMBABWE 2011-2012

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Between October 2011 and March 2012, ~2,750 suspected cases of typhoid fever in two high-density suburbs of Harare (Dzivaresekwa and Kuwadzana) were reported to the Harare City Health Department (HCHD). To prevent outbreak spread, HCHD and non-governmental organizations conducted door-to-door health and hygiene education and distributed point-of-use water treatment (PoUWT) products beginning in October 2011. To evaluate the effectiveness of these interventions, we conducted cross-sectional household surveys in these two affected suburbs in March 2012, including free chlorine residual (FCR) testing in stored drinking water. Reported intervention coverage was high, with 351 (77%) of 458 randomly selected households having received both typhoid fever prevention information and at least one PoUWT product. Of 368 households that received at least one of the three types of chlorine tablets distributed, 326 (89%) reported ever using them, 160 (43%) reported using them daily, and 98 (27%) had stored water that was treated and had FCR ≥0.2mg/L when tested. Only 169 (55%) of 310 household respondents who had chlorine tablets on the day of the survey knew the correct volume of water to treat with their tablets. In univariate analysis, respondents who had higher income, were older, had received PoUWT products or typhoid fever prevention information, and who reported household water treatment before the outbreak were more likely, and respondents who reported boreholes as the primary source of drinking water were less likely, to report water treatment during the outbreak or on the day of the survey, and to have treated stored water with FCR ≥0.2mg/L ($p < 0.05$). The findings highlight: 1) relatively low uptake of PoUWT after free distribution (consistent with other research); 2) the need to improve coordination of NGO response activities through consistent PoUWT product choices and communication about product use; and, 3) the need to emphasize treating drinking water from all sources daily to control and prevent typhoid fever and other waterborne disease outbreaks.

1253

EFFECTS OF ENVIRONMENT ON HUMAN CYTOKINE RESPONSES: ROLE OF URBAN VERSUS RURAL RESIDENCE

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Environment may have a key role in the development of the immune system in childhood and may explain the low prevalence of allergic and autoimmune diseases in the rural tropics. To investigate the effects of urban versus rural residence on the immune response, we recruited 440 school children living in either in rural communities in the Province of Esmeraldas or in the city of Esmeraldas. We collected data on

environmental exposures by questionnaire and on intestinal parasites by examination of stool samples. Whole blood was stimulated with mitogen, parasite antigen and aeroallergens. IFN-γ, IL-5, IL-10, IL-13, and IL-17 were measured in supernatants. Overall, urban children had mothers with greater educational levels, were more likely to have access to piped water (urban 98.7 % vs. rural 1.9%) and were more likely to use latrines or water closets for defecation (urban 94.8% vs. rural 54.7%). Rural children were more likely to be infected with geohelminths (urban 73.5% vs. rural 20.9%). The frequencies of children with .. DDDdetectable levels of cytokines were similar in urban and rural samples except for IL-10 that was significantly more frequent in the urban population when measured as spontaneous production (adjusted OR 2.56, 95% CI 1.05-6.24) and after stimulation with *Ascaris* (adj. OR 2.5, 95% CI 1.09-5.79) and house dust mite (adj. 2.24, 95% CI 1.07-4.70) antigens. Our data do not provide support for a major role for place of residence or geohelminth infections as a major determinant of the cytokine response in childhood. Surprisingly, urban residence that might be considered to be a more hygienic environment, was associated with more frequent production of the immune regulatory cytokine IL-10.

1254

PROGRESS ON MDG 7.C IN THE MILLENNIUM VILLAGES AFTER THREE YEARS: IMPROVED HOUSEHOLD WATER AND SANITATION

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Benefits of improving water and sanitation can influence health, educational, employment, economic and social domains. Since 1990, there have been significant global gains in access to improved water, and slower gains in sanitation. Despite commendable improvements, global progress has been uneven, with sub-Saharan Africa, and rural areas in particular, carrying a disproportionate burden of poor access. This mixed-methods implementation study assesses progress towards MDG 7.C across nine sites in rural sub-Saharan Africa in the first three years of the Millennium Villages Project (MVP), a 10-year multi-sector development project. Details of costs, variability between and within sites, challenges and lessons learned are explored in the study. Across nine MVP sites, the proportion of households not using an improved household water source reduced from 87.3% at baseline (2006/07) to 22.7% at year 3 (2009/10) (64.6% percentage-point change, 95% CI = 60.7-68.6%, p-value <0.0001). This represents a 74% reduction in the proportion of population without access to improved water, and exceeds the MDG target for water at a local level, as well as meeting the sub-Saharan African regional target of less than 25% of the population without coverage by 2015. The proportion of the population reporting not using an improved sanitation facility reduced from 98.1% at baseline to 71.4% at year 3 (26.7% percentage point change, 95% CI 24.6%-29.0%, p-value <0.0001). This represents a 27% reduction in the proportion of the population without access to improved sanitation facilities. Although not yet meeting the MDG for sanitation, if the same rate of change were to continue from today to 2015, sanitation would also be on track to meet local and regional MDG targets. These data provide promising evidence suggesting that with MDG-focused interventions, significant gains can be made in household access to improved water and sanitation facilities in a rural sub-Saharan African setting.

1255

FARM WORKER HYGIENE AND HAND SANITATION IN MEXICO ASSOCIATED WITH CONTAMINATION OF FRESH PRODUCE

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Produce-related foodborne disease outbreaks lead to economic losses, illness, and death, making produce-contamination an important public health concern. In this study we investigated the pathways of produce contamination on Mexican farms, and the sanitation and hygiene practices that may contribute to contamination. We quantified the fecal contamination on produce, hands and environmental samples from 11 farms in Mexico. Produce (cantaloupe, jalapeño, tomato) rinses (161), were collected along with matched irrigation water (89), soil (55), and farm worker hand rinses (106). From these samples, fecal indicators (*E. coli*, Enterococcus, coliforms) and human pathogens (Salmonella, *E. coli* O157:H7) were enumerated. Multivariate regression modeling was used to identify associations. Data were also collected on farm sanitation and worker hygiene through surveys and interviews. Produce was frequently contaminated, 29%-100% of samples were positive for indicators, and the mean concentration of indicators ranged from 10^2-10^6 cfu/fruit. The presence and levels of indicators on soil and water were not significantly associated with those on produce samples. Microbial indicators on hands were significantly higher ($p < 0.05$) than in water or soil. Presence of *E. coli* was significantly associated between hands and produce (OR 7.9, 95%CI [3.3-19.1]). The levels of *E. coli, Enterococcus*, and coliforms (rho=0.4, 0.5, 0.6) were significantly and highly correlated between hands and produce. These data show that hands are a potential source of produce contamination. Hand contamination is likely due to lack of sanitation/hygiene facilities. There were five toilets total available on all 11 farms. Only three had handwashing stations nearby. Evidence of open defecation was observed on two farms. Improved hygiene facilities and sanitation policies on farms could reduce microbial contamination of produce and improve working conditions for employees. Future study aims include the development of training modules on sanitation/hygiene behaviors tailored to farm managers and workers.

1256

WASTEWATER IRRIGATED FARMS AS A COMMON DENOMINATOR FOR MALARIA AND DIARRHEAL DISEASE TRANSMISSION IN URBAN GHANA

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Worldwide, wastewater use in urban agriculture is increasing at a rapid pace due to urbanization and its accompanying stress on limited freshwater resources. The economic and food security benefits associated with wastewater use in urban agriculture are enormous, but so also are the public health burden the practice exerts on the urban population. Wastewater use in urban agriculture is identified as a major risk factor for diarrhoeal disease, particularly among urban farmers and their family members and consumers of wastewater farm products. Malaria mosquito vectors also breed in wastewater farms, thus exposing nearby urban populations to increased malaria risk. In addition, agro-chemicals used in wastewater farms may contribute to development of insecticide resistance in malaria vectors, further exacerbating the malaria problem. So far, little is known of the contribution of wastewater farms to the combined risk of diarrhoea and malaria. Understanding this contribution would be important for developing integrated interventions. The main

aim of this study is to assess the contribution of urban wastewater farms as a common denominator for the transmission of malaria and diarrhoeal disease in urban Ghana. The study is being conducted in Kumasi, the second largest city in Ghana, where more than 12000 hectares of urban vegetable farms are irrigated with wastewater. The outcome of the study will lead to the development of integrated interventions for mitigating malaria and diarrhoeal disease transmission associated with wastewater irrigation.

1257

ASSOCIATION BETWEEN EFFICIENTLY COLLECTED MEASURES AND OBSERVED MEASURES OF HANDWASHING BEHAVIOR IN THE IMPACT EVALUATION OF THE GLOBAL SCALING UP HANDWASHING PROJECT IN VIETNAM

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Handwashing reduces diarrhea incidence but is difficult to measure. Structured observation, a direct approach to measuring handwashing behavior, is costly and time-consuming. Self-report, observation of handwashing materials, and visual inspection of hand cleanliness are inexpensive and timesaving and thus efficient measures. We sought to assess whether these measures are associated with handwashing behavior measured by structured observation. With data from controls in the Impact Evaluation of the Global Scaling Up Handwashing Project in Vietnam, we used multilevel logistic regression to calculate wealth-adjusted odds ratios for associations between efficient measures of handwashing and observed handwashing behavior among caregivers, while accounting for multiple events per caregiver. We examined handwashing events overall and stratified by event type. We examined 1379 events overall; 289 fecal contact events (24% accompanied by handwashing with soap) and 569 food related events (6% accompanied by handwashing with soap). Soap and water at the handwashing places used post defecation (OR= 3.96, 95%CI: 1.61-9.53), and before food preparation (OR= 2.34, 95%CI: 1.17-4.68) as well as a rating of ≥7 on a hand cleanliness scale of 1 to 9 (OR= 3.01, 95%CI: 1.75-5.71) were significantly associated with observed handwashing with soap overall. Self-report of handwashing with soap after fecal contact (OR= 4.29, 95%CI: 1.68-11.01), observation of soap and water at the handwashing place used post defecation (OR = 8.21, 95%CI: 1.12-60.24), and hand cleanliness index ≥7 (OR = 3.48, 95%CI: 1.67 - 7.31) were all significantly associated with observed handwashing with soap after fecal contact events. Self-report of handwashing with soap before feeding a child was the only efficient measure associated with observed handwashing with soap before food related events (OR = 4.00, 95%CI: 1.14-14.02). In Vietnam, self-report of handwashing, presence of handwashing materials, and examination of hand cleanliness were associated with observed handwashing with soap overall, and handwashing after fecal contact. Where structured observation is infeasible due to cost, efficient measures of handwashing may be appropriate for measuring handwashing behavior. More importantly, promotion of handwashing with soap is required in Vietnam to improve hand hygiene at critical times relevant to pathogen transmission.

1258

FAECAL CONTAMINATION OF FOOD, WATER, HANDS AND KITCHEN UTENSILS AT HOUSEHOLD LEVEL IN RURAL AREAS OF PERU

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The aim of this study was to evaluate sources of contamination of child's food and drinking water from rural households in the highlands of Peru. Samples from child meals, drinking water, kitchen utensils, and caregiver and child hands, were analysed for total coliforms and *Escherichia coli* counts using Petrifilm™EC. Thermotolerant coliforms were measured in water using DelAgua® test kit. Diarrhoeagenic *E. coli* were searched by Polymerase Chain Reaction methods (PCR). Thermotolerant coliforms were found on 48% of water samples. *E. coli* was found in 23% of hands, 16% of utensils and 4% of meals. Kitchen cloths were the most frequently contaminated with total coliforms (89%) and *E. coli* (42%). Diarrhoeagenic *E. coli* was found in 33% of water, 27% of meals and 23% of kitchen utensils. There is a need to develop effective hygiene interventions focused to specific kitchen utensils and handwashing, to reduce the contamination of food, water and kitchen's environment in these rural settings.

1259

WATER- AND SANITATION-RELATED RISK FACTORS FOR SOIL-TRANSMITTED HELMINTH INFECTION IN URBAN SCHOOL- AND PRESCHOOL-AGED CHILDREN IN KIBERA, NAIROBI

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Individuals living in urban slums have limited access to city services, including water and sanitation (WS). Some evidence suggests that WS factors affect risk for soil-transmitted helminth (STH) infections, which disproportionally affect school-aged (SAC) and preschool-aged children (PSAC), but further characterization of WS factors and their impact in slum settings is needed to identify intervention points. Households (n=1,192) containing an index PSAC (6-59 months) or SAC (5-14 years) were randomly selected from those enrolled in CDC's International Emerging Infections Program, a population-based surveillance system in the urban slum of Kibera in Nairobi, Kenya. Data collection included a household-level questionnaire and environmental assessment for WS risk factors and index child stool specimens tested for STH ova by the Kato-Katz method. Stools of siblings living with index SACs were also tested. Household WS factors were classified by the WHO/UNICEF WS service level ladders categorizing resources in groups such as improved, unimproved, and shared, and tested for associations with STH infection. Among 130 households with sufficient data for interim analysis, household STH prevalence (≥1 child in the household positive for any STH) was 36.2%. Of all households, 3.1% reported piped water on premises and 96.9% another improved drinking water source; 62.2% (79/127) of these sources were unofficial connections into nearby municipal pipes. Ever having

difficulty meeting household daily water needs was reported by 76.2% of households, most often due to financial barriers (69.8%). Overall, 2.3% of household sanitation facilities were improved, 87.7% shared, and 6.2% unimproved; 2.3% of households practiced open defecation. Sewage observed in the participant's yard was associated with household STH infection (Fisher's Exact Test, p=0.03). Other associations emerging with ongoing data collection will be discussed. The Kibera population faces gaps in water availability and sanitation quality; STH control here and in similar settings requires an integrated approach.

1260

A QUALITATIVE EVALUATION OF HAND DRYING PRACTICES AMONG KENYANS

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Recommended disease prevention behaviors of hand washing, hygienic hand drying, and covering one's mouth and nose in a hygienic manner when coughing and sneezing appear to be simple behaviors but continue to be a challenge to promote successfully and sustain globally. We conducted a formative inquiry to better understand current hand drying behaviors associated with activities of daily living, and mouth and nose covering practices, among Kenyans. We conducted 7 focus group discussions (N=45); 30 in-depth interviews; 10 structured household observations; and 75 structured public observations in rural and urban Kenya communities. Using a grounded theory approach, we transcribed, coded, and analyzed the narrative data. Hand drying with a towel is not a common practice. Most women dry their hands on their leso (rectangular cloth wrapped around the waist) or their clothes when cooking, eating, or cleaning a young child. When men dry their hands, they use their trousers or a handkerchief. Children rarely dry their hands but, if they do, they usually wipe them on their clothes. People drew distinctions between hand drying after sporadic sneezing and blowing their nose during a cold. Many people sneeze into their hands and wipe them on their clothes. Men and women tended to use a handkerchief when they had a cold. Drying hands on dirty clothes and lesos can compromise the benefits of handwashing. Coughing and sneezing into an open hand can help spread disease. Health education and promotion materials and messages should stress hygienic hand drying practices such as using a clean towel or cloth, or air-drying. Messages should be particularly tailored to household activities and should emphasize the potential role of dirty towels or clothes as a vector of disease. The importance of sneezing or coughing into the upper arm or a handkerchief should also be emphasized more prominently. Research into barriers to adopting these simple practices is needed.

1261

POST-IMPLEMENTATION EFFECTIVENESS OF FOUR HOUSEHOLD WATER TREATMENT TECHNOLOGIES IN TYPICAL-USE CONDITIONS IN RURAL KENYA

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Household water treatment technologies are used by about 18 million of the 884 million people without adequate access to safe water. The efficacy of household water treatment technologies has been demonstrated in controlled situations such as laboratory and field trials. However some authors query the sustainability of the efficacy of HWT technologies under real-life situations after the field trials have ended. In view of the dependence of rural communities on highly polluted surface water sources; the sectoral promotion of household water treatment (HWT) systems and the lack of data on their post-intervention effectiveness, it is necessary to evaluate the effectiveness of household water treatment technologies within a real-life context. This study was carried out one and two years after the two implementing organisations had ended their intervention. It examined the microbial efficacy of Aquatab, PUR, Waterguard and ceramic filters by carrying out three unannounced visits between March and April 2010 to each of the 37 HWT user households in five villages in the Nyanza province of Western Kenya. A total of 247 samples were collected from study households' collection and storage containers in order to determine the efficacy of the technologies on water from the 11 unimproved and improved water sources used by the study households. The findings indicate that the four HWTS technologies assessed are able to improve microbial quality of the improved and unimproved water sources. However, based on the observation of inconsistent performance, none of the technologies achieved the minimum expected reduction value or can be classified as a highly protective or protective technology. It is recommended that the drinking water supply and sanitation sector should address the reasons for their reduced effectiveness in the typical-use conditions when compared to laboratory efficacy. These include incorrect usage and inappropriate selection of HWT options for water source characteristics.

1262

VALIDATION OF AN INDEX OF PROXY MEASURES AND SELF-REPORTED HANDWASHING BEHAVIOR IN DHAKA, BANGLADESH

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Handwashing with soap reduces diarrhea, a leading cause of death in young children. Structured observation permits direct measurement of handwashing, but is inefficient and costly. Measures of handwashing behavior can be efficiently collected by rapid observation and self-report, but in isolation, they are not good indicators of behavior. Using data from primary caregivers in a case-control study of pneumonia risk factors in Dhaka, Bangladesh, we sought to develop an index of efficiently collected handwashing measures, and then test the validity of the index by comparing it to handwashing behavior measured by structured observation. We used principal component analysis, a data reduction technique, to generate a handwashing behavior index score for each caregiver based on handwashing demonstration, rapid observation, and self-reported handwashing behavior. We assigned caregivers to handwashing index quintiles and used logistic regression to compare quintiles to observed handwashing with soap after fecal contact in a 5 hour structured observation, accounting for repeated events. We observed 1,958 fecal contact events, of which 773 (39%) were followed by handwashing with soap. Duration of lathering during a handwashing demonstration, use of soap during a handwashing demonstration, presence of soap at a handwashing station, and self-reported frequency of handwashing accounted for 52% of the variance in the handwashing index score. When compared to those in the lowest index quintile of handwashing scores, each quintile except the third was associated with an increased odds of observed handwashing with soap [2nd quintile OR=1.41, p=0.03, 3rd quintile OR= 0.96, p= 0.81, 4th quintile OR=1.34, p=0.05, 5th quintile OR=1.30 p=0.08]; however, there was no significant linear trend (p trend=0.11). The use of principal component analysis to develop a handwashing behavior index did not identify progressive increases in observed handwashing behavior. Alternatives for index construction, such as summing of items, factor analysis, and prediction modeling, may be considered and evaluated against structured observation and health outcomes data. The construction and validation of indices represents only one approach to addressing the pressing need for low-cost, efficient, and reliable handwashing measures for use in modestly funded studies.

1263

EVALUATION OF THE MICROBIOLOGIC SAFETY OF STORED RAINWATER AS AN IMPROVED DRINKING WATER SOURCE FOR COMMUNITIES IN KHON KAEN, THAILAND

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Rainwater (RW) is considered an improved source of drinking water (DW) by the WHO and UN agencies tracking progress towards achieving the safe water access target of the Millennium Development Goals. There are, however, a paucity of data on the microbial quality of RW, making uncertain its safety as a DW source. The objective of this work was to evaluate the microbial quality of stored RW collected in a rural village in Thailand using the WHO DW quality guideline value of <1 *E. coli*/100mL as the basis for safety. In 2011, 59 households in Khon Kaen province, all of which used RW as their primary source of DW, were visited twice, once during dry season and once during rainy season. Observational data related to the physical/sanitary conditions of RW harvesting systems (RWHS) were collected during visits. Sampled containers included each household's main RW collection tank and the refillable container used to store RW for daily consumption. Samples were assayed for *E. coli* by the Colisure Quantitray 2000 method and results were scored as present if *E. coli* was ≥1/100mL. Of all samples processed (collection tank, refillable container), 39% and 82% of households had *E. coli* present in at least one container during the dry and wet seasons, respectively. *E. coli* was present in 21% and 66% of RW collection tanks during the dry and wet seasons, respectively. Initial analysis suggests that no single factor related to RWHS setup (roof, pipe, or tank material) had a statistically significant impact on the presence of *E. coli* in RW collection tanks. These results suggest that stored RW microbiologic quality may be highly seasonal, may not always meet WHO guidelines for safe DW, and that deterioration of the microbiologic quality of stored RW is likely due to a combination of collection and use practices. These results document that the UN Joint Monitoring Program's use of access to improved water supplies as an indicator of progress towards the MDG safe water target results in overestimation because improved sources, like harvested RW, may be microbiologically unsafe.

1264

REDUCTIONS IN DIARRHEA AND CLINIC VISITS FOR DIARRHEA AMONG CHILDREN UNDER THE AGE OF FIVE ASSOCIATED WITH A SCHOOL-BASED WATER SUPPLY, SANITATION AND HYGIENE INTERVENTION IN WESTERN KENYA: A CLUSTER-RANDOMIZED TRIAL

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While many studies have documented reductions in diarrhea incidence in children under five associated with improvements in water, sanitation, and hygiene (WASH) in the domestic environment, the effect of institution-based interventions are not well understood. We conducted a cluster-randomized trial of school-based WASH interventions in 185 public primary schools in western Kenya. Enrolled schools with a nearby water source (<1KM) were randomized into a handwashing promotion and water treatment intervention [HP&WT], HP&WT plus an additional sanitation component [San + HP&WT], or a control group. Schools without a nearby water source were randomized to receive a water supply intervention in addition to the San + HP&WT intervention components or to a control group. Interviews were conducted in a systematic selection of households in the catchment areas of all enrolled schools. Parent reported diarrhea episodes in the past week and clinic visits for diarrhea or vomiting in the past two weeks were recorded for all children under the age of five. Data were collected at baseline (March-April 2007) (n = 4,549) and two years after the start of the interventions (n = 4,392). There was a non-significant 33% reduction in the relative risk (RR) of diarrhea and 51% reduction in the RR of clinic visits among children under five living in the catchment areas of schools receiving water supply improvements compared to control areas (p = 0.185 and 0.075, respectively). Restricting analysis to those children under five living with at least one child attending a school enrolled in the trial increased both the magnitude and significance of this effect (RR diarrhea: 0.53, p= 0.049; RR clinic visits: 0.39, p= 0.03). The HP&WT and San + HP&WT interventions showed no effect on either outcome in both unrestricted and restricted analyses. Our findings suggest that an integrated school WASH intervention that includes the provision of improved water supply at the school can result in substantial reductions in child morbidity even among those children too young to attend school.

1265

EFFICACY OF DISINFECTANTS ON VIABILITY OF FOODBORNE BACTERIA AND ON *CRYPTOSPORIDIUM* AND *CYCLOSPORA*

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E. coli STEC, *Salmonella*, *Cryptosporidium*, *Cyclospora*, and microsporidia are causative agents of diarrheal illness worldwide. Most of the outbreaks associated with contaminated foods have implicated fruits and vegetables that have been consumed raw. The objective of this study was to determine the recovery efficiencies to improve the methodology detection for foodborne parasites to be used in surveillance studies, to determine efficacy of sanitizers used in the food industry on the survival of foodborne bacteria and parasites, and examine cross contamination during produce harvesting. Five *Escherichia coli* O157:H7, five *Salmonella* spp, and one isolate of each *Cyclospora cayetanensis*, *Cryptosporidium parvum*, and *Encephalitozoon intestinalis* were used for these experiments. Four different wash solutions were examined for recovery of 1000 oocysts of *Cryptosporidium* and *Cyclospora* from 25 gr basil leaves: water, 0.1M phosphate buffer, Glycine, and 3%levulinic acid/SDS. Phosphate buffer worked best. Detection of both organisms was done using nested PCR. Experimentally inoculated basil and bean sprouts were blanched (65, 88, and 100C) and determined if this process kills contaminants. The bacterial contaminants were reduced but not eliminated. The times and temperatures effective for pathogen destruction affected the fresh appearance of vegetables. Freezing did not inactivate bacterial pathogens but *Cryptosporidium* and microsporidia were very sensitive to extreme temperatures. Cross-contamination can occur when contaminated water or contaminated coring tools are used during lettuce harvesting. Reduction of contaminants in lettuce and coring tools was achieved when coring tools were rinsed with diluted chlorine (commonly used in agriculture) and more yet when rinsed with 3% LA/SDS. Sequential contamination of lettuce heads with microsporidia was also observed. Because parasites are highly resistant to chemical disinfectants, it is important to prevent crop contamination during harvesting.

1266

CHARACTERIZATION OF THE ETIOLOGY AND EPIDEMIOLOGY OF CENTRAL NERVOUS SYSTEM INFECTIONS IN GEORGIA

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Central nervous system (CNS) infections are caused by a large spectrum of viruses and bacteria, and associated with severe and disabling sequelae. Diagnosis of CNS infections and identification of the causative agents requires a complex combination of laboratory tests. In 2010, a hospital-based surveillance study was initiated in Tbilisi, Georgia to determine the incidence of infectious etiologies of acute meningitis and encephalitis, and to enhance laboratory capacity for the diagnosis of CNS infections. Cerebral spinal fluid (CSF) and acute and convalescent sera were collected for bacterial culture and real-time polymerase chain reaction (RT-PCR) testing for herpes simplex virus (HSV) types 1 and 2, mumps virus, enteroviruses, varicella zoster virus (VZV), *Streptococcus pneumoniae*, Haemophilus influenzae type B (Hib), and *Neisseria meningitidis.* Testing for West Nile virus (WNV), tick-borne encephalitis virus, and Eppstein-Barr virus (EBV) was conducted via ELISA. As of April 2012, 144 patients were enrolled. Of these, 44% were adults and 56% were children < 18 years of age. Female to male ratio was 1:1.14. The majority of the patients (75%) were from urban Tbilisi. In 89.7% of enrolled patients, the discharge diagnosis was meningitis and in 8.8% it was encephalitis. Of the meningitis cases, bacterial meningitis was the discharge diagnosis slightly more frequently than viral meningitis (52.8% and 43.4%, accordingly). *S. pneumonia* was cultured from CSF in five patients. One of the secondary study objectives was to measure the occurrence of HiB *following* the initiation of a nationwide vaccination campaign that began in January 2010, shortly before the initiation of this study. None of the patients were positive for HiB. In 140 CSF samples tested by PCR, enterovirus was the most frequently detected etiology (26%). There were three cases of VZV, one case of HSV-1, and two cases of EBV. Data from this ongoing hospital surveillance study provides valuable etiologic and epidemiologic information regarding viral and bacterial acute meningitis and encephalitis in Georgia.

1267

THIRD CASE OF FATAL YELLOW FEVER VACCINE-ASSOCIATED VISCEROTROPIC DISEASE IN A YOUNG PERUVIAN WOMAN

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Until 2001, yellow fever vaccine was considered to be the world's safest live vaccine. Since then 65 cases of Yellow Fever Vaccine-Associated Viscerotropic Disease (YEL-AVD) have been reported. YEL-AVD resembles yellow fever itself and is frequently fatal. The incidence based on a passive reporting system is 0.3 to 0.4/100,000. However, following an immunization campaign in Peru an incidence of 7.91/100,000 was observed. Known risk factors include age ≥60 and thymectomy as treatment for thymoma. More recently, women of childbearing age have been described as being at increased risk. Nine cases, all fatal, of YEL-AVD in young women have been reported including two from the above Peruvian campaign. Here we describe a third fatal case in a 24 year old Peruvian woman who was vaccinated in preparation for a trip to Australia. The incubation period, day of death following vaccination, and clinical course with multi-organ failure are similar to the other nine cases. RT-PCR demonstrated virus with 100% homology to vaccine virus in serum obtained on the 10th day post-vaccination. The viral load was 45 000 PFU/mL. Of interest is the evidence for concomitant leptospiral infection, lymphopenia, and family history suggesting that the death of a 2 year old male sibling was from meningo-encephalitis. The reasons for the apparent concentration of cases in young women in Peru are unclear. There was no apparent common ethnicity in the three young women. A genetic defect affecting immunity is a plausible explanation and should be investigated with additional research.

1268

UNDERSTANDING TRICHIASIS FROM THE PERSPECTIVE OF THE PATIENT: AN ASSESSMENT OF PREVIOUSLY OPERATED AND NEVER OPERATED PATIENTS TO IMPROVE QUALITY AND EFFICIENCY OF SURGICAL SERVICES IN ETHIOPIA, NIGER AND MALI

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Ethiopia, Niger, and Mali comprise 26% of the trichiasis burden in the developing world and reported 65% of the global trichiasis surgical output in 2009. In 2011, a study was conducted in each country to understand the trichiasis experience from the patient's perspective with the aim of improving the quality and efficiency of the surgical delivery system. In each country, districts were ranked according to surgery output over the past two years and villages selected at random from the top quartile. Within selected villages all operated and un-operated patients were interviewed to a maximum of 25 and additional villages visited until 192 operated patients had been interviewed. A pre-tested standardized questionnaire asked about demographics, knowledge of trichiasis treatments, health seeking behavior, and perception of surgery; a clinical eye exam was conducted by a trichiasis surgeon. A total of 683 operated and 227 never operated cases were interviewed: Ethiopia 296 and 120; Niger 193 and 35; and Mali 194 and 72, respectively. Among those previously operated, the most common reasons for having surgery were pain, fear of vision loss, and inability to work. Post-operative trichiasis was found in 28.7% of patients in Ethiopia, 33.7% in Niger, and 26.4% in Mali; most had minor trichiasis (<5 lashes). Most patients reported satisfaction with the surgery: Ethiopia, 86%; Niger, 93%; Mali, 92%; most had recommended the surgery to others, reported improvement in vision, and no longer felt pain in the operated eye. Of those never operated, knowledge of the opportunity to receive surgery ranged from 25.0% to 73.5%; however, over 80% reported they had never presented for surgery. Major trichiasis (>5 lashes) was common: Ethiopia, 34.5%; Niger 47.1%; Mali 36.1% . The majority had lived with trichiasis for three or more years and reported pain. Study results will be used to increase community mobilization and awareness about trichiasis surgical services, boost surgical access, improve the quality of surgery, and enhance the overall efficiency of the surgical delivery system.

1269

PATTERN OF ACUTE POISONING AND PREDICTION OF MORTALITY: A HOSPITAL BASED SURVEY IN DHAKA, BANGLADESH

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Poisoning is a major cause of morbidity and mortality worldwide. Different substances have variable degrees of toxicity from harmless to fatal. Treating physicians often do not know which poison has been taken. It is important to identify life threatening cases to priorities care appropriately. Few systematic investigations of poisoning have been done in the tropics. A large number of poisoning cases remains unreported due to lack of information and awareness at community level. This study collected baseline information and outcome of poisoning in Dhaka, Bangladesh. All cases of poisoning from 1/4/08-30/3/09 admitted to Dhaka Medical College Hospital (DMCH) were recruited for detailed observation. Details of clinical presentation, social background and outcome were recorded. In total, 5932 cases of poisoning in DMCH were enrolled of which 2108/5929 (35%) were female. Median age was 25 years (IQR 19-35 years). Major substances were Benzodiazepines (12%), including deliberate/"induced", organophosphates/carbamates (OPC) (12%), snake/fish/insect and medications. 36% took unknown substances. Suicidal attempt due to family disharmony was the commonest motivating cause (38%). Overall mortality was 151/5932 (2.6%) with 105/151 (70%) of deaths due to OPC (mortality 16%). Other causes of fatal poisoning included benzodiazepines, rat killer, animal/insect bites and stings, methanol, ethanol, herbal medicine and copper sulfate. Risk factors for mortality by univariate analysis were rural abode, hindu religion, illiterate, farmer, suicide attempt, accidental poisoning, GCS<9, BP<90/60mmHg, HR>100 or <60 bpm and abnormal pupils. Multivariate analysis found GCS<11 to be the best predictor of death with the addition of constricted pupils and non-muslim religion in the OPC group and systolic BP<80mmHg, economic loss/failure to pass an exam in the non-OPC group. A simple scoring system was derived using GCS and BP to predict mortality due to causes other than OPC. Poisoning is a common cause of medical admission in Bangladesh. A wide variety of substances are used. OPC poisoning is common and causes two thirds of the deaths. Those at high risk of fatal poisoning can be predicted from history and examination findings.

1270

RANDOMIZED, DOUBLE-BLINDED, PHASE 2 TRIAL OF WR 279,396 (PAROMOMYCIN AND GENTAMICIN) FOR THE TREATMENT OF CUTANEOUS LEISHMANIASIS CAUSED BY LEISHMANIA PERUVIANA

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In this randomized, double-blind, parallel-group trial, 30 Peruvian patients with parasitologically confirmed cutaneous leishmaniasis (CL) lesions received either WR 279,396 (15% paromomycin + 0.5% gentamicin; n=14) or Paromomycin Alone (15% paromomycin; n=16) topical cream applied once daily for 20 days. Patients were followed for pharmacokinetics (PK), safety, and efficacy for six months. Blood for paromomycin and gentamicin PK parameters was collected from adult subjects after the first days' and last days' drug application. The primary efficacy endpoint was cure of a parasitologically confirmed index lesion, defined as at least 50% reepithelialization of the lesion by Day 63 and 100% reepithelialization by Day 100 with no relapse. At 6 months, final clinical cure of an index lesion occurred in 9/14 (64.3%) subjects in the WR 279,306 group and 11/16 (68.8%) in the Paromomycin Alone group. In pediatric subjects, 9/10 (90%) cured with WR 279,396 and 9/11 (81.8%) with Paromomycin Alone. WR 279,396 appeared to have some benefit over Paromomycin alone as patient's lesions cured at a faster rate when treated with WR 279,396. At 6-months, efficacy of both WR 279,396 and Paromomycin Alone were comparable to historical data for first-line pentavalent antimonial treatments; WR 279,397 cured 5/5(100%) of subjects who had failed prior antimonial therapy. WR 279,396 and Paromomycin Alone creams produced only non-severe application site irritation, without systemic toxicity. PK data showed that there is limited paromomycin and gentamicin systemic absorption thus avoiding drug accumulation and toxicity. Either WR 279,396, or Paromomycin Alone may offer an advantage over first-line antimonial therapies for Peruvian CL, especially in children.

1271

TRICHIASIS SURGEON PRODUCTIVITY IN ETHIOPIA, MALI AND NIGER: WHAT IS NEEDED TO REACH ELIMINATION BY 2020?

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WHO, donors and NGOs are committed to supporting National Trachoma Programs reach their elimination goals by 2020. Trachoma programs train mid-level eye care personnel and general healthcare workers to perform trichiasis surgery during routine services and outreach campaigns. Although much effort has been put into training surgeons, few studies have examined their productivity. In 2011, a standardized questionnaire was administered by phone to currently active surgeons in Ethiopia, Mali and Niger. The questionnaire asked about demographics, work facilities, supervision, training, and surgeries performed the previous year. In addition, an external ophthalmologist collected qualitative data on selection of trainees, training and supervision. A total of 445 surgeons were interviewed: 191 in Ethiopia, 60 in Mali and 194 in Niger; the majority were male (71%). Most surgeons in Ethiopia were trained <5 years ago, while in Mali and Niger, the majority were trained ≥5 years ago. While nearly 75% of Ethiopian surgeons had been retrained, 1% in Mali and 9% in Niger had been. The mean number of surgeries performed per surgeon in 2010 was: Ethiopia, 76.4; Mali, 156.4; and Niger, 55.9. Most surgery was conducted in outreach: Ethiopia, 93%, Mali, 91%; and Niger 67%. Factors significantly associated ($p < 0.05$) with high productivity in Ethiopia included a higher proportion of time dedicated to eye care, no supervisory visit in the previous 6 months, and increasing years since training. In Mali, the only significant predictor was an increasing number of years since training, whilst in Niger predictors included percentage of time dedicated to eye care, being hospital-based, increasing years since training, and higher number of surgeries performed during training. Study results support that training dedicated eye care workers, improving the frequency and quality of supervision, providing refresher training, and

providing more outreach opportunities will support high productivity among trichiasis surgeons to enable national programs to meet their trachoma elimination targets.

1272

CUTANEOUS LEISHMANIASIS AND THE EFFICACY OF AZOLES, A SYSTEMATIC REVIEW

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Cutaneous Leishmaniasis (CL) is endemic to 70 countries with 1.5 - 2 million new cases occurring annually. The majority of cases occur in the Middle East (Old World) although the disease is endemic to parts of South America (New World) as well. Travelers and military personnel are at significant risk of acquiring the disease. Its disfiguring and ulcerative lesions result in a high degree of morbidity for those infected. Despite the wide spread distribution and number of cases, debate persists with regard to the most effective treatment for CL. Antimonials have been the mainstay of therapy, but toxicity and constraints with administering the drug make it difficult to use. In recent years, evidence has been building regarding the efficacy of azole antifungals. In order to better characterize the effectiveness of azole antifungals we conducted a systematic review for azole treatment of CL. The databases used were MEDLINE, EMBASE, and Cochrane Database of Systematic Reviews. Search terms included "cutaneous leishmaniasis," "skin leishmaniasis," "therapy," "treatment," "fluconazole," "posaconazole," "ketoconazole," "itraconazole," and "voriconazole." The references from primary studies, narrative reviews and systemic reviews were reviewed to search for additional primary studies that could have been missed by the electronic search. Two investigators independently screened all citations by title and abstract and made a decision on acceptance. Disagreements were resolved by a third author. Inclusion criteria included a confirmed diagnosis of CL, monotherapy with an azole, availability of azole dosage and duration, at least 2 months of follow-up, and at least 4 patients per study. The results of the systematic review will be presented including a breakdown of effectiveness and side-effects of each azole. The aggregated data supports certain azoles as a choice for treating CL. An alogorithmic approach to the treatment of CL is provided.

1273

BURDEN OF SEVERE DISEASES IN THE FIRST THREE YEARS OF LIFE AMONG A BIRTH COHORT OF 1198 CHILDREN BETWEEN AGES 14 WEEKS AND THREE YEARS IN NORTHERN GHANA

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Reducing under-five mortality by two thirds between 1990 and 2015 according to the Mellenium Development Goals is still quite elusive and efforts to control the cause of the deaths, namely, pneumonia, diarrhea, malaria and malnutrition are being revitalized. Most of these deaths occur in areas with difficulty in gathering accurate data and estimates are the only available means of assessing progress. This study assesses the incidence of serious illness episodes among children aged 14 weeks to 3 years in a cohort of 1198 children recruited into a meningitis vaccine trial in the Kassena Nankana districts in northern Ghana from November 2008 to March 2011. Surveillance teams were set up in all the communities from where children were recruited as well as the health facilities. Serious illness was assessed according to good clinical practices and according to standard clinical criteria. A substantial number had more than one episode of illness during the period under study. Serious illness events were seasonal with over 95% due to infectious diseases. For incidence of severe illness between 14 weeks of age and 3 years, 38.3% of participants experienced at least one episode and the major causes were malaria, acute gastroenteritis and pneumonia which were 20.2%, 7.4% and 2.1% respectively. Proportion of participants who experienced at least one episode of severe illness in the first and second years of life was respectively 130(10.9%) and 175 (16.2%). In the third year of life, 103(9.7%) of participants recorded severe illness and main cause was only malaria with the others being minimal. In all 21(1.8%) of participants died over the 3 years and the main causes of death were respiratory tract infections, malaria and acute gastroenteritis This study confirms a huge burden of preventable infectious diseases among this young age group, where more has to be done in terms of prevention. The relatively low resultant mortality presents a ray of hope that while putting in measures to prevent illnesses, affordable and effective services could be provided to control mortality in children in these age groups in areas of low illiteracy and resource constraint.

1274

MULTILEVEL ANALYSIS OF TRICHIASIS AND CORNEAL OPACITY IN NIGERIA: THE ROLE OF ENVIRONMENTAL RISK FACTORS ON THE DISTRIBUTION OF DISEASE

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The distribution of trachoma in Nigeria is spatially heterogeneous, with the prevalence generally decreasing in a North-South gradient across the country at a larger scale and more local variation observed within these areas. Relative contributions of individual and environmental risk factors to the geographic distribution of disease remain largely unknown. The primary aim of this analysis is to assess the relationship between climatic factors and trachomatous trichiasis (TT) and/or corneal opacity (CO) due to trachoma in Nigeria, while accounting for the effects of clustering and risk factors at other levels. In addition, we explore the relative importance of clustering at different levels and the respective role of individual and environmental factors on these outcomes. Data from the 2007 National Blindness and Visual Impairment Survey were used for this analysis, which included a nationally representative sample of adults aged 40 and above. Data were available from 305 clusters selected using a multistage stratified cluster random sampling strategy. A basic eye examination was given to all participants and the presence or absence of TT and CO recorded. In addition to field-collected data on individual-level variables, remotely sensed climatic data were extracted for each cluster and used to fit Bayesian hierarchical logistic models to disease outcome. As expected, clustering was apparent at both levels in the model and there was evidence that climatic factors independently contribute to increased risk of TT/ CO after accounting for available individual level risk factors. Beyond some well established individual risk factors (age, gender and occupation), there was strong evidence that environmental factors at the cluster-level (aridity, precipitation and global land cover) were also associated with the prevalence of TT/ CO. This study establishes the importance of large scale geographical risk factors for later stages of trachoma, which confirms anecdotal evidence that environmental conditions are associated with increased risk of these outcomes, and highlights potential uses of risk mapping to better estimate their burden.

1275

DEMOGRAPHIC AND CLINICAL RISK FACTORS FOR LASSA FEVER IN SIERRA LEONE

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Lassa Virus (LASV) is the etiologic agent of Lassa fever (LF), an acute and frequently fatal illness endemic to West Africa. Multimammate rats (*Mastomys natalensis*) are the reservoir and are found in abundance throughout sub-Saharan Africa. LF is hyperendemic in the Eastern Province of Sierra Leone where humans are thought to acquire infection via exposure to rodent excreta. The National Lassa Fever Surveillance Program of Sierra Leone is headquartered at the Kenema Government Hospital in Kenema, Sierra Leone. All patients who present to the Lassa Fever Ward and meet criteria for suspected LF cases are evaluated using standard forms. Detailed records are kept of presenting signs and symptoms, diagnostic test results for LF, hospital course and outcome. Blood samples from all patients are tested for the presence of LASV NP antigen at the time of presentation by a recombinant-based antigen capture ELISA. Over a 25 month period (January 2010 through January 2012), 1,158 patients presenting to the Lassa Fever Ward met criteria for suspected LF. Ninety-nine (8.5%) patients tested positive for LASV antigen. Patients with an antigen positive ELISA were more likely to present with bleeding, conjunctival injection, facial or neck edema, sore throat, cough, and confusion relative to those with other diseases. In addition, patients presenting greater than 7 days after onset of illness were more likely to have LF. Outcome was known for 96 patients with LF and 230 patients with other diseases. Having LF was strongly associated with a fatal outcome (OR 4.1 CI 2.4-7.0) with a fatality rate of 64%. A fatal outcome was associated with bleeding and presenting seven or more days after disease onset.

1276

CLINICAL PREDICTORS OF HOSPITAL READMISSION IN UGANDAN CHILDREN WITH CEREBRAL MALARIA

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Cerebral malaria (CM) affects more than 800,000 children each year in sub-Saharan Africa. Determination of clinical symptoms associated with hospital re-admission for children treated for cerebral malaria could help identify those CM children at greatest risk for severe morbidity and mortality. We conducted a study on the pathogenesis of cerebral malaria, and in this substudy, aimed to determine the clinical symptoms associated with greater risk of admission to the hospital in the first 6 months post-discharge in a cohort of Ugandan children aged 18 mo - 12 years. Clinical risk factors were assessed in 165 Ugandan children presenting to the Pediatric Acute Care Unit at Mulago Hospital in Kampala, Uganda, with cerebral malaria who completed 6 months of clinical follow-up. Twenty children (12.1%) were readmitted to the hospital for malaria during 6-month follow-up. Compared to children with CM who were not readmitted, CM children who were readmitted had a higher frequency of measured fever (T≥ 37.5, 85.0% vs. 57.9%; P =0.01) and lactic acidosis (blood lactate > 5.5 mmol/L, 60% vs. 27%, P=0.008) on admission, and were less likely to have received antibiotics during their initial stay at the hospital (55% vs. 80%, P=0.02). Measured fever and lactic acidosis on admission and lack of antibiotics during hospital stay predict risk of readmission in children with CM.

1277

HEALTH SYSTEM STRENGTHENING THROUGH COMMUNITY REFERRAL IN THE MANAGEMENT OF FEBRILE ILLNESS IN NIGERIA

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Use of Community Health Workers (CHWs) in community case management of febrile illness can improve community-clinic continuum of care, health outcomes and referral system. The main objective of this study is to ascertain the level of home visitation carried out by the CHWs, compliance rate for referrals and treatment response. The authors carried out a record review of 12 months of community registers to ascertain the level of home visitation. To determine compliance to referral, all referral slips and clients' cards at the six primary health care centers participating in the on-going Integrated Community case Management of Malaria were assessed. The CHWs made a cumulative overall home visits of 7,282 to pregnant women 4460 (61.2%) and children under-five years of age 2822 (38.8%). The median visitation for pregnant women was 406 compared to children under-five years of age 257. Overall referral was 578; pregnant women 332(57.4%) while children under-five years of age 246(42.6%). The overall median referral was 28; pregnant women (19) compared to children under-five years of age (9). Overall referral compliance rate was 79.1% (457/578) with pregnant women 73.2% (245/332) compared to children under-five years 86.2% (212/246). Median number of days for pregnant women to comply with referral was 4 compared to children under-five years of age 1.5 days. Reasons for referral for pregnant women, ANC attendance topped the list 78.4 %(192/245); malaria treatment 30.6% (75/245) and reactions to medicines Sulfadoxine-pyrimethamine 2.8% (7/245) and Artemisinin Combination Therapy 3(1.2%) while Children under-five years of age malaria treatment topped the list 60.8% (129/212); diarrhea treatment 23.6% (50/212); pneumonia treatment 14.6% (31/212) and reactions to ACT 0.94% (2/212). All cases were treated same day at the health facility. In conclusion we found relatively high compliance in community referral, and care-givers of children under-five years of age are more likely to comply with referral and very early too than pregnant women. Community health education on referral during pregnancy as a component of case management of febrile illness is recommended for program managers and implementers.

1278

SIGNS AND SYMPTOMS AS INDICATORS OF FEMALE GENITAL SCHISTOSOMIASIS

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Female Genital Schistosomiasis (FGS) is a neglected, poverty-related disease. Several studies also indicate higher prevalence of HIV in women with FGS. The co-existence of FGS and sexually transmitted infections (STIs) pose a diagnostic challenge for health care providers. The syndromatic management of STIs are strategies in disease prevention in developing countries. In spite of its public health implications, FGS has never been included in any of these protocols. It is therefore important to explore how self-reported symptoms, signs and behavior can be used as indicators of FGS. A school based, cluster randomized, cross-sectional study was conducted in a *Schistosoma haematobium* endemic area in rural South Africa. A total of 921 young women aged 16-22

were included. They were interviewed and asked about symptoms and behavior and gynecological examination with colposcopy was done. Samples (urine, blood and vaginal lavage) for laboratory analyses for STIs and *S. haematobium* were collected. Girls infected with schistosomiasis (cases) were compared with girls without schistosomiasis (controls). Multivariate regression was used for the statistical analyses. Female genital schistosomiasis may be a differential diagnosis to STIs in schistosomiasis endemic areas. It is of importance that health care workers consider this when adequate laboratory facilities are lacking. Symptoms could be added into an algorithm for a syndromatic approach to diagnosis, the meager effect of treatment and reinfection in this age group will be discussed.

1279

HIV INCIDENCE IN TEENAGE YOUNG WOMEN IN A SCHISTOSOMIASIS ENDEMIC AREA

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Sub-Saharan Africa is severely affected by the HIV (Human Immunodeficiency Virus) epidemic with prevalence higher in women than in men. Young women may be especially prone to HIV infection, but there is still limited knowledge about the reasons for the high prevalence in females. Studies indicate that Female Genital Schistosomiasis (FGS) could be a risk factor. In rural KwaZulu-Natal, South Africa, an area endemic of both *Schistosoma haematobium* and HIV, school-going, sexually active young women were examined twice with an interval of approximately one year. Mean age at first visit was 18.7 years. On both visits the participants went through a detailed interview, including questions regarding water contact, age at sexual debut, alcohol consumption and number of partners. Blood and urine samples were collected, and they were offered a gynecological examination including photocolposcopy. HIV positive and seroconverting young women were compared to the HIV negative individuals. Twenty five high schools of differing schistosomiasis prevalence were included, 921 young women were investigated. Mean age at sexual debut was 16.4 years and 95% reported to have a steady partner when interviewed at first visit. Mean number of lifetime sexual partners was 2 in both groups. The overall HIV incidence was 11.7% and 25% had a genital sandy patches indicating FGS on gynecological examination. The HIV incidence in this schistosomiasis endemic area was unusually high for this age group, however one common risk factor could not be identified. Further analyses for confounders are required. These findings may have implications for the understanding of FGS' role in the different phases of the HIV epidemic.

1280

COMPUTERIZED IMAGE ANALYSIS AS A TOOL FOR IDENTIFICATION OF CLINICAL MANIFESTATIONS IN FEMALE GENITAL SCHISTOSOMIASIS

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The lesions associated with Female genital schistosomiasis (FGS) consist of changes in the genital mucosa that may be described as sandy patches, characterised by their yellow colour. The gold standard for diagnosing FGS is a biopsy from cervical lesions with direct microscopic inspection for ova. However, this is an inappropriate approach in HIV endemic areas. It is therefore necessary to develop alternative methods for non-invasive, objective diagnosis of FGS that can be performed at the point of care without requiring advanced laboratory equipment or training. The image material was acquired in a study on female genital schistosomiasis in KwaZulu-Natal, South Africa. Healthy individuals were used as negative controls. The mean colour of sandy patches was measured in a subsample of colposcopic images. The colour was represented in a range of colour spaces and compared to the values of the surrounding mucosa using the Wilcoxon signed rank test for paired samples. 7 colour channels were chosen based on the significance level in the Wilcoxon test. The mean differences were used to calculate the most appropriate threshold window in each channel. An algorithm was created in which an image is scored based on presence of pixels present in the intersection of the 7 threshold windows. The validity of the algorithm was tested by running it on a random selection of images in which 3 clinicians had agreed on the diagnosis. It was calculated that 69 pathologic images and as many controls would provide statistical significance when assuming sensitivity of 80% and specificity of 65%. This is a novel method in which computerized image analysis can be used to identify genital schistosomiasis based on the lesions' distinct colour. Further analyses should be done exploring other visual aspects of the lesions, such as morphologic features. It is also necessary to control for confounding factors such as STIs and development is required to adapt this method to socially acceptable clinical practice in a third world setting.

1281

SUCCESSES AND SHORTCOMINGS OF POLIO ERADICATION: A TRANSMISSION MODELING ANALYSIS

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Polio eradication is on the cusp of success with only a few regions still maintaining transmission. Improving our understanding of why some regions have been successful and others have not will help both with global eradication of polio and with development of more effective vaccination strategies for other pathogens. To examine past eradication efforts we constructed a transmission model for wild poliovirus incorporating waning immunity, age-mediated vaccination rates, and transmission of oral polio vaccine (OPV). The model produces results consistent with the four country categories defined by the Global Polio Eradication Program: elimination with no subsequent outbreaks; elimination with subsequent transient outbreaks; elimination with detected transmission for more than 12 months; and endemic polio transmission. An analysis of waning immunity rates and OPV transmissibility suggest contrasting effects on transmission. Higher waning immunity rates make eradication harder due to increasing numbers of infectious adults. Higher OPV transmission rates make eradication easier as adults become re-immunized. Given these dynamic properties, attention should be given to intervention strategies that complement childhood vaccination. For example, improvement in sanitation can reduce the reproduction number in problematic regions, while adult vaccination can lower adult transmission.

1282

SUPPORTING THE DEVELOPMENT OF RESEARCHERS IN LOW AND MIDDLE INCOME COUNTRIES IN AFRICA THROUGH PERSONAL DEVELOPMENT PLANNING AND FORMAL MENTORING

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Building a critical mass of researchers in low and middle income countries, who are able to conduct and disseminate high quality research efficiently and effectively, and be able to use results to inform policy and practice in global health is an expectation of funders and institutions. Whilst attention is given to investing in developing the research skills of individuals through fellowship programmes, strengthening systems, infrastructure and institutions, less attention is given to the career planning and

development that is needed to help early-career researchers in these settings become sufficiently established, in order to meet this expectation. Personal Development Planning (PDP) and formal mentoring have been used to support a group of returning African researchers with their career development. The structured and supported process of PDP, assisting in empowering individuals to take ownership of their careers and develop the higher level critical thinking and reflective skills crucial to effective learning, is complemented by formal mentoring where mentors help mentees build confidence towards independence. A participatory action research approach was used to trial and explore how PDP (not used before in this context in sub-Saharan Africa) might help this group of researchers with their career development; in addition to developing an evidence-led PDP model and tools that would work for researchers in Africa. Results showed skills and knowledge gains in research methodology, techniques, communication, networking, updating clinical skills, and developing academic management skills; as well as how these gains were applied effectively in practice. With this same group of researchers, and through a self-selection process of mentees choosing their mentors, a formal mentoring programme was implemented. Whilst mentoring is a long-term process where results and benefits are not always seen immediately, initial results showed that 98% of mentees felt that their mentoring relationship was helping them to progress in their careers, and 85% of mentors were happy with their mentee's progress over the first year. Efforts to make these activities sustainable focus on working with institutions to mentor support groups in PDP and mentoring, and with the aim of embedding these strategies within institutions and programmes. This has the added value of building a network of PDP champions and mentors in Africa.

1283

GETTING HEALTH CARE DELIVERY RIGHT: LEARNING FROM CASE STUDIES

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The future of global health lies in getting delivery right. Medical schools must prepare the next generation of leaders to confront the management challenges of disease and health programs facing an implementation bottleneck. To aid in this endeavor, we created over 25 teaching cases with accompanying teaching notes offering unique lessons and insights to the principles of global health strategy. From this new body of delivery experience, several themes have emerged: 1) context matters_ programs must account for local factors that influence both the health of the populations and the delivery of health care in their design, implementation, and operations; 2) value_the outcomes achieved divided by the resources invested_is the best measure of program performance, and analysis of value using the care delivery value chain can help program managers determine how best to allocate resources and configure program activities; 3) high-value programs address the social, economic, and geographic barriers to health care delivery; they do not see their objective as "offering" services or technology but as ensuring that the population can realize the full value of the services or technology they are providing; 4) measurement should lead to meaningful learning and program improvement; 5) strategy and leadership are essential as managers face shifts in the landscape and increasing burden of disease. The global health delivery case studies are available free of charge via www.ghdonline.org/cases and provide a tool for educators to build global health delivery competency for the next generation of leaders.

1284

COMMUNITY HEALTH WORKERS FOR HOME-BASED COUNSELLING AT HOME TO IMPROVE NEONATAL SURVIVAL

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Access to maternal and newborn health services in developing countries is impeded by shortages in human resources for health. We set out to study whether home-based counselling by community volunteers could change home behaviours critical to newborn survival. The objective of the study was to develop and evaluate a community intervention using village-based volunteers to improve newborn care at home. The method included a formative research involving a review of behaviours that impact on neonatal survival, a baseline survey to assess their prevalence, and qualitative work to assess barriers and facilitators of behaviour change. Key messages for home visits in pregnancy and the early newborn period were agreed with stakeholders based on the findings of the formative research, and focus on early and exclusive breastfeeding, clean delivery, and extra care for low birth-weight babies born at home. Newborn foot size was used as a proxy for birth weight to identify low birth-weight babies born at home. In 2010, over 800 volunteers were trained by district health teams, in a randomly chosen 61 of the 131 wards in the 6-district study area. Supervision was improved to involve community leader and nearby health facility staffs. In 2011, a 5,000-household survey interviewed women aged 13-49 about behaviours critical to newborn survival in control and intervention areas. The results showed that over 75% of women in intervention areas were visited by a volunteer at least once in pregnancy, and almost half received a post-natal visit at home. Key behaviours improved as a result of the intervention: tying the cord with clean thread (70% vs 39%, P=0.002), delayed bathing until 6h or more after birth (81% vs 68%, P=0.005), feeding only breast milk for the first 3 days (83% vs 71%, P=0.001), and putting nothing on the cord (87% vs 71%, P<0.001). In conclusion, home-based counselling has improved behaviours critical to newborn survival.

1285

CLINICAL TRIALS OF THE MEN A CONJUGATE VACCINE CONDUCTED IN WEST AFRICA AND INDIA AMONG INFANTS, CHILDREN AND ADULTS: SHARING ETHICAL CHALLENGES AND LESSONS FOR THE FUTURE

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Application of international ethical guidelines in order to obtain approvals when conducting vaccine trials in diverse local settings can be quite challenging. Since 2005, Meningitis Vaccine Project (a partnership between the World Health Organization and the Program for Appropriate Technologies in Health) in collaboration with the Serum Institute of India has conducted clinical trials on Men A conjugate vaccine across a diverse set of clinical trial sites located in sub-urban and rural communities in India, Mali, The Gambia, Ghana and Senegal. Our collaboration with international, national and local ethics review committees led to the accumulation of huge expereinces on ethical research practices covering aspects of protocol approvals, language and communication in informed

consent, establishing processes for pregnancy testing, supporting health care, obtaining permission and providing feedback to participating communities.

1286

ENHANCING ACCESS TO MEDICINES THROUGH INNOVATIONS IN WORKING CAPITAL FINANCING FOR DRUG SHOPS

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Access to working capital within the different layers of the healthcare distribution network is one of the factors that limit widespread availability of key medicines and also hampers the sustainability of accredited medicine retailers in sub Saharan Africa. In OECD countries a well functioning credit provisioning system exists across the multiple entities involved in pharmaceutical distribution. Such a credit provisioning system is currently lacking in the private sector pharmaceutical distribution networks in low-income countries. The Accredited Drug Dispensing Outlets (ADDOs) in Tanzania is a very successful model for improving access to high quality medicines and similar models are now being planned in Uganda, Zambia and other countries. To ensure greater sustainability of the accredited drug shop business model and to further enhance its ability to increase access to medicines, a cross-sectional study design was employed using a comprehensive survey instrument developed for the study population of ADDO and ADS owners. The study assessed the cash-to-cash cycle, stocking practices and role of working capital credit within the accredited drug shop network. Findings revealed that accredited drug shops struggle to offer the most appropriate stock assortment at optimal levels. Analysis suggests that both drug shop owners and public health in the community would benefit from better training of shop owners on inventory and cash management and from the provision of additional working capital to the ADDO owners.

1287

DENGUE RISK PERCEPTION AND BEHAVIORAL RESPONSES BY LOCAL MEMBERS IN DHAKA, BANGLADESH

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Since 2000, there has been a resurgence in dengue virus in the major cities of Bangladesh. This study aimed to assess the risk perception and mitigation efforts towards dengue by analyzing entomological and socioeconomic risk factors in 12 wards within the City of Dhaka, Bangladesh. Data included in the analysis are: a) two vector surveys [i.e., pupal surveys conducted in 847 households (monsoon season 2011) and 459 households (dry season 2012)]; b) a socio-demographic survey of 300 households; c) 12 focus group discussions (FGDs) and eight key informant interviews (KIIs); and d) constructed Knowledge Models of experts and lay persons. Competent dengue vectors were detected in >40% and 12% of households during the monsoon and dry seasons respectively. The monsoon and dry seasonal pupal index were 0.40 and 0.33 respectively for the selected 12 wards. Vector indices were significantly higher for *Aedes aegypti* in this study compared to others conducted in Dhaka in the past. There are significant variations in dengue risk perception between lower (low and medium) and higher socioeconomic groups (SEG). The low and medium SEGs are concerned more about day-to-day issues than exposure to dengue whereas the higher SEG considered themselves at higher risk of dengue infection. Perceived risk from exposure to dengue virus was lower in female subjects than males. Also, experts ranked dengue risk at a much lower level than lay persons and experts emphasized the need for stronger institutional measures to control dengue outbreaks. These findings in turn signify the link between disease risk perception and preventive responses. In consideration of significant SEG and gender variations, targeted education campaigns, vector control and community mobilization programs should be formulated to mitigate the risk of dengue in Bangladesh.

1288

INCIDENCE, RISK FACTORS, AND COSTS FOR HOSPITALIZATION OF NEONATES BORN TO MOTHERS IN A MATERNAL IMMUNIZATION TRIAL IN BAMAKO, MALI

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Low birth weight (LBW) and prematurity are major causes of neonatal and infant morbidity and mortality worldwide, threatening the achievement of Millennium Development Goal 4. We present the incidences and hospitalization rates of LBW and prematurity among a cohort of infants born to mothers enrolled in a maternal immunization trial, as well as the first cost-analysis of hospitalizations among LBW and/or premature neonates in Bamako, Mali. Women recruited from antenatal care clinics during the 3rd trimester and enrolled after obtaining informed consent were randomly allocated to receive inactivated influenza vaccine (Vaxigrip™, Sanofi Pasteur) or quadrivalent meningococcal conjugate vaccine (Menactra™, Sanofi Pasteur), and followed thru 6 months postpartum. LBW was defined as <2.5kg measured with an infant scale prior to discharge from the maternity center. Prematurity was defined as gestational age <37 weeks by 1st trimester ultrasound if available or otherwise a Ballard exam in the first 7 days of life. All neonatal hospitalizations were identified by 24-hour surveillance. Direct and indirect costs incurred by any hospitalized neonate meeting definitions for LBW or prematurity were recorded daily. From September 2011 thru March 2012, there were 652 liveborn infants, of whom 3.4% were premature and 10.9% were LBW. The rate of hospitalization or death during the 1st month of life was 4.0% among all infants, compared to 17.7% for newborns <2.5kg (RR: 8.2, 95% CI: 3.9-16.8) and 36.4% for newborns <37 weeks (RR: 13.4, 95% CI: 6.3-26.2). Complete cost data were available for 22 hospitalized infants, of whom 14 were LBW and premature, 7 were LBW only, and 1 was premature only. The median duration of hospitalization was 7 days (IQR: 5-11), with a median cost of 94.44 USD (IQR 56.43-157.25). Direct expenditures accounted for 79% of all costs, with medication purchases responsible for the majority. In addition to mortality, prematurity and LBW cause substantial morbidity and economic hardship for families in Mali. Interventions that can reduce the risk for prematurity or LBW are urgently needed.

1289

CAN COMMUNITY HEALTH WORKERS PROVIDE QUALITY INTEGRATED COMMUNITY MANAGEMENT OF FEBRILE ILLNESSES: A CASE STUDY OF COMMUNITY HEALTH WORKERS IN TWO SELECTED LOCAL GOVERNMENT AREAS OF AKWA IBOM STATE, NIGERIA

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The World Health Organization has recommended improved quality of care as key elements in strengthening health systems in poor resource countries, Engagement of Community Health Workers (CHWs) can reduce challenges such as weak public sector, human resource constraints, and variable quality of the private sector. Efforts to improve access to quality case management of febrile illness in Nigeria included the engagement of Community Health Workers (CHWs) to use Rapid Diagnostic tests as a component of home management of malaria, dispense ACTs and manage pneumonia and diarrhea. This current effort monitored and measured the performance of CHWs in providing quality management of febrile illnesses in two selected LGAs. The authors trained one hundred and fifty-two CHWs and developed simple quality performance standards (one-page tool) for CHWs providing community services in Akwa Ibom State, Nigeria. All 152 trained CHWs providing malaria, pneumonia and diarrhea case management were monitored and assessed using the standards. The tool has 37 performance criteria (PC) to measure CHW knowledge, skills and competence in 3 sections: History taking and Examination; Conducting RDTs for Malaria; and Illness Management. Trained assessors observed CHWs providing services. Each correctly performed criterion was scored 1 point. Four rounds of assessments were conducted at an interval of two months from June 2011 - March, 2012. During Round 1 CHWs achieved an average of 19 (52.2%) PC. This rose to 25 (67.5%) PC at Round 2; 28 (75. 6%) at Round 3 and 30 (81.1%) and ($p = 0.00$). PC that needed most improvement included reinforcement on checking RDT expiry date, entering results on records, and safe disposing of sharps. CHWs can provide quality case management of febrile illness in the current efforts to reduce annual deaths of people at risk while contributing to the achievement of targets numbers 4, 5 and 6 of the Millennium Development Goals (MDGs). In conclusion CHW supervisors can use this tool to enhance the quality of services provided by the CHWs and improve CHW training.

1290

REGULATION OF VACCINES TO PROTECT AGAINST GLOBAL INFECTIOUS DISEASES: A ROADMAP TO WORKING WITH THE UNITED STATES FOOD AND DRUG ADMINISTRATION

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Global infectious diseases (GID) such as tuberculosis, malaria, dengue and hookworm affect more than one billion people worldwide. The development of safe and effective vaccines for the prevention of these diseases is of critical importance not only for global humanitarian reasons but also for United States (U.S.) public health. The submission of an investigational new drug application (IND) for a vaccine or biologic to the US FDA can provide sponsors with important scientific and regulatory advice on products that are critical to the advancement of world health. If pursued, U.S. licensure signifies to the global medical and regulatory community that the FDA has made the determination that the vaccine is safe and effective. This finding by the FDA may assist other National Regulatory Authorities in their evaluation of the vaccine. The US FDA recently updated the Guidelines for the Development of Vaccines to Protect Against Global Infectious Diseases. This presentation aims to introduce vaccine developers to these recently updated recommendations and to the regulatory review process at the Division of Vaccines and Related Products Applications (DVRPA) in the Office of Vaccine Research and Review at the Center for Biologics Evaluation and Research (CBER), U.S. FDA. The following issues will be discussed: acceptability and utility of non U.S. studies to support product licensure; the use of clinical bridging studies and how these data may be used to determine interregional acceptance of foreign data; safety monitoring during international vaccine clinical trials. Regulatory issues in the manufacture and pre-clinical testing of new vaccines for global health will be presented. Finally, general principles pertaining to evaluation of vaccine safety and effectiveness, and common concerns related to vaccine manufacturing submissions will be reviewed.

1291

THE WORLD INTELLECTUAL PROPERTY ORGANIZATION (WIPO RE:SEARCH) PARTNERSHIP HUB: GENERATING NEW COLLABORATION OPPORTUNITIES TO ACCELERATE NEGLECTED TROPICAL DISEASE R&D

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Collaborations are a key mechanism to more effectively and efficiently discover and develop new drugs, vaccines, and diagnostics to help the more than 1 billion people suffering from neglected tropical diseases (NTDs), malaria, and tuberculosis. Recognizing the need for more progress in neglected disease research, the WIPO Re:Search Consortium was formed in October 2011. The World Intellectual Property Organization (WIPO) in partnership with BIO Ventures for Global Health and several of the world's leading pharmaceutical companies, renowned academic and other neglected disease research organizations provide access to intellectual property for pharmaceutical compounds, compound libraries, technologies, and importantly expertise and knowledge to support research and development for NTDs, malaria and tuberculosis. WIPO Re:Search aims to expand the number of drug, vaccine, and diagnostic technology candidates for NTDs by sharing these valuable resources and knowledge to accelerate product development. The WIPO Re:Search Partnership Hub facilitates research collaborations among WIPO Re:Search members by fielding requests for specific targets or compounds of interest, identifying collaboration opportunities among key biopharmaceutical and neglected disease research institutions, and providing scientific expertise to proactively match contributions in WIPO Re:Search with members' research program needs. The Partnership Hub establishes mutual interest in exploring a collaboration opportunity and then connects members so that scientists can discuss their research and collaborate. This presentation will focus on the WIPO Re:Search Partnership Hub as an innovative model in global health. We will explain how the Hub has facilitated successful collaborations, and will highlight the impact that the Partnership Hub model has had in accelerating product development for NTDs, malaria, and tuberculosis.

1292

THE DRUG DRUG INHIBITION POTENTIAL OF ANTI-MALARIAL AGENTS

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Therapeutic regimes for malaria involve the co-administration of two or more compounds. The patient can also have additional therapy of which HIV therapy is an example. These combinations have the potential to cause drug-drug interactions (DDIs) leading to drug exposures that vary from that intended. This can be critical when determining combination partners and if the drugs in question have narrow therapeutic indices. A weakness of published IC_{50}s is that they are dependent on experimental

conditions which are often not comparable or able to be transformed into Ki values. The inhibition of the following CYP450 isoforms - 1A, 2B6, 2C8, 2C9, 2C19, 2D6, and 3A4 - were investigated using the metabolism of their specific substrates. Mefloquine, piperaquine, pyronaridine, OZ439, naphthoquine, dihydroartemisinin, primaquine, amodiaquine, chloroquine and lumefantrine were the marketed or MMV proprietary drugs nvestigated. They were investigated in concentration response experiments where the test compound (0.1 µM - 25 µM) was incubated with human liver microsomes and NADPH in the presence of a cytochrome P450 isoform-specific probe substrate. Potent inhibition was considered as IC_{50} < 1 µM, moderate inhibition was considered as IC50 between 1 and 10 µM, and no or weak inhibition was considered as IC_{50} > 10 µM. All of the isoforms tested were inhibited by at least one of the test compounds except for 2C9 which was not inhibited. The majority of the inhibition observed was determined to be moderate except for 1A where primaquine caused potent inhibition. The most affected isoform was 2D6 (4/10 compounds inhibited). Piperaquine, OZ439 and lumefantrine did not cause any inhibition of the isoforms tested. This data can be used as a first guide in forming antimalarial combinations and when combining several therapeutic approaches. Further work will be done to augment these results. This will include widening the set of compounds tested, determining the metabolic pathways of the pathways tested and determining Ki values for inhibition where the IC_{50} values warrant it.

1293

MATHEMATICAL MODELING OF THE EFFECTS OF DRUGS ON MALARIA TRANSMISSION IN LOW TRANSMISSION AREAS

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Malaria eradication is now the ultimate objective of many organizations, including the Roll Back Malaria Partnership, the Bill and Melinda Gates Foundation, and the Global Fund. Achieving this objective will require utilizing the three pillars of malaria control: insecticide-treated bed nets, spraying, and antimalarial drugs. However, the impacts of drugs on malaria transmission are not yet fully understood. We describe the development and application of a new mathematical modeling framework to simulate the effects of dug treatment on transmission in low transmission settings. We find that the addition of gametocytocidal drugs to standard treatment regimens may play an important role in reducing transmission. However, we find that the reductions are not large when only symptomatic individuals are treated. Further, the reductions in transmission from the addition of gametocytocidal drugs can be achieved by other means, such as increasing the fraction of individuals treated and reducing the time to treat. These three methods of reducing transmission can be combined, depending on context of malaria transmission in an area. We also present preliminary results using our modeling framework to predict the effects of artemisinin resistance on treatment outcomes with a variety of artemisinin-based therapies.

1294

HEMATOLOGIC COMPLICATIONS AFTER INTRAVENOUS ARTESUNATE IN PATIENTS WITH SEVERE MALARIA

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Fast acting anti-malarials are essential to treat severe malaria. Existing evidence shows that intravenous artesunate is significantly more effective in parasite clearance but also with respect to survival compared to quinine. Clinical benefits of artesunate appear to be most prominent in patients with high parasitemia. However, previously unknown complications like delayed hemolysis has been described in an increasing number of patients with imported severe malaria. There appears to be an association of post-treatment hemolysis with high parasite levels. We have seen similar hematologic complications after treating three patients with imported severe malaria during a prospective follow-up in our center in Hamburg, Germany. Post-treatment hemolysis occurred in all patients and reached its peak around 14 days after initiating intravenous artesunate. In addition to signs of hemolysis like a second rise in LDH levels, there was a low reticulocyte production index in all patients indicating prolonged impairment of erythropoiesis. We are currently investigating this relevant complication in children with severe malaria in Africa. In addition, pathophysiologic analyses including murine models are underway. Evidence of post-treatment hemolysis, potenial pathogenesis and clinical relevance both for imported as well as endemic severe malaria are discussed.

1295

TRENDS IN U.S. MILITARY HEALTH SYSTEM (MHS) MALARIA CHEMOPROPHYLAXIS PRESCRIBING PATTERNS FROM 2007-2011

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No systematic reviews describing malaria chemoprophylaxis prescription trends within U.S. primary care settings have been conducted. The MHS, with its 9.7 million beneficiaries, represents an enormous pool of potential travelers to be considered for malaria prevention measures. Military force health protection policies target active duty forces but may affect all beneficiaries if providers implement policy guidance comprehensively. In 2009, the Department of Defense released malaria prophylaxis policy guidance limiting the use of mefloquine in deployed personnel. A systematic search of the MHS electronic medical record was performed for all prescriptions of atovaquone-proguanil (AP), chloroquine (CQ), doxycycline (DC), and mefloquine (MQ) to adult patients from 2007-2011. For CQ and DC, search parameters were filtered to target malaria chemoprophylaxis. Absolute and proportional prescribing rates for the total, active duty (AD) and dependent/retiree (DR) populations were assessed for changes over time. Trends for prescriptions originating from primary care (PC) clinics versus specialty travel (ST) clinics were also compared. A total of 624,416 prescriptions (AP 7%, CQ 3%, DC 76%, MQ 14%) were identified during the study period, including 156,150 DR patients (25%). Prescription volume rose from 64K (AP 11%, CQ 8%, DC 45%, MQ 36%) in 2007 to 180K (AP 6%, CQ 1%, DC 89%, MQ 4%) in 2011, with DC representing the majority of the increase ($p<0.001$). MQ use diminished in all clinics over time. Whereas ST clinics predominantly and increasingly prescribed AP (58% in 2007 and 71% in 2011), PC clinics predominantly and increasingly prescribed DC (54% in 2007 and 96% in 2011). Trends were similar for AD and DR populations, suggesting that health policies influence prescription practices in both groups. This study is the first longitudinal systematic review of malaria chemoprophylaxis patterns in the U.S. adult population. Health policies and provider specialty influence malaria chemoprophylaxis choices.

1296

MALARIA KNOWLEDGE AND USE OF MALARIA PREVENTION IN THE UNITED KINGDOM POPULATION AND BY UNITED KINGDOM TRAVELERS TO MALARIA ENDEMIC COUNTRIES

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Few data exist on travellers' knowledge and practices regarding malaria and its prevention, or the numbers of travellers receiving advice or taking chemoprophylaxis when visiting malaria-endemic areas. This study was undertaken to evaluate British adults' knowledge of malaria, and utilisation of anti-malarials through a face to face questionnaires. Two groups were surveyed; a sample of Great Britain's adult population through an IPSOS MORI's Capibus survey of 1,991 adults aged >=15 years old, of whom 548 had previously visited a malaria-endemic country. The 2nd group were 500 passengers in departure areas of Heathrow

Airport departing to a malaria endemic areas, by the airport authority (CAA). All were questioned about their malaria knowledge and used of prophylaxis and other measures. 40% were travelling to W Africa and 38% East and Central Africa. Knowledge and advice scores based on respondents' knowledge of symptoms, seriousness, curability of malaria were calculated. The IPSOS cohort's mean knowledge score was 3.21, versus 2.98 in non- travelled (n=548 & 1443), p<0.001 with a similar score in the CAA travellers of 3.23. The source of advice obtained was categorized scored as a) professional and b) non-professional or no advice. Most had had obtained professional advice - 55% of IPSOS and 61% of CAA travellers - while most travellers not using prophylaxis had not. Prophylaxis use was reported by 77% of Kenyan, 81% of Ghanaian and 49% Nigerian departing passengers. In the CAA travellers, mean knowledge score was similar in those who used prophylaxis or not (3.3 and 3.2 respectively) and the same was true in the IPSOS travellers (mean 3.2 whether used prophylaxis or not). Statistical analysis will be presented of chemoprophylaxis and other factors which may be associated with knowledge of malaria.

1297

DISCOVERY OF A NOVEL TARGET FOR ANTIMALARIAL THERAPY: CYTOPLASMIC PROLYL TRNA SYNTHETASE IS THE TARGET OF HALOFUGINONE IN *PLASMODIUM FALCIPARUM*

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Many current anti-malarial drugs work within the same biological pathways leading to shared resistance mechanism. We have taken the methodology of chemogenomics to identify potential antimalarials that target novel pathways. Understanding the anti-plasmodial mechanism of halofuginone (HFG), a febrifuginone analogue, informs our understanding of parasite biology and directs the future creation of novel therapies. To interrogate mechanism, we selected parasites that are resistant to halofuginone and then used whole genome sequencing to identify the causative mutation (SNPs) and developed high resolution melting (HRM) genotyping assays to follow up those most promising. We found two nonsynonomous mutations in the active site of the cytoplasmic prolyl-tRNA synthetase (*Pf*cPRS) in independent selections. Using a heterologous *S. cerevisiae* model, we have confirmed the sufficiency of *Pf*cPRS to confer sensitivity to halofuginone. In addition, the two nonsynonous SNPs abrogate sensitivity to halofuginone. Amino acid deprivation of *Plasmodium falciparum* activates the amino acid starvation response (AASR) – a highly conserved stress pathway that inhibits cell-wide translation. To determine the involvement of AASR, we then performed western blot analysis of the phosphorylation of eukaryotic initiation factor 2 α (eIF2α) in the presence or absence of excess proline. We have found that halofuginone and febrifugine block *P. falciparum* proline metabolism. Treatment of parasites with halofuginone and febrifugine also results in increased phosphorylation of a *P. falciparum* eIF2α analogue. Furthermore, proline supplementation in the media decreases sensitivity to halofuginone in a dose-dependent fashion. In a similar dose dependent manner, the phosphorylation of eIF2α is dependent on the level of exogenous proline in the presence of halofuginone. Overall, these results demonstrate halofuginone-induced proline starvation via an interaction with *Pf*cPRS leads to translational inhibition. Thus we posit that the potential of amino acid supply and aminoacyl tRNA synthetases as a new promising and potential target for chemotherapeutic intervention.

1298

QUANTIFYING THE ANTIMALARIAL MARKET IN AFFORDABLE MEDICINES FACILITY (AMFM) PILOT COUNTRIES THROUGH ANALYSIS OF IMPORT, EXPORT AND LOCAL MANUFACTURING RECORDS

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The Affordable Medicines Facility for malaria (AMFm), an innovative financing mechanism which aims to increase access to artemisinin-based combination therapies (ACTs) on a multi-national scale through an ex-factory subsidy, launched in July 2010. Since the first private sector order was placed in July 2010, over 158 million ACTs have been ordered by private sector buyers across all active AMFm countries. While these figures likely represent a significant increase in ACT volumes procured in the private sector from pre-AMFm years, understanding the market share and implications for national and global drug forecasts requires better understanding of the total antimalarial market size in these countries. In this investigation, a quantification of the antimalarial markets in 7 of the 9 AMFm pilot countries was performed through a top of the supply chain analysis. For each country, antimalarial import, export, and local manufacturing data were gathered and analyzed. The number of treatment doses procured and manufactured over a three year period was combined and exports deducted to estimate the net market size. The results of this analysis describe the antimalarial market, including ACT market share, before and during initial implementation of the AMFm. For example, an initial analysis of imports, exports and local manufacturing records showed that the total market size in Kenya for 2008 was 43.8M: 25.4M in the private sector and 18.4M in the public sector, representing a significant increase from previous antimalarial demand estimates. This analysis provides context for better understanding the impact of the AMFm and offers a baseline for future analysis of antimalarial demand.

1299

TRACKING MALARIA CASE MANAGEMENT COVERAGE IN THE ERA OF ACT AND RDT SCALE-UP: POSSIBILITIES AND LIMITATIONS WITH USING HOUSEHOLD SURVEYS

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Global malaria control targets focus on coverage of appropriate case management of suspected malaria among children under five. The key indicator for measuring progress towards targets in endemic countries has been presumptive treatment of all fevers among children under five measured using population-based surveys. Access to diagnostic testing and policy change focused on diagnosis before treatment diminish the relevance of the presumptive treatment indicator. This presentation focuses on the value of population-based surveys in the context of scaling up access to treatment and diagnosis. Nationally-representative household surveys focused on treatment-seeking behavior for suspected malaria were conducted in 2012 in Uganda, Madagascar and Nigeria as part of the ACTwatch research program. The timing of these surveys falls at the end of the Affordable Medicines Facility malaria (AMFm) pilot which aimed to increase access to artemisinin-based combination therapy (ACT) in the public and private sectors. Detailed information on treatment-seeking behavior was collected, including where treatment was sought, services and medicines received, and perceived quality of care received at each source. Perceptions regarding dimensions of quality of care were also assessed across local options for fever treatment. Results from questions on type and result of blood testing, and treatment based on test results will be discussed - including issues with respondent recall and respondent awareness of diagnostic test results and treatments received in the context of patient-provider interactions that characterize these settings. While household surveys can provide information on where treatment is sought

and to some extent why, complementary data are necessary to improve case management of suspected malaria and to track progress towards global targets. Methods and measures will be discussed.

1300

REACH OF THE GREEN LEAF: EXPOSURE, AWARENESS, AND REPORTED USE OF AFFORDABLE MEDICINES FACILITY (AMFM)-BRANDED MEDICINES IN THREE COUNTRIES

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The Affordable Medicines Facility - malaria (AMFm) is a global initiative aiming to expand access to affordable artemisinin combination therapy (ACT). The AMFm seeks to reduce consumer prices through price negotiations and a buyer co-payment for which both public and private first-line buyers at the country level are eligible. Reduced prices are expected to extend down the antimalarial supply chain so that effective medicines are available and affordable for consumers. Changes in household treatment-seeking behavior and improved household fever management are expected as access to effective antimalarials increases. Subsidized quality-assured treatments are marked with a green leaf logo to facilitate product promotion and consumer recognition. The first phase of the AMFm began in 7 sub-Saharan African countries in 2010/11. The ACTwatch research program conducted nationally representative household surveys in 2012 in 3 AMFm countries: Uganda, Nigeria, and Madagascar. The studies investigated treatment-seeking behavior for recent fever in children under five. Questions to assess awareness of the AMFm program and the green leaf logo were administered to caregivers in all households with children under five. In households where children had fever in the past 2 weeks, questions on use of antimalarial medicines included recall of the green leaf logo on drug packaging. Information on type, timing, and source of antimalarial treatments obtained was also collected. Results on the reach AMFm communications and the green leaf logo, and implications for treatment-seeking behavior and treatment outcomes will be discussed.

1301

PRIVATE SECTOR DEMAND AND AVAILABILITY OF ARTEMISININ-BASED COMBINATION THERAPIES UNDER THE AFFORDABLE MEDICINES FACILITY FOR MALARIA

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Phase 1 of the Affordable Medicines Facility for malaria (AMFm), a buyer-subsidy program that aims to increase consumer access to artemisinin-based combination therapies (ACTs), launched across nine countries in 2010 and 2011. This program is likely to have a significant impact on the ability of consumers to purchase previously unaffordable ACTs in the private sector, where many seek treatment for malaria-like illness. As of early April 2012, private sector buyers had procured 158M ACTs through the AMFm mechanism. Despite these substantial ACT volumes, it's unclear how well private sector procurement has met consumer demand. Using a dynamic sub-national market forecasting model based on data collected during routine household surveys along with assumptions drawn from the published literature and ongoing operational research, we forecasted private sector consumer demand for antimalarial medicines, including ACTs, following the launch of AMFm. We validated our model using a 2012 analysis of antimalarial import, export, and local manufacturing records in 7 of the 9 AMFm countries. Finally, we estimated the portion of private sector treatments that are likely used to treat true malaria infections and the average cost per treated malaria episode. Results demonstrate that across AMFm countries in 2012, private sector consumer demand for ACTs is significantly greater (165M) than the projected private sector procurement volumes (83M) through AMFm. The mean cost per ACT-treated malaria episode varied widely across the eight AMFm countries, with highest per-infection ACT costs in regions with low malaria prevalence ($6.37 in Kenya) and the lowest in areas with high prevalence ($2.27 in Nigeria). The substantial demand for ACTs in the private sector in AMFm countries suggests that the ACT subsidy's goal of crowding out inferior antimalarial medicines will be difficult to achieve with current procurement rates. In addition, this study provides an indication of the potential cost savings that could result from implementation of improved diagnostic methods in the private sector.

1302

EFFICACY AND TOLERABILITY OF DIHYDRO-ARTEMISININE-PIPERAQUINE (DUOCOTEXCIN*) VERSUS ARTEMETHER-LUMEFANTRINE (COARTEM*) FOR THE TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN SENEGAL: OPEN RANDOMIZED TRIAL

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Malaria remains a major public health problem in Sub-Saharan Africa. Prompt an effective treatment is essential for malaria control. Malaria treatment requires the use of Artemisinin Combination Therapies (ACT). In Senegal, ACT are widely in health care units. In the context of the scaling up of antimalarial treatment, there is a need to monitor ACT efficacy. The study was carried out from November 2011 to January 2012 in Deggo health center within the district health of Pikine (located at 20 from Dakar the capital city). Study end points included (i) PCR corrected adequate clinical and parasitological response (ACPR) at day 28, (ii) ACPR at days 35 and 42, (iii) parasites and fever clearance time, (iv) incidence of adverse events and patients biological profile at day 7. The WHO 2003 protocol for antimalarial drug efficacy evaluation was used to assess each outcome. Overall, 240 patients were randomized to receive either Dihydro-Artemisinine-Piperaquine (Duocotexcin*) (n=120) or Artemether-lumefantrine (Coartem*) (n=120). PCR corrected ACPR at day 28 was at 93.3% in the Dihydro-Artemisinine-Piperaquine group while that was at 97.5% in the Artemether-Lumefantrine group (p=0.21). Therapeutic efficacy was at 100% in Dihydro-Artemisinine-Piperaquine group verus 99% in Artemether-lumefantrine group at day 35 (p=0.44). At day 42 ACPR at 100% was obtained in the two treatments group. The two treatments were well tolerated with similar clinical and biological profile. Abdominal pain, vomit and dizziness were the most frequent adverse event in two treatment group. No serious adverse event was noted in the two study groups. In conclusion, Dihydro-Artemisinine-Piperaquine (Duocotexcin*) and Artemether-lumefantrine (Coartem*) are still efficace and well tolerated and are suitable for the treatment of uncomplicated *P. falciparum* malaria in Senegal.

1303

EFFECTS OF PLASMA PIPERAQUINE LEVEL ON THE ELECTROCARDIOGRAM IN PATIENTS WITH UNCOMPLICATED MALARIA RECEIVING A TWO- VERSUS THREE-DAY COURSE OF DIHYDROARTEMISININ-PIPERAQUINE IN NORTHERN CAMBODIA

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DHA-piperaquine is currently recommended as a first line treatment for uncomplicated *Plasmodium falciparum* and *P. vivax* in Cambodia, and worldwide (WHO 2010). A post-treatment prophylactic effect of DHA-PIP up to 63 days has been reported, making it potentially valuable in malaria eradication efforts. However, cardiotoxicity is an important potential concern with PIP due to QTc interval prolongation. While a 3-day course is widely recommended, the Cambodian military currently employs a 2-day regimen in order to improve compliance. As part of a clinical trial comparing therapeutic efficacy of 2 versus 3 day dosing regimens of DHA-PIP, cardiac safety was evaluated by comparing plasma PIP levels to EKG results. In an open-label clinical trial, 80 patients developing uncomplicated malaria infections of any species were randomized 1:1 to receive a directly observed cumulative dose of 320mg DHA/2880mg PIP divided into either a 2 or 3 day course as inpatients. Plasma piperaquine levels from all volunteers receiving DHA-PIP were collected at pre-dose, 4, 24, 48, 72 hr, 7, 14, 21, 28, 35 and 42 days after the first dose, and on the day of recurrence. Patients had 12 lead EKGs at screening, pre-dose, daily for 3 days and then weekly for 4 weeks if prolongation was more than 10 ms during dosing. Pharmacokinetic analysis is currently in process. Of 35 out of 80 completed subjects with levels measured by high performance liquid chromatography-mass spectrometry to date, there were 8/23 (34.8%) from the 2-day and 2/12 (16.7%) from the 3-day regimen with a positive correlation between the change in QTcB from baseline and the log of the piperaquine concentration. Final results will be presented to determine if either regimen posed a greater risk for QTc prolongation.

1304

STABILIZING SUPPLY AND AVOIDING NATIONAL LEVEL STOCK OUTS OF ACTS IN AN ERA OF WIDE ACT SCALE-UP

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The global market for ACTs has been fraught with volatile demand due to poor planning and supply chain management, uncertainties in funding, and uncertainties in supply due to the long lead times involved in production and the vagaries of an agricultural starting material. Unless appropriately managed these uncertainties have the potential to disrupt global supply of ACTs and hamper the the scale-up efforts achieved in the last few years. Better matching of demand and supply and building resilience in the ACT supply chain may require new approaches. Policy makers have begun to discuss buffer stocks, volume guarantees and other mechanisms to ensure an uninterrupted supply of ACTs to meet the fluctuating demand. However, to date no detailed and rigorous analysis of these mechanisms has been performed to understand their suitability, benefit and cost effectiveness for the ACT supply chain. This paper attempts to address this issue. Models such as a regional ACT buffer stocks, a buffer capital fund, and minimum volume guarantees to ACT manufacturers are discussed for their effectiveness, efficiency and feasibility for this context.

1305

AN EVALUATION OF TREATMENT RESPONSE TO ARTESUNATE-MEFLOQUINE FIXED-DOSE COMBINATION IN CHILDREN DURING A DEPLOYMENT STUDY IN AMAZON BASIN COMMUNITIES OF BRAZIL

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The World Health Organization currently recommends the use of five Artemisinin Combination Therapies (ACTs) for the treatment of uncomplicated malaria. The Drugs for Neglected Disease *initiative* (DND*i*), together with the Special Program for Research and Training in Tropical Diseases (TDR) and the Drugs Technology Institute of the Oswaldo Cruz Foundation (Farmanguinhos/Fiocruz) developed an artesunate-mefloquine fixed-dose combination (ASMQ FDC). The public health impact of ASMQ FDC was evaluated between 2006-2008 in a total of 23,845 patients in the Amazon basin of Brazil, in collaboration with the Brazilian Ministry of Health and RAVREDA-AMI/PAHO. As a large number of these patients (8880;37,2%) were patients under the age of 14, we decided to conduct a post-hoc assessment of treatment outcomes in this specific patient population in the municipality of Cruzeiro do Sul, in order to gather data on the use of ASMQ FDC in children. Cruzeiro do Sul was selected because it is an urban area in which patient follow-up smears are more readily accessible. A total of 584 patients under the age of 14 presented for a follow-up slide until day 40 - the time defined as the interval for recrudescence by the study protocol. Less than 2% of the originally tested patients (8/584;1,4%) had positive thick smears for the malaria parasite, equally distributed among different age categories. Asexual forms of the parasite were detected in a total of 4 cases (0.68%); among which a case with both asexual and sexual forms. These positive malaria smears could represent either re-infections or recrudescence of the initial infection. Our data represent important additional information on the effectiveness of ASMQ FDC in children, and support its use in this specific population. They are consistent with results of other clinical studies, performed in different epidemiological settings and populations.

1306

CLEARANCE OF *PLASMODIUM FALCIPARUM* AS ASSESSED BY RAPID DIAGNOSTIC TESTS, MICROSCOPY AND PCR FOLLOWING ANTI-MALARIAL TREATMENT IN TANZANIAN CHILDREN

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Rapid Diagnostic Test (RDT) has become an important tool for confirmatory malaria diagnosis. Until recently most tests have been based on detection of Histidine Rich Protein 2 (HRP2), a sensitive and stable marker for *Plasmodium falciparum (Pf)* malaria. However, the usefulness of HRP2 based RDT detection of *Pf* is hampered by persistent antigenemia causing false positivity even after successful treatment. Conversely, *Pf*-specific Lactate Dehydrogenase (LDH) based RDT has been proposed to detect only live parasites. Our aim was to study *Pf* clearance as assessed by these two antigens (HRP2 and LDH) in comparison with microscopy and PCR after artemisinin-based combination therapy in Tanzanian children.

Some 50 children <5 years with uncomplicated *Pf* malaria were enrolled. The children were examined on nine occasions during a 42-day follow-up period. At each visit blood was collected for the two RDTs (ParaHit® and CareStart®), Giemsa and Acridine Orange staining of blood slides for microscopy and filter papers for real time-PCR based detection of parasite DNA. A majority of children cleared their parasitemia ≤3 days as accessed by microscopy and PCR. Median HRP2 and LDH positivity time after treatment initiation was 21(range 3-42) and 3 (range 1-7) days, respectively. Due to the remaining HRP2 positivity, this RDT was unable to identify recurrent malaria infections that occurred during follow-up in 10/50 (20%) of the children, whereas the LDH based RDT identified eight of these recurrent infections. The results suggest LDH based RDTs to be more suitable for *Pf* detection in high endemic areas.

1307

HRP2 AND PLDH RDTS COMPARED WITH MICROSCOPY, PCR AND HISTOLOGY FOR DETECTION OF PLACENTAL MALARIA DURING PREGNANCY AND AT DELIVERY IN AREAS OF VARIED TRANSMISSION

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Malaria prevention measures for pregnant women are critical and available, but the effectiveness of intermittent preventive treatment in pregnancy (IPTp) with sulfadoxine-pyrimethamine (SP) is declining with increasing parasite resistance. Diagnostic testing may allow better targeting of efficacious antimalarial treatment to asymptomatic women with demonstrated malaria infection. Light microscopy of peripheral maternal blood misses a large proportion of cases, and PCR is unavailable in routine care. Early data show that detection of parasite antigen in maternal blood may indicate clinically significant infection and predict pregnancy outcomes. Therefore, screening with RDTs may offer a practical way to identify pregnant women who will benefit from targeted therapy for placental malaria infection. We assessed the detection of asymptomatic malaria infection in pregnancy by highly-characterized RDTs in two African clinical settings (Uganda, hyperendemic, and Burkina Faso, seasonal transmission). We enrolled 995 (345 Uganda, 650 Burkina Faso) HIV-negative women in the second or third trimester of pregnancy and followed them to delivery. On the standard IPTp schedule and at delivery, participants' blood was collected for RDTs detecting histidine-rich protein 2 (HRP2) and plasmodium lactate dehydrogenase (pLDH), malaria microscopy and PCR; placental tissue for histology was obtained at delivery. Participants with negative RDT results received SP; those with a positive RDT received artemether-lumefantrine or quinine, and SP. Preliminary data show that 130 (38%) and 112 (32%) participants were positive by HRP2 and pLDH, respectively, at enrollment in Uganda; 134 (21%) were positive by either RDT at enrollment in Burkina Faso. Quality controlled interpretation of peripheral and placental blood microscopy, PCR and histology samples is on-going. Data will be presented on the accuracy of these diagnostic testing methods for detection of asymptomatic malaria during pregnancy and on the potential utility of RDT screening for management of such infections.

1308

ABSOLUTE QUANTIFICATION AND DETECTION OF *PLASMODIUM* PARASITE BY QPCR

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Determining precise parasite quantification in real time PCR has been a challenging aspect in malaria diagnostics. In general, quantification of *Plasmodium* by qPCR is done by serially diluting a standard of a known parasite density were the source can be from a cultured parasites or clinical samples. The parasites density is determined by microscopy and described in parasite/µl which is known as a relative standard. A relative standard can lead to incorrect quantification because it may have difference on the source, parasite culture or clinical samples. It relies on microscopy being performed accurately and consistently. Absolute quantification is based on known concentration of DNA standard molecules such as plasmid DNA. We have developed an absolute quantitative multiplex qPCR for detection of *Plasmodium spp., P. falciparum* and *P.vivax* described in parasite/µl. Plasmids DNAs are constructed for qPCR assays by amplifying PCR fragments from genomic DNA from either clinical samples or cultures and cloned into TOPO TA vectors. The concentration of each plasmid DNA was determined in genomic equivalence (GE) and was used for subsequent experiments. All of the absolute qPCR assays performed with efficiency of more that 94%, R^2 values greater than 0.99 and the STDEV of each replicate was <0.167. Correlation of genomic equivalence to parasite/µl was established using standard clinical samples and or cultures. One copy of plasmid DNA was established to be equivalent to 0.12 parasite/µl for *Plasmodium spp.* assay, 0.54 parasites for *P. falciparum* assay and 0.16 parasite/µl for *P.vivax* assay. From this data, absolute qPCR can be expressed in parasite/µl. An absolute quantitative qPCR assay is better than a relative qPCR because it is more accurate and consistent. Plasmid DNAs are stable, can be easily produced in large quantities and stored for a long period of time. In addition, plasmid DNA production and quantification can be highly standardized ensuring more uniform quantification. Accurate quantification of parasites can have great impact on malaria diagnosis in clinical trials as confirmatory method to microscopy.

1309

DEVELOPMENT OF LOCAL EXPERTISE FOR PLACENTAL MALARIA HISTOPATHOLOGY IN TORORO, UGANDA: FROM COLLECTION TO INTERPRETATION

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Placental histology is a valuable technique to evaluate malaria epidemiology and control during pregnancy. Histology laboratories and expertise are usually confined to urban tertiary care hospitals and facilities are rare in Africa. The process of preparation requires specialized skills and training. As part of a study of malaria rapid diagnostic tests (RDTs) in pregnancy, placental specimens were collected in rural eastern Uganda (n=267) and southwestern Burkina Faso (n=548), areas of high and seasonal transmission, respectively. Specimens were fixed in 10% neutral buffered formalin and after 24 hours transferred to 70% ethanol for shipping and storage (4-8°C). On site in Uganda, paraffin blocks were

manually generated, and sections were Giemsa stained. Concurrently 30 biopsies per site, 30 paraffin blocks and stained slides were shipped to Seattle, USA, for external quality control (QC). Tissue was well preserved with no formalin pigment artifact. Blocks and stained slides were well prepared with minimal artifact. Processing and staining problems detected early were rapidly addressed. After one-on-one training on reference slides, study samples were interpreted by two trained technologists, with cross-checking against placental blood smears. A subset of all positive cases and 10% of negative cases were reviewed at University of Washington. The majority of supplies were locally available, however a microtome was imported by another research group at the same site, and microtome knives, charged slides and paraffin wax were imported. Challenges included exposure to alcohol, xylene, and formalin, and the physical distance to the nearest experienced histopathologist. The exercise demonstrated development of placental malaria histopathology expertise with robust QC in an inexpensive laboratory in a rural district hospital, showing successful implementation of capacity-building for a highly skill-dependent activity critical to study success, and providing potential for long-term reference-level histology for pregnancy studies in Africa.

1310

POINTING OUT MALARIA INFECTIONS WITH LASER POINTERS

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Detecting the presence of malaria parasites primarily relies upon; the highly accurate but time consuming method of PCR, the use of expensive and subjective RDT card tests, or the inexpensive but slow (up to 1 hr) microscopy-based methods which can yield false positives for 36% of samples and false negatives as high as 18% of the time. Inter-operator error in creation, staining, and visual analysis of the slides may contribute to this high error rate. Therefore, there is a need for novel malaria diagnostic techniques to identify which samples are potentially infected and help confirm negative diagnoses. Through a multidisciplinary effort we have designed an inexpensive, rapid malaria detection device (3 minutes) that detects the presence of hemozoin, a parasite byproduct of hemoglobin digestion. We place blood samples into the path of a polarized light beam in the presence and absence of a magnetic field. When the partially magnetic malaria hemozoin is present, it aligns with the magnetic field and acts as a reflector thus decreasing the amount of light reaching a light level detector on the far side of the sample. This decrease in light is directly proportional to parasitemia (R2=0.996) which can be detected at parasitemias as low as 0.00033% (17 parasites per microliter) which exceeds detection levels for microscopy without the need for staining or trained microscopists. Our long term goal is to translate this technology into a field ready, low-cost device, which can be used in malaria-endemic regions to enable rapid malaria diagnosis at the point-of-care.

1311

TRENDS IN PRESCRIBER ADHERENCE TO MALARIA TESTS IN HEALTH FACILITIES RECEIVING JOINT CLINICAL AND LABORATORY SUPERVISION VISITS

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During 2008-2012, the President's Malaria Initiative made considerable investments towards improving malaria diagnostics to promote the rational use of anti-malaria drugs in health facilities in sub-Saharan Africa. Through the Improving Malaria Diagnostics (IMaD) project, Ministries of Health in Benin, Ghana, Malawi, and Zambia, implemented quality assurance programs based on Outreach Training and Support Supervision (OTSS). In Ghana, laboratory supervisors implemented routine supervision and observed laboratory specific topics such as malaria microscopy (MM) and RDT performance. The same laboratory supervisors addressed prescriber compliance during their visit. In Benin, Malawi, and Zambia, supervision was implemented together by a laboratory and clinical supervisor. Laboratory supervisors focused on MM and RDT performance while clinical supervisors addressed fever diagnosis and prescriber adherence. Standardized checklists were used during each visit and improvements were tracked using a Microsoft Access database. In countries implementing joint supervision, general positive trends in prescriber adherence to microscopy and RDT results were observed: Benin 38% (MM) and 39% (RDT) percentage point improvement between visits 1-7, Malawi 17% (MM) between visits 1-3, and Zambia 23% (MM) and 21% (RDT) improvement between visits 1-4; In Ghana, where supervisory visits were implemented by laboratory supervisors only, no discernible trend was observed in prescriber adherence to negative tests: changes of 3% (MM) and -7% (RDT) were observed. Trends in prescriber adherence from OTSS data show that a joint approach to supervision had a greater impact on prescriber adherence to negative blood slide and RDT results than supervision conducted by laboratory supervisors alone, provider confidence improves when laboratory results are quality assured, and communication between the two cadres is strengthened. It should be noted that not all facilities have received a full cycle of visits due to the staggered nature of the roll-out of the OTSS program.

1312

CLINICAL SIGNS AND SYMPTOMS OF *PLASMODIUM FALCIPARUM* MALARIA INFECTION (PATENT AND SUB-PATENT) IN PREGNANT WOMEN LIVING IN AN AREA OF HIGH SEASONAL TRANSMISSION

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Malaria in pregnancy is a major public health concern in endemic countries. There a paucity of data on the association between peripheral parasitaemia and the presence of signs and symptoms of malaria during pregnancy. The objective of this study is to document the frequency of the attendance of pregnant women at health facilities with clinical complaints suggestive of malaria and to assess their parasitological status. To attend this objective, a hospital-based descriptive study at the maternity clinic was conducted in the rural district of Nanoro in Burkina Faso. A total of 600 pregnant women attending the antenatal care (ANC) were recruited, 200 pregnant women with signs and symptoms suggestive of malaria and 400 others without signs and symptoms were control group. The women were matched by gestational age and parity. For each woman, a capillary blood sample was taken for rapid diagnostic test, microscopy and hemoglobin test. A multivariate model was used to access each predictor of malaria. The overall prevalence of malaria was 42.6% (256/600) using the microscopy while anemia was found in 60.8% (365/600). Nearly a half (49.5%) of the women who displayed symptoms was parasitaemic and 39.5% of the asymptomatic women were parasitaemic. The most frequently encountered signs and symptoms were fever 36% (72/200), history of fever 29% (58/200) and headache 52% (104/200). The predictive positive values for fever were 53% (95%CI 41-64), history of fever 58% (95%CI 37-63) and headache 51% (95%CI 41-61). Signs and symptoms suggestive of malaria are quite frequent in pregnant women in intense transmission area. A large number of asymptomatic but parasitaemic women were found. For a better management of malaria in pregnancy, active case detection of all pregnant women attending the ANC should be performed to detect and treat earlier malaria infection.

1313

BLOOD SMEAR TEST FOR MALARIA CONFIRMATION AT THE COMMUNITY LEVEL: FEASIBILITY AND LESSONS LEARNED FROM SARAYA HEALTH DISTRICT, SENEGAL

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Following Rapid Diagnostic Test (RDT) and ACT introduction in 2009, health units were asked to confirm all malaria cases; this has been partially scaled up to the community level where Community Health Workers (CHWs) were trained to use RDT. Smear blood tests were realized only in the laboratories with laboratory technicians. In Saraya district thick and thin blood smears were introduced to confirm all malaria cases in 24 villages involved in a Seasonal Malaria Chemoprevention research project. The objective of the study was to assess the feasibility of smear blood tests at the community level. Saraya district is located in South East Senegal, bordering Mali and Guinea Republic. Health staff was very limited, and with a strong network of community health workers and malaria village volunteers called DSDOM. Twenty four CHWs and malaria volunteers were trained by staff from the medical school, parasitology laboratory for 3 days to perform Blood smears with practical sessions. They were asked to complete RDT, thick and thin blood smears for all patients under 10 with fever. Blood smear tests were kept in a box and collected by supervisors. Close follow up were made by supervisors, mainly in the first month for continued training and improvements. Slides were read at the Medical school, parasitology laboratory. CHW performed 1635 blood smear tests between July and November 2011; 68.47% were positive, 31.47% negative, 0.06% not readable. Parasite density mean was 22.8 [13, 521] and nearly all malaria cases were due to *Plasmodium falciparum* (98%) with only 2% of malaria due to *P. malariae* and *P. ovale*. Blood smears can be performed at the community level by lower educated personnel with formal training and close follow up; this would be helpful to be more accurate on malaria diagnosis and non malaria febrile illnesses as well in remotest under served areas.

1314

A REVIEW OF MALARIA RAPID DIAGNOSTIC TESTS (RDT) GUIDELINE IMPLEMENTATION IN A DISTRICT HOSPITAL IN GHANA: HAS RAPID TESTING BEEN PRIORITIZED?

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Rapid diagnostic tests (RDTs) can improve timeliness and accuracy of malaria diagnosis. This can help to slow down the development of antimalarial drug resistance by promoting appropriate treatment. Since 2009, revised malaria management policies in Ghana promote testing by microscopy or RDTs, before treating all suspected malaria cases aged five years and above. In this study we reviewed the use of RDTs in a district hospital over a two-year period following the implementation of 'test-before-treat' policies for malaria in Ghana. A random sample of 500 malaria cases recorded at the Nkawie-Toase District Hospital from January 2010 to December 2011 were identified and reviewed**.** For reference visits where the clinician made a differential diagnosis of malaria, only 3.6% (95%CI: 2.2-5.7) of reviewed cases were tested with RDTs compared to 13% (95%CI:10.2-16.3) by microscopy. For cases with repeat visits within 6 months of the reference attendance, percentage RDT-use decreased to 0.6% (95%CI: 0.01 - 3.5), while testing by microscopy increased to 26.1% (95%CI:19.3-33.8). RDT use ranged from 1.6% to about 4% (p=0.09), from low to high malaria incidence months. Testing with microscopy appeared strongly associated with seasonality of malaria, almost doubling from 10% in non-peak, to 19.3% (p=0.005) in peak malaria incidence months. Testing by microscopy was more frequent than RDT use during the period under review. These findings suggest that rapid malaria testing was poorly implemented in this district hospital over the study period, despite existing policy revisions in Ghana. Investigating RDT utilization in similar referral level facilities is essential to understand and to improve the implementation of current malaria testing guidelines in Ghana. This information will be useful to advise investments in rapid diagnostics for malaria, and to improve their application in limited resource settings.

1315

RAPID DIAGNOSTIC TEST (RDT) PERFORMANCE OF THE MALARIA GOLD MINING PROGRAM IN SURINAME: A COMPARISON BETWEEN RDT AND BLOOD SMEAR RESULTS

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Currently malaria infections occur mainly among persons (ca. 15,000) engaged in small-scale gold mining and related activities. The mining areas are remote from the existing healthcare services. To address this problem, a system of quick diagnosis and treatment was established by training lay persons (e.g shopkeepers) in gold mining areas to perform malaria diagnosis and to treat uncomplicated malaria. They are called Malaria Service Deliverers (MSD). Also in the city, in the gold miners' neighborhood, the Tourtonne laboratory was established to provide similar services. For each RDT performed a blood smear is taken and examined by Tourtonne Laboratory (TL) for quality control of the RDT. Good RDT performance is the cornerstone of the MSD system. The RDT results from 2007 through the first quarter of 2012 were compared with the Blood Smear results. 4489 slides received from the MSD were readable for comparison. The overall sensitivity was 83.3% (81.2 - 85.2%), and the overall specificity 91.8% (90.8 - 92.7%); a PPV of 80.8% was calculated. For the TL 6761 RDT results were available for comparison. The sensitivity was 81.3% (79.7 - 82.8%), the specificity was 96.2% (95.6 - 96.7%) and the PPV was 92.7%. Looking specifically at the performance of RDT related to *Plasmodium falciparum,* the sensitivity, specificity and PPV were respectively 83.1%, 94.2% and 62.6% for MSD versus 84.8%, 95.6% and 82.7% for Tourtonne laboratory. The sensitivity of both the MSD system and TL were lower than the expected sensitivity (95.3%) calculated by the manufacturer. The specificity of both on the other hand was according to expectations. As the accurate diagnosis and treatment for especially *falciparum* malaria is of paramount importance, false negative tests should be avoided. Further research e.g. parasitaemie level, storing conditions, reader variability is needed to explain the difference found between the expected and found sensitivity.

1316

INVESTIGATING THE OPTIMAL SAMPLING SCHEME FOR MEASURING PARASITE CLEARANCE WITH THE PARASITE CLEARANCE ESTIMATOR

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The emergence of artemisinin resistance in South East Asia threatens the efficacy of artemisinin derivatives (AD). Since the pharmacodynamic hallmark of AD is rapid parasite clearance, the clinical phenotype of slow clearance characterises resistance. Frequent parasite counts are needed to define clearance rate but it is uncertain what sampling frequency is required to ensure reliable estimates. We selected 2841 parasitaemia-time profiles from clinical studies in which 6-hourly parasite counts were available in the first 48 hours (h). Patients were treated with an artesunate alone or in combination with a partner drug. WWARN's Parasite Clearance Estimator estimated the median (range) parasite half-life (HL) as 3.2 (0.7 - 17.5) h. Four measurement schedules (at 0,6,12,24 or 0,6,18,24 or 0,12,18,24 or 0,12,24 h and then every 12h) were investigated. The median (range) for the difference between the original HL estimate and that from the 4 schemes were -0.02 (-3.4 to 3.8), -0.06 (-3.3 to 3.5), -0.09 (-3.6 to 3.4), -0.15 (-5.0 to 3.6) h, respectively. The overestimation of the HL by the restricted schemes was greater for profiles with short reference HL. Bootstrapping was then used to estimate the sampling distribution of HLs for two subsets of the population with: (A) fast clearance (20% of reference HL>3h) and (B) slow clearance (50% of reference HL>3h). In both subsets, the median HL was overestimated by the 4 schemes (A:91 -100%, B: 79-97% of bootstrap samples), but by ≤0.5h for nearly all samples. The schemes overestimated the proportion (%) of profiles with HL >3h, on average by 39, 44, 54, 72% (A) and 6, 7, 9, 12% (B), relative to the scheme with 6 hourly measurements, respectively. The proportion of profiles with HL longer than 6h in bootstrap samples was very similar for all restricted schemes. Our data indicate that HL can be best estimated by including samples at 6 and 12h while every 12h counting is satisfactory in patients with slow clearance.

1317

WORLDWIDE ANTIMALARIAL RESISTANCE NETWORK (WWARN) TOOLKIT: PROMOTING HARMONIZATION OF ANTIMALARIAL RESISTANCE EPIDEMIOLOGICAL OUTPUTS

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To meet the known threat of parasite resistance to artemisinin-based therapies, the WHO's Global Plan for Artemisinin Resistance Containment stresses the need for more quality-assured antimalarial efficacy data. The WorldWide Antimalarial Resistance Network (WWARN) response is an online Toolkit that guides research scientists to collect the reliable, comprehensive evidence needed by the public health community to identify and contain antimalarial drug resistance. The Toolkit contains a growing portfolio of essential tools and services to promote high-quality antimalarial efficacy and laboratory testing. These include guidance on study protocol design, tools for data collection and analysis and technical procedures, supported by proficiency testing and reference material programmes, training workshops and online courses. The Toolkit will assist researchers - particularly those working in resource-limited environments - in the design, conduct and interpretation of their studies, thereby facilitating high-quality prospective data collection, and reducing data heterogeneity. The standardised Toolkit data outputs from *in vivo* studies and laboratory tests can be pooled - across studies, time, and place - uncovering subtle trends or sub-population effects with higher statistical certainty. Increasing the ease and potential for data mining in turn allows complex issues, like antimalarial resistance, to be understood more quickly and cost-effectively and with less duplication of effort. We will describe the components of the Toolkit and present the 'roadmap' that guides scientists progressively through the steps to plan and run antimalarial resistance research projects and how to use the various tools and services.

1318

LONGITUDINAL STUDY OF SULFADOXINE-PYRIMETHAMINE (SP) RESISTANCE IN TURBO, COLOMBIA

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Pyrimethamine was introduced in the 1950s in South America as a mass treatment in Venezuela. By 1968, pyrimethamine-resistant parasites were found in Colombia and resistance rapidly disseminated in the Amazon and Orinoco basins. However, SP resistance in Colombia is unevenly distributed, showing high resistance in the Amazon basin to moderate levels in the Caribbean, the Cauca Valley and northwestern regions. In this study we characterized a total of 145 *Plasmodium falciparum* samples from Turbo, a port town in Antioquia Department, collected during years 2002 to 2009 and characterized point mutations in two genes that have been implicated in resistance to SP, dihydrofolate reductase (dhfr) and dihydropteroate synthase (dhps). The treatment given to the patients in this area during 2002 until 2006 was a combination therapy of amodiaquine and SP, which was changed to artesunate and mefloquine in 2007 and then to Coartem in 2008. We found that pyrimethamine-resistant double mutants (50C/51I/59C/108N/164I) are nearly fixed in the population, while both sensitive and resistant sulfadoxine genotypes (436S/437G/540K/581A/613A) were present in the population. We also assayed neutral microsatellite markers around the dhfr (chromosome 4) and dhps (chromosome 8) loci to get an idea of the strength of selection. According to the microsatellite haplotypes for the dhfr and dhps SP-resistant alleles, the dhfr double and dhps single mutants seem to have a single origin. Further studies are required to determine the increased in frequency of SP sensitive parasites, as well as to characterize the gene flow between the southwestern populations, where SP is still efficacious, and the northwestern populations of Colombia where moderate resistance has been documented.

1319

WORLDWIDE ANTIMALARIAL RESISTANCE NETWORK (WWARN) *IN VITRO* PROFICIENCY PILOT PROJECT: DETERMINATION OF THE INTERLABORATORY VARIABILITY OF IC_{50} ESTIMATES IN *PLASMODIUM FALCIPARUM* REFERENCE CLONES

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In vitro testing is a key component of resistance surveillance as drug susceptibility can be tested without the influence of human confounders, such as immunity and pharmacokinetic parameters. Furthermore the

effect of single compounds in a combination therapy can be evaluated. Currently a wide range of methodologies and conditions are being used to perform drug susceptibility testing in the global community of *in vitro* laboratories. Although there is no gold standard *in vitro* protocol that is suitable for all drugs in all different settings, several aspects of *in vitro* methodology can be standardised to reduce variability. In this study we assessed whether the inter-laboratory variability of drug susceptibility testing could be minimized by introducing simple standardisation measures. Fifteen participating laboratories used their established methodology to test the drug susceptibility of *Plasmodium falciparum* reference clones 3D7 and W2 on several occasions. WWARN provided the following measures to improve standardisation: 1) genetic validation of reference clones by microsatellites and *pfmdr1* gene copy number at the start and close of the pilot project; 2) validated test drugs – chloroquine, mefloquine, desethylamodiaquine and dihydroartemisinin – supplied from the WWARN Reference Material Programme; 3) standardised data analysis using the WWARN *In Vitro* Analysis and Reporting Tool (IVART). Comparing data from different laboratories improves understanding of the range of variability encountered with different *in vitro* readout methods when other parameters have been standardized. These results will be presented and used to design a Proficiency Testing programme to improve standardisation of *in vitro* assessment across the malaria community.

1320

GENOMIC APPROACH FOR TARGET IDENTIFICATION OF ANTIMALARIAL CYCLOPROPYL CARBOXAMIDES

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One of the current antimalarial drug discovery approaches is focused on whole cell screening. One of the greatest challenges working with compounds identified in phenotypic screenings is the complete lack of knowledge of the molecular target responsible for antimalarial activity. Cyclopropyl carboxamides (CCAX), a chemical class not described previously as antimalarial drugs, have been identified recently from a whole-cell screening as potent inhibitors of *Plasmodium falciparum* drug-sensitive and resistant strains, as reported previously. Moreover, this series shows a promising *in vivo* oral efficacy in *P. falciparum* mouse models. This might indicate an antimalarial mode of action different from already known resistant mechanisms, although only the identification of the target responsible for the antimalarial activity could confirm it. Despite a potent *in vitro* activity, further characterization of the molecules has revealed an unusual propensity to develop resistance. The frequency of spontaneous resistance is one order of magnitude higher than in the case of atovaquone when using W2 strain. To investigate the resistance mechanisms of this series and to achieve the identification of the cyclopropyl carboxamide antimalarial target we have selected seven independent pure clones that have been extensively characterized. Despite of the high level of resistance (two orders of magnitude) none of them shows sensitive differences in terms of growth rate compared with the parental strain. We have purified genomic DNA of the different clones and started a full genome sequencing approach in order to identify the determinants responsible of CCAX resistance. The identification of this target would help to the progression of this chemical series and to a better understanding of antimalarial resistance.

1321

PHARMACODYNAMICS OF ARTEMISININ-BASED COMBINATION THERAPIES (ACTS) IN A RODENT MODEL OF ARTEMISININ-RESISTANT MALARIA

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The emergence of the delayed parasite clearance phenotype in *Plasmodium falciparum* parasites present in the Greater Mekong subregion, as reported previously, highlights the urgent need to identify antimalarial therapies and regimens that can adequately treat these infections. In this study, we have employed an animal model to monitor the course of rodent malaria infection after treatment with Artemisinin-based Combination Therapies (ACTs). In addition to providing insight into the pharmacodynamic properties of existing ACTs when used against artemisinin-resistant strains, our work shows that the artemisinin-resistant *Plasmodium berghei* SANA strain can be treated successfully with an artesunate-pyronaridine combination therapy at similar drug concentrations that are curative for infections with the parental drug-sensitive N strain. The 30-day outcomes indicate that SANA resistance to artemisinin can be overcome with the combination of pyronaridine-artesunate. Piperaquine, which is currently used in combination with dihydroartemisinin in Southeast Asia, also proved to be effective in clearing parasite infections after three doses in the animal model. Of the five partner drugs tested, pyronaridine was the most effective at suppressing the recrudescence of SANA parasites.

1322

EFFICACY AND EFFECTIVENESS OF ARTEMETHER-LUMEFANTRINE AFTER FIVE YEARS OF WIDE SCALE USE IN TANZANIA

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Artemether-lumefantrine (AL) is the most widely adopted ACT in sub-Saharan Africa. The recent emergency of artemisinin resistance in *Plasmodium falciparum* malaria in South East Asia, characterized phenotypically by slow parasite clearance following ACT treatment, highlights the need for both detailed follow up monitoring of efficacy/effectiveness of AL, including detailed assessment of parasite clearance time, and surveillance . identification of molecular markers of resistance to AL as an early warning system. This study is conducted in two rural sites in Bagamoyo and Kibaha Districts, We are conducting a randomised clinical trial to assess the efficacy and effectiveness of AL in children with uncomplicated malaria, including parasite clearance time, selection of molecular markers of resistance, identify factors associated with poor adherence after five years of wide scale use in Tanzania. We are enrolling patients 6 months-10 years with confirmed malaria by finding parasites in blood samples. Patients are randomly allocated to either supervised (admitted to the health facility for 3 days) or unsupervised (at home) artemether-lumefantrine (Coartem®) treatment, and then they are reviewed every week for 42 days, to monitor treatment outcomes. Study nurses make home visits to assess treatment adherence through parent/caretaker interview and blister pack pill count. Standardized procedures recommended by WHO are used to accurately detect and document drug resistant malaria and lumefantrine drug levels on day 7. Data collecting will be completed August 2012.

1323

PLASMODIUM FALCIPARUM IN VIVO EARLY RESPONSE TO ARTEMETHER-LUMEFANTRINE THERAPY IS ASSOCIATED WITH ABC TRANSPORTER TRANSCRIPTS

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Increased expression of ABC transporters has been associated with decreased clinical drug response in several different pathologies. *Plasmodium falciparum* malaria is no exception and increased copy number of the parasite P-glycoprotein homologue has been associated with resistance against several antimalarials, including lumefantrine and artemisinins. The aim of this work was to investigate the expression of *P. falciparum* ABC transporters in a clinical setting, upon treatment with artemether-lumefantrine (AL), the most used treatment against *P. falciparum* malaria. The clinical trial was conducted at Fukayosi Primary Health Care Centre, Bagamoyo District, Tanzania in 2006. A total of 50 patients: age 1-10 years were included, hospitalized and treated with standard 6 doses of AL. Venous blood samples were taken at 0,2,4,8,16,24,36,48,60,72 hour and preserved for nucleic acid extraction.. RNA was extracted and quality controlled at Karolinska Institutet. For the time points up to 24h cDNA was synthesised and analysed by Real-time PCR for relative quantification of pfmdr1, pfcrt, pfmrp1, pfmrp2 using the endogenous control seryl-tRNA synthetase (PF07_0073). Gene expression relative to the control was calculated using the ΔΔCt method. After initiation of AL treatment the expression of pfmrp1 increased significantly whereas the expression of pfmdr1, pfcrt and pfmrp2 significantly decreased. Our results emphasises the likely importance of pfmrp1 in artemisinin combination therapy drug resistance.

1324

EVALUATING THE ROBUSTNESS OF PARASITE CLEARANCE RATE MEASURES USING HERITABILITY

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Measurement of malaria parasite clearance rates following artemisinin treatment requires sequential parasite counts taken at intervals following treatment. Frequent sampling is ideal, but extremely labor intensive, and the optimal strategy for obtaining robust clearance rate estimates while minimizing sampling effort is poorly understood. We evaluate a variety of metrics (24 and 48hr parasite reduction ratios, time to parasite clearance, clearance half-lives ($t_{1/2}$) measured using 6-24 hourly sampling until clearance or for the first 48 hrs only). We also evaluate the effect of different slope fitting procedures (ignoring or incorporating time lags) on the robustness of clearance estimates. We perform these analyses using parasite clearance data collected from 1731 hyperparasitemic patients from a region of emerging resistance on the Thai-Burma border. All parasites were genotyped using 96 single nucleotide polymorphisms, and we used heritability (the proportion of variation attributable to parasite genetics) to evaluate the robustness of each measure. Our assumption is that the most robust measure will show the highest heritability, while less useful measures will show lower heritability due to measurement error. The results of these analyses will be presented.

1325

SUPPRESSING ANTIMALARIAL DRUG RESISTANCE WITH COMPLEMENTARY INHIBITORS: CATCHING *PLASMODIUM FALCIPARUM* BETWEEN A ROCK AND A HARD PLACE

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Managing drug resistance is a core problem in anti-malarial drug therapy. Resistance has arisen to all drugs in clinical use. Combination therapy is a key tool for delaying the development and spread of resistant parasites. Most combinations use two drugs with different mechanisms of action. We explore here the possibility of using two agents acting on the same target - one drug that selectively inhibits the wild-type target, and a complementary partner drug that selectively inhibits the most likely drug-resistant mutations. Selection of malaria parasites resistant *in vitro* to either alkylthiophene- or triazolopyrimidine-based Dihydroorotate Dehydrogenase (DHODH) inhibitors resulted in mutations in the drug-binding pocket of DHODH, which had been previously determined through crystallography and biochemical characterization of purified protein. Using those mutants resistant to both the alkylthiophene and triazolopyrimidine inhibitors we re-screened a library of DHODH inhibitors. This screen yielded several compounds 10-100-fold more potent against the mutants than the wild-type parasites. Subsequent selection of resistance to these selectively potent compounds in the alkylthiophene-resistant strains resulted in reversion of the DHODH gene back to wild-type, confirming the key role of the mutation. Modeling and molecular dynamics simulations are being used to probe the mechanisms of heightened sensitivity to different compounds. Combination therapy that exploits target reciprocity traps malaria: escape from the primary drug results in increased sensitivity to the secondary drug. Selective pressure on the partner compound is predicted to be reduced; it acts only against the small population of parasites that become resistant to the primary compound. In targets that tolerate few mutations, the fitness costs of becoming resistant to both complementary inhibitors may provide a path for suppressing drug resistance.

1326

EMERGING COARTEM RESISTANCE ASSESSED BY DAY THREE PARASITEMIA IN SURINAME

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In 2004 Suriname changed its first line treatment for *Plasmodium falciparum* malaria to an artemisinin based combination therapy (ACT), introducing Coartem.. This and other measures resulted in a more than 90 percent decrease of malaria. Currently malaria cases are mainly seen in gold miners in the interior. In this population adherence to treatment is poor and also the use of counterfeit medication is widespread. Following WHO recommendations, the efficacy of the treatment was evaluated in 2006 and found to be adequate. A study was undertaken to assess Coartem efficacy in patients with *P. falciparum* malaria. Consenting patients with *P. falciparum* malaria mono-infection were enrolled and followed to assess the course of clinical symptoms and parasitemia. Because of the current low number of cases available for a 28 days follow up, in this assessment we also included day 3 parasitaemia, as a clinical endpoint The treatment was directly observed; patients were followed until parasite clearance plus one day and then on day 7, 14, 21 and 28. 67 Patients were enrolled, of whom 9 were withdrawn because of protocol violations. There were no reports of serious side effects. From the

remaining 58 patients, 5 were lost to follow up before parasite clearance. Only 11 patients were followed for the full 28 days period, none of whom had recurrent parasitaemia.. From the 53 patients that were followed at least until parasite clearance, 15 (28.3%) had still parasites on day 3. From 11 patients that were followed until day 28 only 1 had a positive slide on day 3, which became negative on day 4. Comparing these results to those of 2006 we found that at that time the incidence of day 3 parasitaemia was 1.6 percent, with 95% of cases with a negative slide on day 28. We conclude that the rate of day 3 parasitaemia has significantly increased in 2011 ($p < 0.001$). This may be an indication for emerging resistance to Coartem. It is suspected that this may be due to the (improper) use of counterfeit ACT.

1327

MALARIA AS A CAUSE OF ACUTE FEBRILE ILLNESS IN AN URBAN PEDIATRIC POPULATION IN GHANA

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Annually about 500 million people become severely ill with malaria. Over 90% of cases and deaths occur in Sub-Saharan Africa with children under five years and pregnant women being affected mostly. In Ghana, about 3.7 million cases of malaria were reported in 2010 out of which only 26% were confirmed by laboratory testing. Most febrile cases are treated as malaria sometimes with fatal consequences though they may not be malaria. This could explain the high proportion of funds that the Ghana Health Insurance Authority spends on the treatment of malaria. This study sought to determine the proportion of acute febrile illness in children under five years due to malaria. A hospital based surveillance system recruited children less than five years who reported at the out-patient department of an urban hospital with fever ≥ 37.5°C at the time of visit from February 2009 to February 2010. Parents/guardians who consented were interviewed using a structured questionnaire and the child examined by a clinician. Capillary blood through a finger prick was used for a thick blood film using Giemsa and viewed under the microscope for malaria parasites. Out of the 605 children with fever whose blood samples were taken for microscopy, only 68 were positive for malaria, giving an overall positivity rate of 11.2%. Malaria was equally distributed among males and females, the proportion malaria cases increased as age increased. Of the 492 children whose reports were available, 80% of the children were diagnosed by clinicians as having malaria either alone or in combination with other diseases and were treated with anti-malarials. The treatment of febrile cases based solely on clinical symptoms has been shown to be less cost effective than confirming the diagnosis with a laboratory test and also promotes the occurrence of drug resistance.. Clinicians should look out for other causes of fever rather than treating almost all febrile cases as malaria. The National Malaria Control Programme has intensified efforts to increase laboratory testing before treatment.

1328

A COMPREHENSIVE RISK MAP FOR MALARIA IN KINSHASA, DEMOCRATIC REPUBLIC OF CONGO

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The Democratic Republic of Congo (DRC) is the second most malarious country in the world. However, there is a paucity of epidemiological data on the risk pattern of malaria. In 2009 (dry season) and 2011 (end of the rainy season) two two-stage cluster sampling malaria surveys were conducted in the capital city Kinshasa with the twofold aim of (1) assessing malaria parasite prevalence, anemia and associated malaria risk factors, and (2) producing a malaria risk map using a geographic information system (GIS). A total of 6410 children aged 6-59 months (3058 in 2009 and 3352 in 2011) were tested for both malaria (using rapid diagnostic tests) and anemia (by HemocueTM). Nine health zones (HZ) were sampled in 2009 with an average prevalence for malaria and anemia of 6.6% (95% CI 5.8-7.5) and 66.0% (64.5-67.4) respectively, while in the 25 HZs in 2011 the prevalence was 17.0% (15.7-18.3) and 64.2% (62.6-65.9). Overall, the prevalence rate for both surveys was 11.9% (11.2-12.8) for malaria and 65.1% (63.9-66.7) for anemia. To ensure comparability of the results between surveys, two HZs from 2009 were resampled in 2011. Prevalence for malaria in 2009 and 2011 was: Ngiri Ngiri 1.0% versus 0.8% and Selembao: 14.1% versus 26.8%. Prevalence for anemia was: Ngiri Ngiri 62.5% versus 55.4% and Selembao: 67.1% versus 61.4%. In a multivariate analysis of the 2011 data significant protective factors for malaria risk were: educational level of the respondent (OR = 0.12, 95% CI: 0.03 - 0.56) and sleeping under an ITN (OR = 0.52, 95% CI: 0.43 - 0.63). All key parameters were mapped to the level of the HZs (n=35). Malaria parasitemia, anemia and fever prevalence were found to be much lower in the city center than in the peri-urban suburbs, where transmission rates remain high. ITN usage showed the opposite pattern. These maps provide for the first time a comprehensive picture of the epidemiology of malaria in Kinshasa and provide solid baseline information for planning future malaria control interventions.

1329

LINKING THE INCIDENCE AND AGE PATTERNS OF CLINICAL MALARIA TO PARASITE PREVALENCE USING A MATHEMATICAL MODEL

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Estimating the changing burden of malaria disease remains difficult due to limitations in health reporting systems in those countries with the largest burden of disease. Methods extrapolating from parasite prevalence data are therefore often employed. We present an approach to estimating disease incidence from prevalence data accounting for the changing age distribution of cases that occurs as transmission declines. We use a transmission model to capture the shifting age-pattern of disease at different transmission intensities through dynamically modelling the acquisition and loss of immunity. The model is fitted to age-stratified data on the incidence of uncomplicated malaria due to *Plasmodium falciparum* from 24 sites in 9 sub-Saharan African countries. We used Bayesian methods, and accounted for variation in treatment rates and reporting methods (active versus passive case detection). We estimate that passive case detection picks up 32% (95% credible interval: 18-56%) as many cases as daily active detection, and weekly detection 77% as many (95% CrI: 63-88%). However, there was wide variation in incidence between studies that cannot be explained by differences in case-finding or case definitions such as parasitaemia thresholds, and so substantial uncertainty remains in the incidence at any given transmission intensity. We estimate that at a parasite prevalence in 2 to 10 year-olds of 60%, 50% of cases occur in under-fives and 10% in over 15s; at a prevalence of 20%, 21% are in under-fives and 38% are in over 15s; and at a prevalence of 5%, 11% are in under-fives and 59% in over 15s. As our transmission model includes the principal control measures, these results will allow us to predict the impact of interventions on the incidence of clinical malaria.

1330

RATIONALE AND DESIGN OF CCM IN SARAYA DISTRICT: RESULTS AND IMPLICATIONS FOR POLICY IN RURAL SENEGAL

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Following the Abuja conference, malaria control strategies improved malaria patterns in Senegal with 3% of prevalence in outpatients in 2009. Figures hid disparities between Northern regions where morbidity was low and the southeast with high malaria incidence. Furthermore the data came only from health units only. To fill the gap in most under served areas, Community Case Management (CCM) was scaled up in rural Senegal with Community Health workers (CHW) and village volunteers called DSDOM. The objective of the study was to evaluate CCM at larger scale and identify policy implications for malaria. The project was held in Saraya district located in south east Senegal. It covers 6837 km2 for 102 villages with an estimated 40000 population; 70% lived at more than 15 km of the nearest health unit. CCM was done in 47villages with CHW or village volunteers, trained in malaria diagnosis and treatment. A Community Households cluster survey with Knowledge, Attitude, Practices (KAP) and Treatment seeking behavior were completed, quality of data assessed and traditional healers involved from June 2010 to December 2011. 683 heads of household were interviewed. Mosquito as the malaria vector was recognized by 81% of household heads, 93% cited mosquito nets as protective, RDT was well known (71%) and ACT as well (62%); 33% of respondents knew of potential adverse events, and use of LLINs the night before the survey was reported by 80% of respondents. In 73% of households 1 member was ill during the last 15 days, 91% had fever, and first visits were done by CHWs (34%), nurses (17%), DSDOM (15%) and traditional healers (7%); 44% of patients completed consultations within 24 hours. ACT was administered to 55% of patients. Few patients (N=52) sought second treatment mainly from CHW (36%) and traditional healers (18%). 15.491 visits were documented. 80% were reported by CHWs, about 50% were children under 10 years; 11.479 RDT were completed and 74% were positive; referrals (2005) were made and 6 deaths recorded. 36 traditional healers were visited by 67 patients. They referred 65 patients, 63% were RDT positive. The study revealed the central place of CHWs and the need to re-evaluate policy in under served areas, especially CHW status, the training curriculum and discussions on incentives to sustain filling the gap.

1331

IMPACT OF *PLASMODIUM FALCIPARUM* INFECTION ON HEMATOLOGICAL PARAMETERS IN CHILDREN WITH ABNORMAL HEMOGLOBIN LIVING IN A MALARIA ENDEMIC AREA OF BURKINA FASO

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Malaria is the most common cause of childhood morbidity in Africa, having varied haematological consequences. The high prevalence of HaemoglobinS/C is associated with the protection against malaria during childhood. Much less is known about the effect of HbS and HbC on malaria infection and haematological parameters. Susceptibility of the human host to malaria infection and haematological parameters has been reported to be influenced by some genetic factors as abnormal haemoglobin.The aim of this study was to evaluate haematological parameters in children less than fifteen years of age with abnormal haemoglobin genotypes and malaria infection. The study was conducted in 2008 in rural villages. It consisted of a combination of 2 cross-sectional surveys during the low and high malaria transmission. During each survey, each child was clinically examined, and thick and thin blood films were prepared for malaria diagnosis. The full blood count was performed with a haematology analyzer and an additional blood specimen was taken to determine the haemoglobin genotypes by PCR. In total, 406 children were recruited, 176 and 230 during the high and low seasons, respectively. Prevalence of Hb genotypes during the high and low season was: normal haemoglobin AA (76.7 and 65.7%) and abnormal haemoglobin (22.2 and 25.3%). There was no difference between the two groups in terms of leucocyte count and haemogloblin level if the subject was infected or not. However, during the low season, abnormal haemoglobin children without parasitemia tended to have higher lymphocyte counts (p=0.02), monocyte counts (p=0.02), red blood cell counts (p=0.03) and neutrophil counts (p=0.01), as compared to normal haemoglobin group. The platelet counts differed between the two groups for healthy children during the high season (p=0.002).The comparison of haematology parameters within haemoglobin type showed that basophil, lymphocyte and monocyte counts were significantly lower during high season. Basophil, eosiniphil, red blood cells, haematocrit, haemoglobin and monocyte counts in the malaria-infected normal haemoglobin group were significantly lower. In conclusion, these findings suggest that malaria parasites may affect the haematopoiesis of children living in malaria endemic area. Genetic factors, such as abnormal heamoglobin genotype, also influenced haematological parameters if subjects were not infected.

1332

ANTHROPOMETRIC GROWTH TRENDS DETERMINED BY WHO (2006) AND CDC (2000) GROWTH CRITERIA FOR CLINICALLY WELL INFANTS ENROLLED INTO A TRIAL OF SINGLE DOSE FANSIDAR FOR PRESUMPTIVE TREATMENT OF MALARIA IN RURAL NORTHEASTERN GHANA

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Current benefits of Fansidar-based intermittent preventive treatment for malaria during pregnancy (IPTp), and infancy (IPTi), prompted us to look retrospectively for evidence of a positive Fansidar effect on growth in clinically well young children that were randomized into a placebo-controlled, single-dose Fansidar trial at the peak of malaria season in rural Ghana. Growth charts from CDC (2000) and the newer WHO (2006) growth standards were used to determine within- and between-group differences at treatment baseline (Aug. 2001) and follow-up endpoint (Jan. 2002) among girls (n = 261) and boys (n = 237) enrolled in that four month trial. Weight-for-Age Z (WAZ) and Weight-for-Height Z (WHZ) scores derived from CDC growth charts were significantly lower (worse) than those derived from the WHO growth standards, while Height-for-Age (HAZ) Z scores were significantly higher by the CDC scale. Consequently, frequencies of underweight and wasting based on the CDC growth curve were greatly inflated over those derived from WHO standards and estimates of stunting were much lower by the CDC scale. Surprisingly, baseline and endpoint comparisons between sexes for mean WAZ, WHZ, and HAZ scores by both CDC and WHO growth criteria revealed better, more normative growth for girls-significantly improved over boys for all

three indices according to WHO criteria whereas by CDC criteria only HAZ was significantly improved. Endpoint comparison based on the newer WHO growth standards found no change in either sex for WAZ and % underweight, but WHZ and % wasting were improved significantly in boys. In boys and girls HAZ and % stunting worsened significantly; a result reflected by the two growth scales but which disappeared when sexes were combined. Analyses that combined sexes found no significant differences in growth indices that were associated with Fansidar or bednet effect, but comparison with a non-enrolled age-, sex-, and location-matched control revealed highly improved growth indices, by both CDC and WHO standards, for the cohort that had been subjects during this brief study.

1333

COMMUNITY HEALTH WORKERS AS AN EFFECTIVE CHANNEL FOR DELIVERY OF CHILD HEALTH INTERVENTIONS: EXPANDING THE KNOWLEDGE BASE

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A systematic literature review was conducted to assess the published and unpublished evidence on the effectiveness of strategies to improve community case management (CCM) of malaria. Specific objectives were to investigate interventions to (i) increase the coverage and quality of services provided by community health workers (CHWs) responsible for malaria case management; (ii) strengthen referrals from community to facility-based providers; (iii) increase the capacity of health systems (HS) to support CCM; and (iv) integrate malaria diagnosis and case management with other health services at the community level. Thirty-six studies were included in the review, the majority (32) reporting reasonably standard indicators of CHW performance. Findings show that CHWs are able to provide good quality of care, including performing simple procedures such as rapid diagnostic tests. Appropriate training and regular supportive supervision are important facilitating factors. However, crucial to the sustainable success of CHW programmes is the strengthening of HS capacity to support commodity supply, supervision, and appropriate treatment of referred cases. The little evidence available on referral systems from the community to health facility level suggests that this is a priority area that needs attention. There are few published studies on integrated CCM, although this is now the direction that policy and programmes are moving. Adding additional tasks does not reduce the quality of malaria CCM, provided sufficient training, supervision and support is maintained. However, with the exception of pneumonia treatment, reporting on the quality of delivery of additional interventions is limited. Amongst included studies, 11 reported on referral, 11 on HS capacity and 9 on iCCM; however not all data was quantitative and indicator definitions varied, making direct comparisons challenging. There is a need to encourage implementers to evaluate programmes robustly using standardised indicators and share their findings with other programmes to enable broader lesson learning.

1334

RISK FACTORS ASSOCIATED WITH MULTIPLE MALARIA INFECTIONS IN BANGLADESH

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Malaria is endemic in Bangladesh. In 2011, there were 51,773 episodes and 36 deaths attributed to malaria with most cases occurring in the Chittagong Hill Tracts (CHT), Bangladesh. This study identifies environmental and socioeconomic malaria risk factors and determines the spatial distribution of malaria in an endemic area in CHT. Longitudinal data on malaria incidence were collected from 1634 households in 54 villages (total population 7922) from January 2009 to December 2010. Hydrological, topographic, climatic and socioeconomic risk factors were used as potential predictors for malaria infection. Spatial malaria patterns were observed. Relative risk ratios were calculated to identify possible reasons for zero, one and >1 malaria infections in the study population. There were 509 malaria cases (6.4%) during the study period. These were distributed heterogeneously between villages. Children were most vulnerable to malaria infection. About 21.8% of homesteads accounted for all the malaria cases in the study area. The multivariate analysis with socioeconomic risk factors showed that bed net ratio (number of nets per person per household), ethnicity, house wall construction material, and household density had significant relationships with malaria incidence. Among the topographic and hydrological risk factors, households within two kilometers of a 4th order streams were at highest risk of malaria infection. In multinomial analysis belonging to the Bengali ethnic group, house walls made of mud and high household density were associated with a high risk for multiple malaria infections. High bed net ratio, belonging to the Tripura ethnic group, household heads having a non-specific ('other') occupation were associated with a low risk for multiple malaria infections. No clear relationship was observed between climatic and topographic parameters and malaria. Prioritizing the risk zones and identified risk factors will assist in cost effective targeting of malaria interventions and may contribute to a further reduction in malaria burden in the region.

1335

HEMOGLOBIN C TRAIT PROVIDES PROTECTION FROM CLINICAL FALCIPARUM MALARIA COMPARABLE TO THAT PROVIDED BY HEMOGLOBIN S TRAIT IN MALIAN CHILDREN

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Hemoglobin (Hb) C trait, like Hb S trait, appears to protect against severe malaria in children. Recent work examining whether Hb C trait also protects against uncomplicated malaria has produced conflicting results. We hypothesized that children with Hb C trait would have a longer time to the first clinical malaria episode than children with Hb AA in a cohort study of malaria incidence in Bandiagara, Mali. Three hundred children aged one to six years were enrolled in a longitudinal follow-up study of malaria incidence that included scheduled monthly blood smears and unscheduled follow-up for sick visits. Hb electrophoresis was measured at baseline. Excluding those participants with mutations for glucose-6-phosphate dehydrogenase deficiency, 216 children had Hb AA, 35 children had Hb AC, nine children had Hb AS, three children had Hb SC, and two children had Hb CC. Children with Hb AC had a longer time to first clinical malaria episode than children with Hb AA (P=0.002; 309 mean malaria-free days versus 227 days). Children with Hb AS also had a longer time to first clinical malaria episode than children with Hb AA (P=0.03; 334 mean malaria-free days versus 227 days). Children with Hb AC had fewer episodes of clinical malaria in a single season than did children with Hb AA (0.2 episodes versus 0.7 episodes, P=0.002). However, children with Hb AC or AS experienced the same number of anemia episodes (Hb<8.4 g/dL) as children with Hb AA. Children with Hb AS experienced more asymptomatic malaria episodes (1.44 episodes versus 0.57 episodes, P=0.009) and a lower cumulative parasitemia than children with Hb AC (P=0.02). Thus, while both Hb C and S traits exerted a protective effect

against clinical malaria episodes, they appeared to do so by distinct mechanisms that differentially affected a subject's response to infecting malaria parasites.

1336

HIGH BURDEN OF MALARIA IN UGANDAN ADULTS AND INCREASED RISK OF SEVERE MALARIA AND DEATH IN ADULT MEN AS COMPARED TO WOMEN

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Malaria prevention has targeted young children and pregnant women because of the disproportionate burden of disease in these populations. Few studies have assessed the frequency and severity of malaria in adult males. To examine the relative impact of gender and age on malaria outcomes, we conducted a chart review of all individuals admitted with a primary diagnosis of malaria to three Ugandan public hospitals between January 2000 and June 2005. The hospitals included Kabale Hospital (hypoendemic malaria transmission), Mulago Hospital (mesoendemic), and Soroti Hospital (holoendemic). 45,176 charts were reviewed. Adult males ≥14 years accounted for 17.2% (Kabale), 8.1% (Mulago) and 8.6% (Soroti) of all malaria admissions, but 35.3% (Kabale), 14.5% (Mulago) and 16.5% (Soroti) of all deaths in persons admitted with malaria. Among persons <14 years, there was no difference in the risk of severe malaria or death in males as compared to females in any hospital. In contrast, among persons ≥14 years of age, males had a significantly higher risk of severe malaria and death than females at two of the three hospitals (risk expressed as odds ratio, 95% confidence interval): Mulago (severe malaria, 1.31, 1.11-1.55, P=0.001; death, 1.54, 1.25-1.90, P<0.0001) and Soroti (severe malaria, 1.56, 1.34-1.82, P<0.0001; death 2.15, 1.72-2.68, P=<0.0001). Among persons admitted to Mulago Hospital with a primary diagnosis of pneumonia, risk of death was also higher in males than females in persons ≥14 years (1.50, 1.23-1.83, P<0.0001) but lower in males than females for persons <14 years (0.86, 0.76-0.98, P=0.02). Among persons ≥14 years of age hospitalized for malaria in Uganda, males have a significantly greater risk of severe disease and death. Given that adult males are largely neglected in malaria control and prevention efforts, further study is needed to understand the reason for this observation.

1337

GAMETOCYTE DYNAMICS IN AN AREA WITH SEASONAL MALARIA TRANSMISSION

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Renewed interest in malaria elimination has underscored the need for understanding malaria transmission. Gametocytes, the sexual stage of malaria parasites, are responsible for disseminating malaria infection. To better comprehend the epidemiology of gametocytes, including transmission reservoir and "hotspot", in an area with seasonal malaria transmission, we recruited 250 individuals, from ages of 3 months to 50 years, in Bancoumana, Mali. During one year, study subjects were surveyed for parasite carriage every 4 weeks. Children aged 5-15 years had gametocytes more frequently (8%) during their monthly visits compared with individuals in younger or older age groups (2.5-4%). This might be a consequence of a higher proportion of visits with infection in this age group (24% in 5-15 age group compared 7-13% in other age groups) as opposed to a higher probability of having gametocytes when infected. At the beginning of transmission season (July and August), individuals between 5 and 20 years of age were more likely to carry gametocytes than other individuals. However, at the peak of transmission (September through November), individuals from different age groups also presented gametocytes during scheduled visits. There was a strong correlation between proportions of visits with infection in a compound and proportions of visits with gametocytes in the same compound (P<0.01). There was evidence for familial aggregation of gametocyte positivity during follow-up (odds ratio 4 [95% CI 1.04 - 15.3]) but we could not rule out that this might be solely due to infection aggregation. Three compounds that represented 12.4% of the study population had 35.5% of all visits with gametocytes, mostly because of chronic infections in asymptomatic young children. Taken together, the data suggests that children aged between 5 to 15 years carry gametocytes more frequently; whether this is related to longer average duration of infection or to higher incidence of infection in this age group still requires further investigation. Similarly, the identification of factors present in compounds where most gametocyte positivity clusters would guide studies to understand malaria transmission dynamics and possibly the design of clinical trials to test transmission-blocking interventions.

1338

PLASMODIUM FALCIPARUM MALARIA HAS INCREASED IN BISSAU IN RECENT YEARS, MAINLY AMONG OLDER CHILDREN

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Plasmodium falciparum malaria was holo-endemic in Guinea-Bissau during the early 1990's.We have undertaken back to back clinical trials at the Bandim Health Centre in Bissau since 1994. The health centre serves the population living within the Bandim Demographic Surveillance Site and has been well staffed throughout the study period. The annual number of children aged <15 years, seeking medical attention with at least 20 *P. falciparum* per 200 leukocytes from 1994 to 2011 (except 2009) were as follows: 116, 214, 211, 172, 125, 377, 266, 301, 256, 343, 180, 172, 109, 40, 141, (no data 2009), 316 and 362. Data are lacking Jan-May 1994 (prior to study start), July, August and Nov 1998 (due to civil war), October 2006 (in-between studies) and the whole of 2009 (laboratory staff were not available). The median age of children the same years were; 48, 58, 47, 46, 43, 58, 61, 65, 57, 68, 60, 64, 61, 59, 79, (no data), 107 and 115 months. There was a significant increase of age between 1994 and 2007 as well as between 2007 and 2011 (non parametric test for trend p<0.0001 for both). The number of children aged 5-15 years with malaria were 188/377 (50%) in 1999, 20/40 (50%) in 2003 and 307/362 (85%) in 2011. The annual total rainfall varied with peaks of 1980 mm and 1839 mm in 2003 and 2010, respectively and a trough of 1085 mm in 2007. The overall decrease of malaria after the war in 1999 until 2007 (377 to 40 cases) is in line with findings in neighbouring The Gambia. The decrease was not due to artemether-lumefantrine, as the drug did not replace efficacious high dose chloroquine until 2008. Contrary to the situation in The Gambia, the number of children with malaria has increased ~9 fold since 2007 (40 to 362 cases). The increase consisted of a doubling (20 to 40) of cases amongst children under 5 years of age and a 15 fold (20 to 307) increase in children aged 5-15 years.

1339

MALARIA IN PREGNANCY IN SOUTHERN PROVINCE, ZAMBIA

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Malaria in pregnancy (MIP) in areas of stable malaria transmission is responsible for maternal anemia and adverse pregnancy outcomes such as low birth weight and preterm birth. In recent years, Zambia has received substantial funding for malaria and has scaled up activities to control MIP. This study assessed institutional capacity to prevent MIP and the utilization of MIP services in Southern Province of Zambia. We conducted comprehensive health center (HC) surveys in Southern Province, Zambia. Pregnant women recruited at the same HCs during routine antenatal care to participate in a neonatal study (ZamCAT) were interviewed at the time of recruitment and 4 days post-delivery about their use of MIP services. Of the 90 primary HCs surveyed, only 25.6% had a functional microscope, 16.7 % had supplies to prepare blood smear and 94.4% had rapid diagnostic tests (RDTs). In terms of antimalarials, 96.6% had oral quinine, 96.6% had artemether-lumefantrine and 87.8% had sulfadoxine-pyrimethamine (SP) for intermittent preventive treatment (IPTp) in stock on the day of the survey. Among 9,816 women interviewed, 55.3% reported sleeping under an insecticide-treated net (ITN) on the night before recruitment and 62.7% reported sleeping under ITN with the baby the night before the post-delivery interview. The average number of antenatal visits made by the women was 3.3; however only 52% received the Zambia Ministry of Health -recommended 3 doses of SP during pregnancy. Women who attended facilities that had SP available were 1.2 times more likely to have completed 3 doses of SP during pregnancy compared to women who attended facilities without stock (95% CI: 1.03, 1.40). Despite appropriate stocking of SP and an adequate number of antenatal visits by pregnant women, many women did not receive the recommended number of doses of IPTp, a situation of missed opportunities. An evaluation of factors responsible for the missed opportunities is needed to improve IPTp coverage.

1340

USING HEALTH MANAGEMENT INFORMATION SYSTEM DATA ON PARASITOLOGICALLY-CONFIRMED MALARIA CASES TO EVALUATE THE EFFECT OF VECTOR CONTROL COVERAGE

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Routine health management information system (HMIS) data are an under-utilized source for evaluating the effect of malaria control program intensity on the malaria morbidity burden. Since 2009, facilities in Zambia have reported both clinical and parasitologically confirmed (by RDT or microscopy) malaria through the HMIS on a monthly basis. We sought to evaluate the association between vector control coverage and monthly confirmed malaria cases at the district level in Zambia for the period 2009-2011. We first used Bayesian geo-statistical models to create smoothed estimates of insecticide-treated net (ITN) ownership from MIS data and to estimate differences in fever treatment-seeking behavior by district from 2009-2011. We incorporated programmatic data on the distribution of ITNs and indoor residual spraying (IRS) to improve district-level coverage estimates. We included mean monthly rainfall and temperature from remote sensing data to control for climate variability, and additionally controlled for differences in reporting and testing by district and month. We then modeled the association between confirmed cases and vector control coverage with conditional autoregressive models in a Bayesian framework to account for spatial and temporal correlation. After adjusting for reporting, total malaria outpatient cases increased from 3.3 million in 2009 to 4.3 million in 2010, and decreased to 3.8 million in 2011. Confirmed cases represented 29% of total cases in 2009, the first year parasitological confirmations were recorded in the HMIS, 30% in 2010, and 48% in 2011. After controlling for reporting, testing, climate, and district level factors influencing treatment seeking, we estimate that an increase in district level ITN coverage of 1 ITN per household is associated on average with a 19% reduction in population-standardized confirmed case incidence. We did not find an association with IRS. HMIS data, if improved through comprehensive parasitologically confirmed-case reporting, can become an important data source for evaluating associations between malaria program scale-up and spatial and temporal trends in disease burden.

1341

CLINICAL AND LABORATORY FEATURES OF SEVERE MALARIA IN PERU

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Malaria is a vertor-borne disease considered as one of the main public health concerns by the World Health Organization (WHO). It cause close to 1 million deaths annually. For this reason, WHO established criteria to define severe malaria (SM), in order to reduce the morbidity and mortality. These criteria were based on features of severe malaria cases due *Plasmodium faliparum*, however reports of SM due to *P. vivax* have being rising during the last years. Vivax ans *falciparum* malaria are endemic in Latin America, but the features and prognosis of SM caused by them are poorly characterized. We describe the epidemiological, clinical and laboratory features of patients with SM, in a national reference center of an endemic area of malaria as Peru. Case reports. Patients admitted at Hospital Nacional Cayetano Heredia (HNCH), from 2005 to 2011, with diagnosis of SM according to the 2000 WHO guidelines. The inclusion criteria was to have a full medical record. We identified 40 cases of SM, from which 34 full medical records were available. The mean age for these patients was 39 years (range from 14-64 years) and the male/female ratio was 2.8. Most of the cases came from the Amazon region (47.1%), and few imported cases from Africa (14.7%). *P. vivax* was the most common agent identified in 70.6% of our patients, followed by *P. falciparum* (17.6%) and mixed infection (11.8%). Among the criteria of severity showed by this group of patients, 54.2% (13/34) presented with jaundice and hyperbilirubinemia, followed by 47.1% (16/34) with severe thrombocytopenia, 32.4% (11/34) with hyperpyrexia and 14.7% (5/34) with shock. Only one patient, with renal failure, respiratory insufficiency and multifactorial refractory shock, died of SM caused by *P. falciparum*. No fatal case of *P. vivax* was reported. *P. vivax* is a frequent cause of severe malaria in countries of the Latin America region such as Peru, even if it is not the most common agent reported in the worldwide. The most common complications were liver injury, severe thrombocytopenia, hyperpyrexia and shock.

1342

COSTING A LARGE-SCALE IMPLEMENTATION OF SEASONAL MALARIA CHEMOPREVENTION IN CHILDREN DELIVERED THROUGH COMMUNITY HEALTH WORKERS IN SENEGAL

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Seasonal Malaria Chemoprevention in children (SMC) is a new strategy for malaria control in areas where transmission is strongly seasonal. In Senegal, a pilot implementation of SMC was conducted by four district health teams from 2008 to 2010 in order to evaluate the feasibility of delivering SMC, its safety and effectiveness, when administered on a large scale using community health workers (CHWs). In 2010, SMC was delivered by 46 health-posts to a rural population of 175,000 children under 10 years of age in 1097 villages, and costing data were collected from each health facility in order to estimate the financial and economic costs of delivery. Delivery was coordinated by the head nurse in each health-post who assigned CHWs to a circuit of villages to visit over a 5-day period in September, October and November, to deliver SMC house to house to all children 3-120 months of age. Tools were developed to collect data on costs and resource use at four levels: the project, the district, the health post, and the CHW. Data was collected from both "top-down", and "bottom-up" (using facility-based costs and extensive interviews on resource use). Data were collected from all 46 health-posts after each round of administration. The study takes a provider perspective with a focus on costs of SMC at the district level. Each health-post employed from 4-68 CHWs and delivery each month took from 2-5 days. High coverage was achieved with about 90% of eligible children treated each month. When the financial cost of delivery was estimated, it cost $233,714 to administer SMC to a population of 175,000 children under 10 years of age at a cost of $0.50 per course. The main cost driver was the incentives paid to CHWs (44%). High coverage of SMC can be achieved at moderate cost. As SMC is now a recommendation from the World Health Organization and each year CHWs may visit households a number of times for distribution of Vitamin A, bednets, mass vaccination and other programs, this will be an opportunities for economies of scope by combining SMC with delivery of other interventions.

1343

OPERATIONAL METHODS TO OBTAIN GEOLOCATION INFORMATION TO TRACK COMMON DISEASES FOR PATIENTS PRESENTING AT HEALTH FACILITIES IN AREAS WHERE ADDRESSES ARE NOT AVAILABLE: A CROSS-SECTIONAL SURVEY IN FIVE HEALTH FACILITIES IN THE WESTERN KENYAN HIGHLANDS

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The spatial distribution of cases is an important component of understanding the epidemiology of diseases, including malaria, and is valuable in planning and evaluating disease control. Spatial surveillance of cases facilitates targeted control, monitoring for potential epidemics, and evaluation of spatially heterogeneous transmission levels. In countries where no organized network or geocoded database exists, locating where patients come from can be problematic. Obtaining individual coordinates for a health facility attendee is operationally unattractive and less labour intensive methods should be developed that can accurately locate individuals. Such a system would facilitate research and enable disease control interventions to be targeted. To do this, we explored operational approaches to geolocate health facility attendees and determined their relative accuracy. We conducted a cross-sectional survey for malaria in 5 health facilities in the Western Kenyan highlands in October 2011. Of the 1659 people sampled, approximately 30% were followed-up to their compound with coordinates recorded. Information on various geolocation strategies was collected: 1) nearest landmarks to the compound as indicated by the patient, 2) patients identifying the names of heads of compound and 3) of nearest neighbours as well as 4) asking patients to indicate their area of residence on a poster sized satellite image. The effectiveness of the methods was assessed using ArcGIS to create zones around the landmarks where people are more likely to come. A database from previous studies in the area was used as a baseline and the proportion of participants followed-up during the health facility survey that were correctly located was calculated. Preliminary results indicate that of the names of the head of compound and nearest neighbors that were matched, 60% of patients were geolocated to within less than 500 meters of their compound. The results on the optimum approach, or combination of approaches to achieve the most accurate method to the finest possible resolution as assessed by spatial area and population density will be discussed.

1344

CHANGING OF MALARIA PREVALENCE AND AGE OF INFECTED CHILDREN IN DIFFERENT AREAS OF GABON FROM 2005 TO 2011

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In Libreville, the capital city of Gabon, a reduction of malaria prevalence and a trend towards a higher risk of *Plasmodium falciparum* infection in children aged more than five years have been reported after new malaria control strategies implementation. With the support of Global Fund allocations, Malaria national control program organized the deployment of bednets and Artemisinin based combination therapeutic within the country from 2005. The aim of the study was to estimate the disease burden among children and to characterise malaria transmission intensities based on $PfPr_{2-10}$ in various areas of Gabon. Prospective cross sectional surveys were conducted at the Malaria Clinical Research Unit in Libreville and in four public health facilities at Melen, Port_Gentil, Oyem and Owendo. Febrile pediatric patients, aged less than 11 years old were screened for malaria using microscopic examination. A total of 14293 febrile children were enrolled; 78.5% were less than five years. Between 2005 and 2008, there was a significant drop of malaria prevalence from 35.1% to 16.8%; followed by a raise reaching 26.6% in 2011. Before 2011, $PfPR_{2-10}$ was low in urban areas: 20% at Libreville in 2005 and 2008 and under 5% in 2005 at Port-Gentil. In the rural and semi urban areas of Oyem and Melen, it was above 40.0%. The mean age of infected patients, increased from 37.0 to 48.0 months between 2005 and 2008. From 2008, children above 5 years old were the most infected in all sites. The risk of being infected in this group was 3.21 fold to 5.05 fold higher in urban areas. These data confirm a shift in the age of infected patients towards older children and a large heterogeneity of malaria epidemiology suggesting the need to maintain malaria control strategies in Gabon; and to redefine their implementation throughout the country.

1345

THE IMPACT OF ACADEMIC DETAILING ON PRESCRIBING AND ACCESSIBILITY OF ACTS IN THE PRIVATE SECTOR IN MADAGASCAR

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Madagascar is participating in the first phase of the Affordable Medicines Facility for malaria, a multi-national subsidy for artemisinin-based combination therapies (ACTs). It is unclear, however, whether subsidized drugs will reach rural areas of the country without additional intervention. We piloted a supporting intervention to encourage prescribing, stocking and purchasing of subsidized ACTs in rural areas of Madagascar by employing "academic detailers" to share scientifically accurate knowledge about ACT effectiveness with doctors and shopkeepers. Baseline cross-sectional surveys on factors related to prescription practices and antimalarial stocking were conducted in five regions of Madagascar in July 2011, covering 160 medical providers and 234 outlets. Additionally, exit interviews were conducted with antimalarial drug shoppers at 128 outlets to identify drug choice. Doctors and outlets in intervention regions were visited by academic detailers with educational ACT materials from October 2011 to March 2012. At baseline, 80.9% of urban outlets were stocked with subsidized ACTs compared to 50.3% of rural outlets. About 80% of providers reported ever prescribing ACTs. Of 279 customers interviewed at outlets that stocked subsidized ACTs, only 27% purchased them. Logistic regression models suggested purchase decisions were predicted by ACT awareness, urban versus rural location, and whether or not the outlet was visited by a representative for subsidized ACTs in the six months prior to the interview. These results suggest the potential for a low-cost intervention involving academic detailers to improve the proportion of treatment-seekers who receive effective antimalarial drugs.

1346

MALARIA AND ANEMIA PREVALENCE AND INSECTICIDAL NET OWNERSHIP AND USE IN PLATEAU AND ABIA STATES, NIGERIA (2010): RESULTS FROM REPRESENTATIVE HOUSEHOLD SURVEYS

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There have been few recent surveys of malaria prevalence and net coverage in Nigeria. In September 2010, The Carter Center worked with the ministries of health of Abia (Southeast Nigeria) and Plateau (North Central Nigeria) states to conduct a modified Malaria Indicator Survey prior to mass LLIN distribution. In 58 systematically selected clusters (census enumeration areas or segments thereof) of 25 households per state, the average household size was 4.4 persons in Abia (1305 households, 5754 persons) and 6.2 in Plateau (1337 households, 8312 persons). All children <10 years of age were tested for malaria and anemia, and persons of all ages in every third household were tested for malaria. The percentage of households owning ≥ 1 net was much lower in Abia (10.2%) than Plateau (34.8%). The majority of nets were LLIN: 68% (N=123) in Abia and 89.6% (N=489) in Plateau. The percentage of persons using nets the previous night were: Abia: 3.4% of all ages, 6.0% of children under 5 years and 3.6% of pregnant women; Plateau: 14.7% of all ages, 19.1% of children under 5 years, and 21.0% of pregnant women. Crude malaria prevalence by RDT was 36.2% in Abia (95% CI 30.5-41.8, N=2619) and 40.5% in Plateau (95% CI 33.7-47.7, N=4242). Age specific prevalence peaked in the 5- 9 year age group at 47.5% in Abia and 58.9% in Plateau, with second highest prevalence among 10-14 year-olds (Abia 43.0%, Plateau 50.1%). The percentage of children <10 with moderate to severe anemia (hemoglobin < 8 g/dl) was higher in Abia (13.2%, 95% CI 10.3-16.8%, N=1556) than Plateau (5.1%, 95% CI 3.9-6.5%, N=2835). The results reveal high malaria prevalence in these states, and low baseline net ownership. Additional work is needed to explain the fact that anemia prevalence is higher in Abia, though malaria prevalence is comparable to Plateau. A possible contributing factor could be differences in treatment coverage for neglected tropical diseases. Nigeria's universal coverage distribution policy and on going national LLIN distribution campaigns should increase access to LLIN among children 5-14 years of age, but other determinants of use in this age group remain poorly understood.

1347

LOOKING FOR GOLD, FINDING MALARIA: 2011 MALARIA SURVEILLANCE IN GOLD MINERS' COMMUNITIES IN SURINAME

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Despite the marked reduction of malaria incidence in Suriname, malaria continues to affect the migrants' population (n= 15,000) involved in gold mining. Miners have been trained in the use of RDTs and treatment of

uncomplicated malaria to provide services in their communities. Blood films are prepared for the quality control of all RDTs performed. They report to the Tourtonne laboratory (TL). The TL in the epicenter of the Brazilian gold miners' community in the city is the other component of malaria surveillance in gold miners' communities. The TL staff executes Active Case Detection Campaigns on a regular basis in gold mining areas. The surveillance data serves as the basis of this paper. In 2011, 646 cases were recorded, representing a decrease of 54% (p< 0.0002) from the 1403 recorded in 2010. *Plasmodium falciparum*, *P. vivax* and *P. malariae* were identified in 42.7%, 49.8% and 2.5% of cases respectively. 5.0% had a mixed infection. 484 (75%) cases were imported; the 162 autochthonous cases signify a reduction of 66.3% compared to the 480 reported in 2010. Of the autochthonous cases, 83 (51.2%) were acquired in the Lawa region, 48 (29.7%) around the Lake, Tapanahoni had the lowest number of casses 1 (0.6%). The 162 cases were dispersed over 44 locations. Only 3 (6.8%) locations, all on the Lawa River had more than 10 cases; 47.7% of the locations had only 1 malaria case in 2011. The mean prevalence measured during ACDs was 1.9% (0% - 6.0%). The SPR was 15.1%, ABER 19.9% and API 10.8 per 1000. 97.4% of the infections occurred in Brazilians. 22 cases were reported in pregnant women of which 6 were *P. vivax* relapse. One possible explanation for the tremendous reduction in malaria cases from 2010 to 2011 could be the fact that LLINs have been distributed in 2010 among the populations at risk in the gold mining areas. We have to find innovative ways including cross-border cooperation to deal with the high incidence of imported cases in Suriname.

1348

THE ESSENCE OF ACCURATE SURVEILLANCE IN A LOW INCIDENCE ERA: REASSESSING AUTOCHTHONOUS CASES THROUGH MALARIA CASE INVESTIGATION IN SURINAME

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The persistent low prevalence measured at any time during 2010 and the low incidence through 2010 of autochthonous cases recorded by the malaria notification points in mining areas brought the authors to the hypothesis that malaria transmission in Suriname is lower than is being captured by the regular surveillance system at the Tourtonne laboratory (TL) in the city. The Malaria Case Investigation form (CI) used by the Bureau of Public Health extended with questions relevant to gold miners was introduced at the TL in February 2011. The CI form captures amongst others, a detailed travel history. The travel history, malaria endemicity and the incubation period for the different species were taken into account to classify the cases either as imported or autochthonous. To test the hypothesis the classification by CI was compared to the classification by general surveillance. 415 malaria cases were diagnosed at TL from February through December 2011. According to the regular surveillance 94 cases were classified as autochthonous. 376 forms were completed, representing 47.2% of all cases (n=797) diagnosed in Suriname. 53 (n=376) cases were classified as autochthonous based on the CI forms. The proportion of autochthonous cases 14.1% based on the CI form was lower (p< 0.003) than the proportion (22.7%) calculated from the general surveillance data. Several possible explanations might account for this difference, including the fact that *Plasmodium vivax* with the ability to relapse if not treated radically, is the predominant infection in Suriname. Self-medication could suppress clinical symptoms and favors the relapse of *P. vivax* and recrudescence of *P. falciparum*. If the patient acquiring an infection abroad stays long enough in Suriname and develops symptoms, the infection might erroneously be recorded as an autochthonous case. Since information on the possible location of transmission is identically captured by all the malaria surveillance systems in Suriname, the authors assume that the over estimation of autochthonous cases is country wide.

1349

MALARIA CASE INVESTIGATION AT THE TOURTONNE LABORATORY

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Malaria incidence in Suriname has decreased tremendously in the past decade. In order to get a better understanding of the malaria epidemiology and the habits of the persons at risk the need for detailed information on every case becomes more pressing. The Malaria Case Investigation form (CI) used by the Bureau of Public Health extended with questions relevant to gold miners was introduced at the Tourtonne Laboratory in February 2011. The CI form captures amongst others, detailed information on the travel history, symptoms and medical history. Also basic knowledge on malaria prevention is evaluated and the health seeking behavior is assessed. 415 malaria cases were diagnosed at TL from February through December 2011. 376 (90.6%) forms were completed, representing 47.2% of all cases diagnosed in Suriname. Vendors (35%) and gold miners (21.4%) were the groups most affected; CSW 3% were the group least affected. The mean interval between onset of symptoms (Sx) and testing was 5.9 days. The mean interval between onset of Sx and treatment was 6 days. 54.8% (n=312) of the patients used self-treatment. 34.1% (n=314) did not know that malaria is transmitted by a mosquito. 49.4% did not know how to protect oneself against malaria. 13.3% (n=369) used a bed net. Conveying the importance of adhering to appropriate preventive measures for malaria and seeking early detection and effective treatment is a prerequisite to sustain the reduction of malaria. The use of ACT for self-treatment is a concern since it contributes to the emergence and spread of resistance.

1350

MALARIA IN PREGNANCY IN RWANDA AS THE COUNTRY TARGETS PRE-ELIMINATION

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Rwanda has made strides toward lowering malaria transmission with universal coverage of long-lasting insecticide treated nets and easy access to artemisinin based combination treatment. National prevalence is estimated at 1.4% among children 6-59 months and 0.7% among women aged 15-49 years according to the 2010 DHS. Slide positivity rates from the national health management information system continue to drop and yet malaria persists. Pregnant women thus remain vulnerable even as prevalence drops. While Rwanda no longer practices IPTp it is concerned it is interested in offering the best malaria protection to pregnant women. In order to plan appropriately, there is need for a malaria in pregnancy revalence study. Pregnant women were studied at first antenatal care registration visit in low, moderate and relatively higher transmission areas using rapid diagnostic test and microscopy. Ethical clearance was provided by the ethical review board within the Ministry of Health. ANC staff were trained to obtain data during normal client visits. Among nearly 4000 women studied, prevalence with RDT was 2.4% ranging from 6.6% in the higher border districts in the east to 0% in the areas designated as low transmission based on the HMIS. For microscopy the overall prevalence was 1.6% and also varied from 4.5% to 0.1%. RDT positivity showed reducing trend with increasing parity and with LLIN use the night before the interview. Results show need to continue to protect pregnant women and their unborn children in Rwanda through increased use of LLINs and identification and tracking women of low parity.

1351

PLASMODIUM FALCIPARUM PARASITE CLEARANCE IN PATIENTS TREATED WITH ARTESUNATE-AMODIAQUINE VS. COMPARATOR GROUPS, SUB-SAHARAN AFRICA

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Monitoring response to artemisinin combination therapy (ACT) worldwide is particularly important now that artemisinin resistance is reported in Southeast Asia. Delayed parasite clearance is considered the best practical surrogate for artemisinin resistance. Artesunate-amodiaquine (ASAQ) is the second most widely used ACT. We analysed 11,570 patients (81% children under 5 years of age) from 41 sites from 20 countries sub-Saharan. The median parasite clearance on ASAQ by site varied from one to two days; a third of the patients cleared their parasitaemia on Day 1; the decrease in mean log parasitaemia between Day 0 and Day 1 was -58% (range: -44% to -83%); between Day 0 and Day 2 it was -96% (-77% to -100%). Using multivariate logistic regression with random effects and controlling for treatment, the risk for a delayed parasite clearance (still parasitaemic on Day 2) was higher in children under five (AOR 1.34, 95%CI 1.10-1.63, p=0.004) as well as in patients with higher parasitaemia at enrolment (AOR 2.56, 95%CI 2.26-2.90, p=0.001). No difference was detected between ASAQ and other ACT (artemether-lumefantrine, dihydroartemisin-piperaquine, AS+SP), but non-ACT (AQ, AQ+SP, chloroquine+SP) carried a higher risk of delayed parasite clearance ($p<0.005$ for all comparisons vs. ASAQ). The analysis provides a platform for future comparisons of antimalarial performance across sub-Saharan Africa.

1352

THE DEMOGRAPHICS OF WITHIN-COUNTRY POPULATION MOVEMENT NETWORKS IN EAST AFRICA: IMPLICATIONS ON MALARIA TRANSMISSION AND CONTROL

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Human population movement plays an important role in the transmission and importation of malaria. Movement between areas of differing transmission may risk importation of infection from high to low transmission zones. Different demographic and socioeconomic groups are likely to have different movement patterns and infection rates and therefore different risks of importing infections upon travel. It is therefore relevant to quantify and compare movement patterns between varying transmission areas, for different sub-populations. At a national level, household surveys and population census data provide records for individual-level migration. Together with malaria endemicity maps, population distribution maps, mathematical models and network analysis tools, Kenyan, Uganda and Tanzanian migration data was analysed to construct within country population movement networks, useful for malaria importation assessment. The models were further stratified for different demographic and socioeconomic groups to identify and compare movement patterns relevant for malaria importation. Network characteristics, such as cumulative degree distributions and network diameter, were calculated to quantify and compare network structure. Movement networks were different between countries and between demographic and socioeconomic groups. Some demographic groups however, were had similar network characteristics. For example, children under 10 years and adults between 15-24 years had overlapping cumulative degree distributions, illustrating that children were likely to move with their parents. After including malaria in the movement analysis, certain population groups were more likely to contribute to imported infections in certain geographical locations. Census and survey data include migration and demographic data useful for nationwide population movement assessments. Together with national malaria maps and quantitative techniques, malaria importation estimates provide a unique evidence base to inform control policy.

1353

COMMUNITY ACCEPTANCE OF LARVICIDING FOR MALARIA CONTROL IN RURAL TANZANIA

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Larval source management, including the application of larvicides, is a lesser-used intervention for malaria control yet holds promise as a safe, effective, and environmentally sustainable component of a successful integrated vector management strategy. Recent research has supported the feasibility and effectiveness of larviciding in the urban setting of Dar es Salaam, but its application in rural areas remains understudied. One key element of determining feasibility of larviciding in a rural setting is community acceptance of the method. Community acceptance of larviciding in rural east-central Tanzania was assessed through a range of methods in April-May 2011, including surveys of 962 randomly-selected households from 24 villages, 12 focus group discussions, and in-depth interviews with local leaders and community health workers in each village. The household survey found that the majority of household heads surveyed (82.3%) were not familiar with larviciding as a way to control mosquito larvae in water bodies. Most households (93.8%) indicated that they would grant permission for larvicide to be applied in water bodies where mosquitoes breed near their homes based on a brief standardized description of the process. There was a high level of trust in the safety (74.6%) and efficacy of larviciding, both to control mosquitoes around the home (92.6%) and to reduce the risk of malaria infection (92.9%). Survey questions following up on these attitudes using a Likert scale allow for a more nuanced interpretation of villagers' perceptions. Also, in structured key informant interviews, respondents indicated that community members would be receptive to larviciding in the area, but that community sensitization efforts should be a key component of such an intervention. Household surveys indicated a willingness among community members to make a nominal household contribution (1800 TZS on average, or $1.20 USD) every 3 months. Overall the results of the assessments indicate a receptive environment for future efforts directed at larviciding for malaria control in a rural setting in Tanzania.

1354

EFFECTIVE PARTNERSHIP DURING HOUSEHOLD CAMPAIGN WITH HANG UP OF INSECTICIDE TREATED NETS MAKE NETS AVAILABLE TO PEOPLE IN GHANA

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Malaria continues to be the cause of significant morbidity and mortality in the country. In 2011, there were Cases and attributed to malaria in Ghana. Insecticide treated nets (ITNS) have been shown avert about 50% of malaria cases. In order to increase ownership and use of ITNs, a household door-to-door campaign to distribute ITNS and hang them in households was instituted. A partnership made up the government, multilateral, bilateral, non-governmental agencies, private sector, political heads, chiefs and elders and the community was formed to ensure the implementation of this campaign. To paper is to describe the partnership at play during the hang up campaign. Partnership started right from the planning stage through the implementation to the post implementation evaluation stages. Looking at the financial and technical capabilities of partners, roles were agreed on and assigned to ensure a coordinated activity. Some partners procured specific quantities of ITNS to cover particular sections the country; others provided funds for procuring other logistics, whilst others provided technical support for quantification, registration, supervision and evaluation. The Chiefs and political leaders contributed through advocacy, conflict resolution and transporting the logistics to the needed sites for distribution. The household campaign, which involved community sensitization, training, registration of households, actual hanging, supervision and monitoring has been undertaken in nine out of ten regions in the country. By end of April 2011, 9,683,160 ITNs had been hanged in the homes of 19,366,320 in the country. Through the effective partnership at play, Ghana is likely to achieve universal coverage of ITNS by July 2012. Resources can be mobilized with the appropriate partnership to achieve health targets and objectives.

1355

INVESTIGATING MALARIA VECTOR-PARASITE GENOTYPE-GENOTYPE INTERACTIONS AND HOW THEY MIGHT INFLUENCE THE USE OF GENETICALLY-MODIFIED MOSQUITOES IN MALARIA CONTROL

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Typically, malaria transmission models do not consider genotypic structure in the mosquito or the *Plasmodium* population. However, there is evidence that the interaction between malaria parasites and their *Anopheles* vectors is dependent on the specific genotype-genotype (g2g) combination. Here, we initially develop a simple 2 vector - 2 parasite malaria model to explore the impact of g2g interactions on transmission dynamics. This model is then extended to include a greater number of vector and parasite types. In particular, we assume that transmission (from human to vectors) and vector mortality rates are specific for each vector-parasite g2g combination. Motivated by results from experimental infections, we consider whether there is an evolutionary trade-off between transmission and virulence (to the vector) for each vector-parasite combination, and the conditions under which both parasite and vector types can co-exist. The more complex model is used to investigate how introducing a genetically-modified (GM) *Anopheles* population into the system affects the abundance of the other mosquitoes and parasites. Specifically, we consider the case where the GM mosquito is refractory to most, but not all, of the parasite strains. We investigate under which conditions it would be possible for the GM mosquito to replace all other *Anopheles* of the same species, or the conditions under which all malaria parasites could be eliminated. Applications of this work will be helpful to assess the feasibility of using GM mosquitoes to reduce or eliminate malaria in the presence of genotype interactions.

1356

HOW MANY BEDNETS PER HOUSEHOLD NEED TO BE DISTRIBUTED? EVALUATION OF A UNIVERSAL COVERAGE BED NET DISTRIBUTION CAMPAIGN IN FOUR DISTRICTS IN SOFALA PROVINCE, MOZAMBIQUE

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Malaria remains a priority public health problem in Mozambique. The National Malaria Control Program (NMCP) is conducting universal coverage (UC) distribution campaigns for long lasting insecticide-treated nets (LLINs), among other control measures. However, given the lack of a standard UC distribution model, countries embarking on UC distribute different number of LLINs per household. Setting a fixed number of LLINs per household (HH) is a common strategy, with the risk of these being insufficient or excessive to cover all family members or sleeping spaces. The NMCP piloted a new UC distribution model in 4 districts in Sofala Province (Central Mozambique), using information gathered from the community on the HHs composition (sex, age and relation among each HH member) to determine the number of LLINs to be allocated to each HH based on assumed sleeping patterns. The objective of this model was to maximize the efficiency of the LLIN campaign and cover all sleeping spaces. We conducted an evaluation of these sleeping patterns assumptions, the coverage of sleeping spaces with LLINs (ownership coverage) and the individual use of LLINs by household members (usage coverage). A community-based two-stage cluster random cross sectional survey, including 35 clusters and 32 households per cluster, was performed in May 2010, shortly after the LLIN distribution, and one year later, in June 2011, in the area where the UC campaign had been conducted. Informed consent was obtained from the head of each selected HH and a standardized questionnaire was filled out with information on the LLIN ownership and frequency of use as well as the number of HH members and their sleeping patterns. Analysis of data is ongoing and results will be available in October. This information will be used to validate the assumptions of the distribution model (assumed sleeping patterns within a HH) and to evaluate the effectiveness of the model in covering all sleeping spaces with an LLIN right after the distribution campaign and one year later, as well as to assess other uses of the nets.

1357

INCREASING ACCESS TO MALARIA PREVENTION IN SOUTH SUDAN BY INTEGRATING NET DISTRIBUTION AND INTERMITTENT PREVENTIVE TREATMENT WITH ANTENATAL CARE AND IMMUNIZATIONS

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Increasing access to malaria prevention by integrating malaria control and prevention services with existing services like antenatal care and routine childhood immunizations is feasible in a post-conflict country like South Sudan where malaria is the leading cause of morbidity and mortality. Pregnant women and children under five are especially at risk. Sleeping under a long-lasting insecticide-treated net (LLIN) may reduce child mortality by as much as 20%. Intermittent preventive treatment

(IPT) reduces the risk of malaria during pregnancy, which may cause complications such as anemia or illness for the mother, and low birth weight or spontaneous abortion for the fetus. In 2010, a household survey reported only 51.6% of pregnant women received one dose of IPT, and only 22.7% received the two doses (IPT2) recommended by the World Health Organization. The second phase of the USAID-funded Sudan Health Transformation Project (SHTP II) targets children and pregnant women by distributing LLINs during antenatal care (ANC) visits as well as during routine immunization for children under five. Between April 2010 to December 2011, SHTP II-supported facilities distributed 79,885 LLINs during routine immunization and ANC visits. There were corresponding increases in ANC1, DPT3 (immunization indicator), and LLIN distribution, culminating in a 97% increase in net distribution. Although an influx of refugees during the 2011 referendum resulted in intermittent stock outs of LLINs, numbers of ANC and DPT3 immunizations continued to rise, demonstrating a consistent increase in access to services and awareness. To prevent malaria during pregnancy, IPT2 services were integrated into ANC visits. IPT2 services increased by 38%, from 4,815 to 6,636 after the first two years of the project. A corresponding increase during this time period was noted in both ANC1 and ANC4 visits (7,638 to 10,301, a 35% increase, and 3,284 to 5,743, a 75% increase, respectively), showing a greater access to primary care as well as an increase in the perceived importance of ANC as well as malaria prevention. The conclusion is that is possible to make significant progress on malaria prevention in a challenging post-conflict, fragile state like South Sudan, by focusing on key interventions that can be integrated with existing services like antenatal care and immunization programming.

1358

BIOINFORMATICS SYSTEMS FOR UNDERSTANDING MALARIA TRANSMISSION AND CONTROL

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Innovative control strategies that target the entire mosquito life cycle may be required to achieve malaria elimination. Researchers need to analyze huge quantities of ecological data collected from multiple experiments to understand malaria transmission for the development of control strategies. The data preparation process for analysis is very time consuming. We present a bioinformatics system for understanding malaria transmission and control that integrates mosquito densities, infectious status, phenotypic observations, and sample archiving with capabilities to securely store and share data. A relational database schema is designed based on commonly used procedures by mosquito entomologists, which are experiment design followed by sample sorting, observation, constitution, and archiving. Our system handles the data preparation process by providing users with the ability (1) To upload raw data using standardized customizable templates, (2) To download cleaned data for analysis, (3) To generate summarized scientific reports, and (4) To archive and share data locally and globally. Our secure bioinformatics system reduces data preparation time, thus increasing research output. The system provides researchers with field and lab mosquito data rich in information such as densities, species type, and infectious status to address different scientific questions. Researchers upload data using customizable templates that handle data collected using different portable or paper based field collection forms but adhering to standardized terminologies. Users can download cleaned data linking a sample from the field, to the lab, and to a storage location with a data dictionary for analysis. Also, researchers are able to share data and/or to generate quick summaries such as mean catches per mosquito species per infectious status. The system is securely accessible online, but users may opt to run the system locally for data uploading, cleaning, and linking. Our relational schema is extensible to store and link other data such as environmental data and easily can be linked to other databases e.g., demographic surveillance systems (DSS). An extensible bionformatics system for understanding malaria transmission and control is developed to increase research output. Our system allows users to store field- and lab-based mosquito data, download them for analysis, and share them, with an ability to generate quick reports.

1359

EVIDENCE-BASED BEHAVIOR CHANGE COMMUNICATIONS (BCC) ENHANCE LONG-LASTING INSECTICIDAL NET (LLIN) UPTAKE AND UTILIZATION IN SOUTHEAST NIGERIA

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Long-lasting insecticidal net (LLIN) ownership is often the strongest determinant of net use, but having a net does not guarantee use. Net distribution should be accompanied by evidence-based interventions to address other key determinants. To inform the development of BCC strategies, we asked about social and behavioral determinants of net use during a 2010 survey of 1290 adults in 1192 households located within randomly selected clusters in Imo and Ebonyi States (Southeast Nigeria). Knowledge that mosquitoes transmit malaria was widespread (83%), but 66% reported that malaria was only a risk during the rainy season, and 65% that malaria is caused by eating certain foods. Though 72% reported that LLINs protect against mosquito bites, only 15% mentioned malaria prevention as a benefit. When asked about disadvantages of LLINs, 42% said there are none, but 15% said they were hot and 5% that they cause allergies. More people agreed with the statement that LLINs are safe to sleep under (90%), than that it is safe to hang them where you store food (54%). Only 2.4% knew that LLINs do not need re-treatment. Nets have some negative connotations: 39% agreed that they are "old fashioned;" 33% that they are for poor farmers; and 27% that they are a Western plot to reduce African populations. Low literacy (46%), limited comprehension of languages used for malaria communications, and widespread distrust of many sources of information suggested that home visits by trusted community members would be the most appropriate channel for BCC. The data informed the development of a community-based net monitoring and BCC intervention piloted in six sentinel villages in Ebonyi State in 2011 to improve LLIN ownership, use and care. We stressed the safety and effectiveness of LLINs for both malaria and lymphatic filariasis prevention, and taught skills to make it easier to hang nets at the appropriate height, over any sleeping space. Messages were tailored to fit household behaviors and barriers to use. After six months, 100% of households owned ≥ 1 net (N=1240); 95% of nets were hanging and 94% had been used the previous night (N=2982). 97% of people reported net use (N=5912). All were statistically significant improvements from baseline. Household net data collected by community volunteers provided motivation and direction for an LLIN "mop-up" campaign. This strategy can be modified for implementation by community directed distributors of treatments for neglected tropical diseases.

1360

THE AFFORDABLE MEDICINES FACILITY-MALARIA (AMFM): ARE REMOTE AREAS BENEFITING FROM THE INTERVENTION?

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In most cases, remote areas are less likely to be covered by health interventions despite often exhibiting the worst health indicators. One aim of the Affordable Medicines Facility - malaria (AMFm) is to ensure that people in remote areas have access to effective and affordable malaria treatment by making subsidized quality - assured artemisinin-based combination therapies (ACTs) available in these areas. AMFm, hosted by the Global Fund to Fight AIDS, Tuberculosis and Malaria, is a financing mechanism which subsidizes quality-assured ACTs for distribution to the public and private sectors, complemented by supporting interventions to promote rational drug use. AMFm has been in operation since mid-2010 in eight national-scale operational pilots in Ghana, Kenya, Madagascar, Niger, Nigeria, Tanzania mainland, Uganda and Zanzibar. By March 2012, over 220 million co-paid ACT treatment doses had been ordered. The Independent Evaluation of AMFm Phase 1 was commissioned by the Global Fund to assess the impact of AMFm on availability, price, market share and use of quality-assured ACTs in all the operational pilots. The assessment is based on a pre- and post-test design with detailed documentation of the implementation process and context, treating each pilot independently. In each pilot, a nationally representative survey of outlets stocking antimalarial medicines was conducted at the baseline (2009/10) and the endline (2011). At the endline, an additional sample of outlets was selected in remote areas in Kenya and Ghana, where availability, price and market share of quality-assured ACTs were measured. Areas were classified by remoteness based on an index computed from estimated travel times to three levels of service centers. The composite index was computed from the sum of the standardized travel times which was used to generate remoteness quintiles with areas in 4th and 5th quintiles considered remote. The number of outlets screened in non-remote and remote areas, respectively, was 501 and 194 in Ghana and 9,980 and 2,353 in Kenya. We compare remote and non-remote areas in each country with respect to availability, price and market share of quality-assured ACTs. The significance of the differences is assessed using Chi-squared tests for proportions and Wilcoxon rank tests for price indicators, expressed as medians.

1361

INSULIN SIGNALING IN THE MOSQUITO: UNDERSTANDING AKT PHYSIOLOGY IN THE FAT BODY REGULATION AT THE MOLECULAR LEVEL

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Lifespan is a key factor in determining the transmission efficiency of mosquito borne diseases. Finding a novel mechanism affecting mosquito lifespan could be a valuable tool to control mosquito-borne disease transmission. In mosquitoes, the insulin/insulin growth factor 1 signaling (IIS) cascade regulates lifespan, reproduction, and innate immunity. To better understand the impact of IIS in mosquitoes we induced IIS in the fat body of transgenic Anopholes stephensi mosquitoes. To accomplish this we used the vitellogenin promoter to express a myristoylated form of An. stephensi Akt (AsteAkt), a key component of the IIS cascade. Myr-AsteAkt transcript and protein expression occurred as expected with expression only in the fat body, following a bloodmeal. We characterized how changes to IIS specifically in the fat body effects egg production during multiple reproductive cycles and the impact is has on mosquito lifespan. Although myr-AsteAkt expression had little effect on total egg production, lifespan was significantly extended in the transgenic mosquitoes, an effect that was opposite of the anticipated result. Ongoing work on this transgenic mosquito may yield unique insights into how IIS regulates lifespan in mosquitoes and other eukaryotes.

1362

CHARACTERIZATION OF CARBONIC ANHYDRASES AND ION REGULATORY PROTEINS IN *AEDES AEGYPTI* FEMALE MOSQUITOES PRE- AND POST-BLOOD MEAL

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Mosquitoes represent a major threat to human health due to their capacity to spread diseases, such as malaria and dengue, to both humans and livestock. Blood feeding plays an important role in reproduction and pathogen transmission. Blood meals represent a significant challenge to digestive and ion regulatory processing mechanisms due to the protein, ion, water, and carbon dioxide-rich nature of the blood. This nutrient rich meal needs to be processed during and shortly after a blood meal to facilitate post-blood-meal flight and prevent toxic levels of sodium and CO_2 from remaining in the mosquito. This research determines the respective roles ion transport proteins and carbonic anhydrases play in ion transport and pH maintenance post-blood meal, specifically in the female midgut and hindgut tissues. The ion transporters analyzed include sodium proton antiporters, sodium dependent anion exchangers, and chloride-bicarbonate exchangers. The carbonic anhydrases analyzed fall into the alpha carbonic anhydrase family, with two genes at the focus of our studies. Immunohistochemical analyses reveal that CA9 is localized to the anterior and posterior midgut of the adult, while CA10 is localized to the nervous system and the hearing organ, the Johnston's organ. Immunocytochemical analyses also indicate that the sodium proton antiporter, NHA1, is localized to the apical membranes of the ileum and the stellate cells of the malpighian tubules. We hypothesize that if a female mosquito is fed blood, the expression of ion transport proteins and carbonic anhydrases in the gut will modulate in such a way as to maintain the alkaline pH within the posterior midgut and rapidly transport sodium out of the gut lumen and into the hemolymph. Also, we hypothesize that if ion regulatory or carbonic anhydrase gene expression is perturbed via reverse genetics, the gut and ion regulatory systems will not be able to properly digest the blood meal and regulate ion secretion, thus reducing reproductive capacity and fitness.

1363

CLIP-SERINE PROTEINASE CLIPB8 SUPPLEMENTS A SRPN2/CLIPB9 REGULATORY UNIT THAT CONTROLS MELANIZATION IN AFRICAN MALARIA MOSQUITO, *ANOPHELES GAMBIAE*

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Melanization immune response encapsulates and kills invading pathogens in insects and other arthropods. Melanization is regulated by the activation of prophenoloxidase (PPO), which is controlled by a proteinase cascade and its serpin inhibitors. To date, the molecular composition of this system is partially understood especially in mosquitoes. Recently, a regulatory unit of melanization in *Anopheles gambiae* was documented comprising an inhibitory serpin-clip-serine proteinase pair: serpin2-CLIPB9. Partial reversion of SRPN2 phenotypes in melanotic tumor formation and adult survival by SRPN2/CLIPB9 double knockdown suggests other target proteinases of SRPN2 in regulating melanization. Here we report that

CLIPB8 is identified as a target proteinase of SRPN2 and supplements SRPN2/CLIPB9 regulatory unit in controlling melanization in *An. gambiae*. Heterologously expressed SRPN2 forms a complex with activated recombinant proCLIPB8 and directly inhibits CLIPB8 activity *in vitro*. Similar as CLIPB9, double knockdown SRPN2 and CLIPB8 also partially reversed the pleiotrophic phenotype induced by SRPN2 silencing both in adult survival and melanotic tumor formation. Differently, CLIPB8 does not cleave and does not activate PPO *in vitro* as CLIPB9 did either by using purified *M. sexta* PPO or *M. sexta* plasma. Biochemical analysis showed that CLIPB8 and CLIPB9 can not activate each other *in vitro*. In addition, reverse genetic analysis by triple knockdown of SRPN2, CLIPB8, and CLIPB9 did not show accumulative effect in reverting the pleiotrophic phenotype by SRPN2 silencing. These results suggest CLIPB8 is on the further upstream of CLIPB9 in activation of melanization.

1364

A MULTIPLEX REAL-TIME PCR ASSAY FOR DETECTION AND QUANTIFICATION OF *PLASMODIUM* SPP INFECTION IN MALARIA VECTORS

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The enzyme linked immunosorbent assay specific for circumsporozoite protein (CSP-ELISA) is the gold standard method for the detection of malaria parasites in the vector despite several limitations. Here, we developed a new multiplex PCR-based method to detect and quantify the mixed infection rates of *Plasmodium* species in the African malaria vectors to better estimate the level of parasite infection in field populations and to ensure more accurate evaluation of the level of transmission following the implementation of vector control interventions. TaqMan duplex real-time PCR was first evaluated using different ranges of plasmids. The efficiency of real-time PCR was compared with the CSP ELISA using field caught *Anopheles gambiae* and *An. funestus* mosquitoes collected from two localities in southern Benin. Finaly, quantification of DNA of *Plasmodium* spp was performed and normalized using a housekeeping gene RS7. A total of 200 mosquito samples (100 An.gambiae and 100 An.funestus) were used to develop and validate the RT-PCR method. The validation of these oligonucleotides this technique on the mosquito homogenates showed that RT-qPCR was more sensitive than the ELISA-CSP for the detection of *P. falciparum* (RT-PCR RT-PCR= 97% and CSP (RT-PCR RT-PCR= 97% and CSP-Elisa=87%). These results indicated high specificity of the multiplex real-time PCR to detect the other *Plasmodium* species (notably *P. malariae* and *P. ovale*) in anophelinae mosquitoes. The relative quantification shows that the amount of DNA varies between 3 and 90 copy number/ng per samples. The average number of copies / ng in *An. gambiae* is (28.35767) and (7.16700) in An. funestus (p-value = 0.1045). This study describes a new method for the detection and quantification of the four *Plasmodium* species in the African malaria vectors. This will ensure a better diagnostic of malaria parasite's infection in field populations and allow for new basic research on the fitness cost associated with malaria infection during the life of the mosquito.

1365

PARAQUAT FEEDING FOR STUDY OF MOSQUITO DEFENSE CAPACITY AGAINST OXIDATIVE STRESS

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Anautogenous female mosquitoes take blood meals for egg production. Digestion of hemoglobins is accompanied with heme associated oxidative stress, which is potentially detrimental to lipids, proteins and DNA. As an adaptation, mosquitoes have evolved certain antioxidant mechanisms to cope with this oxidative stress. However, little is known about the extent of defense capacity that mosquitoes have. Paraquat is an herbicide known to causes extensive damage to the mitochondria through the production of free radicals and oxidative stress. In this study we used Paraquat feeding to add extra oxidative stress to the mosquitoes, and examine the anti-oxidant capacity in the gut ecosystem. Mosquitoes were fed on sugar diet with different concentration of Paraquat (2mM, 10mM and 20mM) after emergence. Paraquat causes mosquito death in a dose dependent manner. Interestingly, blood feeding increased the mortality of mosquitoes that had been fed on 2 mM Paraquat, suggesting that the bloodmeal increased the stress to a level that exceeds the defense capacity of mosquitoes. The expression patterns of mosquito and bacterial catalase and SOD, and bacterial AhpC, Paraquat inducible protein A and B genes were assayed by qPCR. These mosquito and microbial anti-oxidant genes responded to the stress in various settings, such as blood-fed, Paraquat-fed and Paraquat-plus blood-fed mosquitoes. The data suggest that gut redox homeostasis is managed collaboratively by both mosquito and its microbial community.

1366

TRANSCRIPTIONAL MEDIATORS KTO AND SKD ARE INVOLVED IN THE REGULATION OF THE IMD PATHWAY ANTI-*PLASMODIUM* DEFENSES IN *ANOPHELES GAMBIAE*

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Malaria is responsible for the deaths of over one million people annually. *Anopheles* mosquitoes are the main vectors for the malarial parasites. We have shown that the IMD pathway is the most important arm used by the mosquitoes to resist infection with the human malaria parasite *Plasmodium falciparum*. In this study, we showed that the transcriptional mediators Kto and Skd are involved in the regulation of the IMD pathway. Transcriptional mediators serve as transcriptional co-activators, which are a group of evolutionally conserved proteins that can form complexes to bridge regulatory regions to the RNA polymerase II initiation complex in eukaryotic cells. Studies with *Drosophila*, zebrafish and *Caenorhabditis elegans* have shown that Kto and Skd are required for several specific developmental processes. Here we show that knocking down Kto and Skd in the *Anopheles gambiae* cell line down-regulate the expression level of Cec1 which is controlled by the IMD pathway. However, Kto and Skd are not transcriptional co-activators of Rel2 and are not involved in the transcription of the main IMD pathway components. Silencing the two genes *in vivo* would lead to increased susceptibility of mosquitoes to bacterial and *P. falciparum* infection, but not to infection with P. berghei. Together the results suggest that Kto and Skd are involved in the regulation of the IMD pathway, which is crucial for the mosquito's defense against *P. falciparum*.

1367

FRAGMENTATION MECHANISMS OF ARGININE ISOBUTYL ESTER APPLIED TO ARGININE QUANTIFICATION IN EXCRETA FROM INDIVIDUAL *AEDES AEGYPTI* FEMALES

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Our laboratory is interested in uncovering the metabolic regulation of argininolysis and uricolysis in mosquitoes. For this purpose, it is necessary to have a rapid and efficient method to monitor arginine (Arg) levels in excreta from individual *A. aegypti* females. Thus, the fragmentation patterns of the isobutyl esters of Arg and $^{15}N_2$-Arg (labeled at the guanidino group) were studied by electrospray ionization-tandem mass spectrometry, and fragmentation pathways not described before were characterized. In addition, Arg, $^{18}O_2$-Arg, $^{15}N_2$-Arg and $^{15}N_2$-$^{18}O_2$-Arg were analyzed to elucidate some of the minor fragments in greater detail. Mosquito excreta from individual females were collected before and at different times after feeding a blood meal, mixed with $^{15}N_2$-Arg, an internal standard, and derivatized as isobutyl esters. Based on the fragmentation mechanism of Arg standards studied by MS^2 and MS^3, Arg levels in the mosquito excreta were analyzed by multiple-reaction monitoring (MRM) in a triple-quadrupole mass spectrometer. Arg excretion was quantified at 1, 6, 12, 18, 24, 36, 48, 72, 96 and 120 h before and after feeding female mosquitoes with a bovine blood meal. As expected, Arg is not present in the sugar-fed female excreta and only a very small amount is observed in blood-fed female excreta at the beginning of the time course. At 12 h, the Arg concentration is approximately 20 nmol/female mosquito. This value increases significantly during the time course, reaching the highest levels between 36 and 48 h (about 60 nmol/female) and remains constant through the end of the time course (120 h after a blood meal). These data correlate well with the periods of intense blood meal digestion and maximal excretion of nitrogen compounds in the blood-fed females. The quantification of Arg by mass spectrometry provides a rapid, sensitive and accurate method to investigate the metabolic regulation of nitrogen wastes in individual *A. aegypti* females.

1368

MOLECULAR AND FUNCTIONAL CHARACTERIZATION OF INWARD-RECTIFYING POTASSIUM (KIR) CHANNELS IN THE 'KIDNEYS' OF MOSQUITOES: TOWARDS THE DEVELOPMENT OF NEW INSECTICIDES

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The evolution of insecticide resistance in mosquitoes has led to an urgent need to develop new chemical control agents with novel mechanisms of action. In the present study, we evaluate the inward-rectifying potassium (Kir) channels of mosquitoes (Aedes aegpyti) as potential insecticidal targets by characterizing their molecular and functional expression. We focus our study on the Kir channels expressed in the Malpighian tubules, because this renal epithelium is a key component of the mosquito excretory system and has not been exploited as a physiological target for controlling mosquitoes. We show that (1) Malpighian tubules express a combination of at least 3 different Kir channel genes that is distinct among mosquito tissues, (2) at least two of the Kir channels encode barium-sensitive potassium channels when expressed in Xenopus oocytes, and (3) injecting a small-molecule antagonist of Kir channels into mosquitoes elicits desirable sub-lethal effects that are consistent with perturbed Malpighian tubule function.

1369

THE ROLE OF APOPTOSIS IN DENGUE-2 INFECTION OF THE MOSQUITO VECTOR *AEDES AEGYPTI*

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Aedes aegypti is the primary vector for dengue virus (DENV). An understanding of host-pathogen interaction is important in understanding what factors contribute to vector competence. Our previous global transcriptional analysis has suggested the induction of apoptotic proteins in the involvement of resistance and susceptibility to DENV infection. However the mechanism through which is happens is largely unknown. Here we analyze the possibility that programmed cell death is actively involved in the defense of *A. aegypti* host cells to DENV infection. The effector caspase, *CASPSL2*, has been previously shown to be part of the core apoptotic pathway involved in the response to drug and UV-induced DNA damage in *A. aegypti*. Here we use siRNA interference to show that *CASPSL2* is also involved in apoptotic signaling for DENV-2, and that silencing of this gene affects virus titer at early and late points of infection. Silencing of *CASPL2* also affected dissemination and transmission of the virus. In addition, we investigate the possibility that by delaying programmed cell death in susceptible individuals, DENV-2 can manipulate this process for its benefit.

1370

P38 MAPK SIGNALING IN *ANOPHELES STEPHENSI*: A MECHANISM FOR TOLERANCE OR RESISTANCE DURING PARASITE INFECTION?

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Among the mitogen-activated protein kinases, p38 MAPK-dependent signaling is critical to the regulation of the balance between resistance and tolerance to infection. However, little is known about the functional biology of p38 MAPK signaling in vector mosquitoes. Our data demonstrated that inhibition of *Anopheles stephensi* p38 MAPK signaling can reduce malaria parasite development, including oocyst burden in the midgut as well as infection prevalence. Further, p38 MAPK signaling regulates a wide variety of known mosquito anti-parasite effector genes in patterns that suggest a balance between tolerance and resistance. This work indicates that the essential roles of p38 MAPK signaling identified in mammals are conserved in mosquitoes. More importantly, however, our data provide new insights into regulatory mechanisms that can be manipulated to control suites of anti-parasite genes as the basis for a novel strategy for the development of transgenic, parasite-resistant mosquitoes.

1371

CHARACTERIZATION OF A G PROTEIN COUPLED RECEPTOR (GPCR) THAT BINDS TO THE ANTI-*PLASMODIUM* IMMUNE FACTOR FBN9

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In *Anopheles gambiae* mosquitoes, the fibrinogen related protein family (FREP, also known as FBN) is the largest group of pattern recognition receptors. We have previously reported that one of its members FBN9, interacts directly with various species of bacteria and also exhibits anti-*Plasmodium* activity. To further understand the role of FBN9 in the mosquito's innate immune system, a yeast two hybrid screen was performed to identify novel binding partners. In addition to a number of other interacting proteins, we have discovered a G protein coupled receptor (GPCR) that binds to FBN9. Interestingly, this GPCR has been

identified as a rhodopsin receptor (GPROP10) and has no previously described immune related function. Rnai studies show that GPROP10 may also participate in controlling *Plasmodium* development in the mosquito midgut. Here we describe the characterization of GPROP10 and its potential function in the innate immune system of *An. gambiae* mosquito's defense against pathogens.

1372

TRANSCRIPTOMIC COMPARISON OF LABORATORY AND GEOGRAPHICALLY DISTINCT FIELD-DERIVED *AEDES AEGYPTI* POPULATIONS TO IDENTIFY GENES THAT REGULATE VECTOR COMPETENCE FOR DENGUE VIRUS2

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Dengue virus (DENV) is the most important mosquito-borne virus affecting humans today, and is vectored primarily by the mosquito *Aedes aegypti*. Since no vaccine against DENV is currently available, there is interest in transmission control strategies that target the mosquito vector. Most studies of mosquito immune responses have been performed with the laboratory strains of *Ae. aegypti*, which have been maintained under insectary conditions for decades. As compared to natural mosquito populations, laboratory mosquito strains are exposed to lower doses and a much narrower range of microbes; this together with the genetic bottleneck of a small initial parental population size often results in a loss of genetic variability. Although most field studies have focused on genetic polymorphisms, natural and laboratory mosquito populations are also likely to differ in their transcriptomic responses to pathogen infection, either in terms of the magnitude of gene regulation or in the subsets of regulated genes. We established field colonies of *Ae. aegypti* from geographically-distinct dengue-endemic regions, spanning South America, the Caribbean, and Southeast Asia, and evaluated their and vector competences for DENV2. This analysis identified both refractory and susceptible strains to DENV2 infection. A genome-wide gene expression microarray was then performed to compare the transcriptomes of field-derived strains to our laboratory Rockefeller strain. Several candidate genes were identified that may regulate vector competence in field-derived strains; we are currently functionally testing the role of these genes through RNAi-mediated gene knockdowns. This study will not only provide valuable information about immune gene regulation and usage in natural mosquito populations, but will also allow us to identify novel pathogen recognition receptors and effector genes that control DENV in field mosquitoes.

1373

HIGH PREVALENCE OF HTLV-1 AND HTLV-2 INFECTIONS IN PERUVIAN AMAZONIAN COMMUNITIES

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HTLV-1 and HTLV-2 infections are distributed worldwide and endemic to regions of Japan, sub-Saharan Africa, the Americas, Melanesia, and the Middle East. Peru has reported HTLV-1 and HTLV-2 infections in indigenous Amazonian populations and among African-Peruvian and mestizo populations. To assess the prevalence, risk factors and neurological manifestations associated with HTLV-1 and -2 infections, we conducted a cross-sectional study of 878 adult participants, ages 15-64 years, from 14 indigenous communities near Pucallpa. 94 (10.7%) participants were infected with HTLV: 56 (59.6%) had HTLV-1, 35 (37.2%) HTLV-2, and 3 (3.2%) were infected with both HTLV-1 and HTLV-2. Seven patients had indeterminate test results and were excluded from further analysis. The median age for all participants was 34 (SD ± 13.8) years. HTLV positive participants were older than HTLV negative participants (mean 43.1 vs. 32.9 years ($p<0.0001$). HTLV-1 and -2 infections increased with age ($p<0.0001$) but decreased for participants aged 50 years or older. Factors significantly associated with HTLV infection included age ≥ 38 years ($p<0.0001$, OR: 3.07), female gender ($p=0.008$, OR: 1.82), illiteracy ($p=0.002$, OR: 2.85), education of 7 years or less ($p<0.0001$, OR 2.22), having had a relative with gait difficulties affecting both legs ($p=0.036$, OR 2.4), prior episode of chronic scabies (significant only for males; $p=0.046$, OR: 2.1), and being pregnant more than four times ($p=0.027$, OR 1.88). Surprisingly, no participant had clinical evidence of HTLV-associated myelopathy/tropical spastic paraparesis (HAM/TSP). To our knowledge, this is one of the highest reported prevalences of HTLV infection among native Amazonian ethnic groups. Although not measured in this study, the high prevalence of helminthic coinfection reported among Amazonian inhabitants may potentially attenuate immune responses and thus impede the development of HAM/TSP.

1374

AUSTRALIAN ARBOVIRUSES AND A NOVEL RHABDOVIRUS IN ANOPHELINE MOSQUITOES IDENTIFIED USING METAGENOMICS

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Identifying viruses as etiologies of human and animal disease is an important initial step in preventing and treating illness. The success of many previous virus identification strategies has been impeded by the requirement for prior knowledge of the viral genome, which dictates the testing assay. Deep sequencing, by contrast, is a cutting-edge metagenomics technique that detects and characterizes known and unknown viruses in a specimen nonspecifically and with high sensitivity, without prior knowledge of the viral genome. We used deep sequencing to identify virus genomes in pools of mosquitoes from New South Wales, Australia, that were antigenically negative for known Australian flaviviruses and alphaviruses. Full genome characterization and phylogenetic analyses revealed sequences of several viruses in a least one pool each: 1) strains of Liao Ning virus (LNV, *Reovirus*, *Seadornavirus*), heretofore only detected in Indonesia and China where it is the etiological agent of encephalitis in humans, 2) strains of Stretch Lagoon virus (SLOV, *Reovirus*, *Orbivirus*) a mosquito-borne virus that infects livestock and has previously been isolated only in Northern Australia and once in Sydney, and 3) a novel rhabdovirus in *Anopheles annulipes* that diverges by ≈40% at the amino acid level compared to other members of the *Vesiculovirus* genus and probably represents a new species. To our knowledge, this is the first report of LNV outside China, and we extend the distribution of SLOV to central New South Wales. This study highlights the power of metagenomics for identifying novel RNA viruses in field-collected mosquitoes. The new rhabdovirus may eventually be linked to human or veterinary disease, and follow-up epidemiological arbovirus studies will address this possibility.

1375

LASSA FEVER OUTBREAK INVOLVING HEALTHCARE WORKERS IN TARABA STATE, NIGERIA: MARCH 2012

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Lassa fever is an acute, highly infectious viral haemorrhagic illness caused by Lassa fever virus - a single stranded, RNA virus belonging to the virus family Arenaviridae. The reservoir is Mastomys natalensis. The disease is endemic in West African sub region causing 300,000-500,000 infections annually, with about 500 deaths. In March, 2012, we investigated a reported outbreak of Lassa fever in Taraba State, Nigeria to confirm the outbreak, determine its extent, characterize the outbreak, institute public health actions and make appropriate recommendations. We reviewed hospital records and used IDSR standard case definition for Lassa fever to identify and line-list cases. A suspected case was defined as "any person with severe febrile illness not responsive to the usual causes of fever in the area with or without sore-throat and at least one of the following: bloody stools, vomiting blood, bleeding into the skin, unexplained bleeding from the nose, vagina or eyes". A standardized line-listing form was developed to capture socio-demographic and clinical information of the cases. Various exposure factors including age, gender, occupation and contact history were examined. A total of 35 cases were recorded. Nine of 35 cases were laboratory confirmed (25.7%). Altogether, 14 deaths were recorded giving a case fatality rate of 40%. Majority of the cases belonged to the age group 25-34 years (40%) with females constituting 51% . Most of the cases were healthcare workers (22.9%). The commonest presenting features were fever (85.7%), cough (28.6%), bleeding from orifices or into skin (25.7%) and headache (20%). In addition, the State's Epidemic Management Committee was non-functional resulting in uncoordinated response to the outbreak. There were many exposure factors to Lassa fever such as over-crowding, drying of food items along high ways and bush burning and there was low index of suspicion of Lassa fever among health care workers. Community sensitization and sensitization of health workers in Taraba State on Lassa fever were carried out. There was a confirmed outbreak of Lassa fever in Taraba State mostly affecting healthcare workers. It was recommended that the State should reactivate its Emergency Management Committee, surveillance of Lassa fever should be strengthened, Public/Health workers sensitization activities should be scaled up and records keeping should be improved.

1376

DIARRHEA INCIDENCE BEFORE AND AFTER ROTAVIRUS VACCINE INTRODUCTION IN NICARAGUA: A PROSPECTIVE, POPULATION-BASED STUDY

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Nicaragua was the first GAVI-eligible country to introduce the pentavalent rotavirus vaccine in 2006. Prior evaluations of the vaccine's effectiveness in developing countries have been performed in health facilities; however, the majority of rotavirus cases are treated in the community. The goal of this study was to examine changes in childhood diarrhea incidence in the community before and after vaccine introduction. We conducted active surveillance for diarrhea episodes using the Health and Demographic Surveillance Site, León to provide simple random population-based samples. Two open cohorts of children were followed, one in the pre-vaccine period, 2001-2003, and the other in the post-vaccine period, 2010-2011. Home interviewers visited households to record each child's characteristics and returned every 2 weeks to record numbers of diarrhea episodes. Poisson regression models were used to compare the incidence rate of diarrhea in the pre- and post-vaccine periods, stratified by age. Because laboratory data were not available for comparison, a "rotavirus-specific" diarrhea surrogate definition was used, based on the literature: greater than 4 stools per 24 hr period with either vomiting or fever or both. We anticipated a decline in rotavirus-specific diarrhea incidence in the post-vaccine period. A total of 726 children were enrolled in the pre-vaccine cohort and were followed for 249 person-years (py); 826 children were enrolled in the post-vaccine cohort and were followed for 563 py. Overall unadjusted diarrhea incidence was lower in the post-vaccine period than in the pre-vaccine period. Rotavirus-specific diarrhea incidence showed a greater decline from the pre-vaccine to the post-vaccine periods: among infants from 0.38 to 0.14 cases per py (p=0.026), among 12-23 month old children from 0.35 to 0.07 cases per py (p=0.001), and among 24-59 month old children from 0.10 to 0.02 cases per py (p=0.002). In conclusion, substantial declines in the incidence of rotavirus-specific diarrhea were observed in the post-vaccine period in this community setting.

1377

MOLECULAR DETERMINANTS OF MOUSE NEUROVIRULENCE AND MOSQUITO INFECTION FOR WESTERN EQUINE ENCEPHALITIS VIRUS

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Western equine encephalitis virus (WEEV) is a naturally occurring recombinant virus derived from ancestral Sindbis and eastern equine encephalitis viruses. We previously showed that infection of CD-1 mice with WEEV McMillan (McM) and IMP-181 (IMP) isolates resulted in high (~90-100%) and low (0%) mortality, respectively, when virus was delivered by either subcutaneous or aerosol routes. However, relatively little is known about specific virulence determinants of WEEV. We additionally observed that IMP infected Culex tarsalis mosquitoes at a high rate (app. 80%) following ingestion of an infected bloodmeal but these mosquitoes were infected by McM at a much lower rate (10%). To understand the viral determinants generating these phenotypic differences, we characterized the pathogenic phenotypes of McM/IMP chimeras. Exchanging the arginine present at IMP E2 glycoprotein position 214 for the glutamine present at the same position in McM ablated mouse mortality. However, the reciprocal exchange did not confer mouse virulence to the IMP virus. Mosquito infectivity was determined by multiple loci one of which was the same E2-214 amino acid identified above as the mouse virulence determinant . Replacing either IMP E2 amino acid 181 or 214 with the corresponding McM amino acid lowered mosquito infection rates to McM-like levels. As observed during our study of mouse neurovirulence, neither reciprocal exchange conferred mosquito infectivity. The identification of WEEV E2 amino acid 214 as necessary for both IMP mosquito infectivity and McM mouse neurovirulence indicated that they are mutually exclusive phenotypes and suggests an explanation for the lack of human or equine WEEV cases even in the presence of active transmission.

1378

PROBING THE ROLE OF CD4+ AND CD8+ T-CELLS IN CONTROLLING EARLY INFECTION WITH THE CHIKUNGUNYA CHIKV/IRES CANDIDATE VACCINE AND PROTECTING AGAINST CHIKV CHALLENGE

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Recently, Chikungunya virus (CHIKV), a mosquito-borne alphavirus, re-emerged in Africa and spread to islands in the Indian Ocean, Indian subcontinent, SE Asia and Italy. Viremic travelers have also imported CHIK to the Western hemisphere, which highlights the risk of CHIKV in naïve populations. In addition to the great burden of arthralgic disease, which can persist for months or years, epidemiologic studies estimated case-fatality rates of ~0.1%, principally from neurologic disease in older patients. There are no licensed vaccines or effective therapies. Using the La Reunion strain as the genetic backbone we inserted a picornavirus internal ribosome entry site (IRES) that functions poorly in insect cells, and inactivated the subgenomic promoter which drives overexpression of the structural proteins, to develop a live-attenuated CHIKV vaccine (CHIKV/IRES). This vaccine is highly attenuated yet immunogenic in mouse models and non-human primates, and is incapable of replicating in mosquito cells. In an effort to understand better the contribution of host response to CHIKV/IRES replication at the initial stages of virus infection we are currently conducting a series of studies to determine whether $CD4^+$ and/or $CD8^+$ T cells control virus replication in the A129 mice (deficient in the IFN α/β response). Prior to CHIKV/IRES infection, T cell subsets are depleted and the replication of the virus in the serum and various tissues (brain, spleen, muscles, liver) are monitored over a period of 12 days.In addition, isolated T cell subsets collected on days 0, 3, 6, 9 and 12 are characterized by intracellular cytokine staining using flow cytometry. Furthermore, we are conducting a series of adoptive transfer studies to determine which T cell subset is contributing to the protection afforded by the vaccine against wt CHIKV challenge. Understanding the role of T cell immunity in controlling infection and its contribution to protection will assist our efforts in designing an effective vaccination strategy against CHIKV.

1379

VACCINE SAFETY AND THE DEVELOPMENT OF A RODENT MODEL OF PERSISTENT CHIKUNGUNYA VIRUS INFECTION

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Chikungunya virus (CHIKV) is a positive sense single stranded RNA virus in the genus Alphavirus and the etiologic agent of several epidemics in Africa, and recently, the Indian subcontinent and Southeast Asia. CHIKV causes a syndrome characterized by rash, fever, and debilitating arthritis. In the more recent outbreaks, CHIKV has begun to manifest more neurologic signs of illness in the elderly and those with co-morbidities. The syndrome is often self-limited; however, many patients develop persistent arthralgia that can last months or years. These characteristics make CHIKV not only important from a human health standpoint, but also from an economic standpoint. Currently, there is no licensed vaccine. Many studies have begun to elucidate the pathogenesis of CHIKV, the mechanism of arthralgia persistence and the role of the adaptive immune response that is poorly understood. In this study, Rag1 KO (Recombination activation gene 1 knockout) mice were inoculated subcutaneously or in the foot pad with 3 log10 PFU of the La Réunion strain of CHIKV (CHIKV-LR) or varying doses of our vaccine candidate CHIKV/IRES (1, 3, or 5 log10 PFU) or the U.S. Army vaccine strain 181/clone25 (3 or 5 log10 PFU). Mice were bled on days 1-8, 14, 28, 42, 56 and 70 after infection and weighed on days 1-14. Tissues were harvested on days 2, 4, 7, 14, 28, 42, 56 and 70. None of the subcutaneously inoculated mice demonstrated clinical signs of illness (e.g., weight loss, lethargy, or scruffy fur). Mice inoculated with CHIKV-LR developed persistent infection. Viremia reached a peak of 4 log10 PFU on day 6 after infection, gradually decreasing to 2 log10 on day 28; no viremia was detected after day 28 post infection. CHIKV-LR also persisted in several organs up to day 42 after infection, but no virus was detected by plaque assay in the organs after day 42 post infection. The brain had inflammation 28 days post infection. These findings are in contrast to both vaccine strains, which never produced detectable viremia or viral persistence in the organs. This study has two key findings: 1) the adaptive immune system is critical for clearance of CHIKV, and 2) the newly developed CHIKV/IRES vaccine strain does not persist even in the absence of T/B cells. The latter point is very important when considering vaccine safety, because many people in developing countries that are exposed to CHIKV are also immunosuppressed due to various conditions (e.g., HIV, malnourishment).

1380

DEVELOPMENT, VALIDATION, AND FIELD PERFORMANCE OF A FIVE-PLEX REAL-TIME QPCR ASSAY TO DETECT DIARRHEAGENIC RNA VIRUSES

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Several viruses and bacteria are known to cause diarrheal disease in the developing world. Traditional methods such as microscopy or culture are either inappropriate or impossible when dealing with these pathogens. In addition, ELISA-based tests may not possess the sensitivity required to detect these pathogens from stool samples. PCR-based methods are generally more sensitive compared to both traditional and ELISA-based methods. In this work, we developed and validated a 5-plex TaqMan-based real-time qPCR assay targeting diarrheagenic RNA viruses including: Astrovirus, Norovirus GII, Rotavirus, and Sapovirus (types 1,2,4, and 5), and includes an extraction/amplification control based on a non-coding region of MS2 phage. In addition, we also developed a 4-plex TaqMan-based real-time qPCR panel targeting Campylobacter, Salmonella, and Vibrio bacterial species with the Glycoprotein B gene from Phocene Herpes Virus as the extraction/amplification control. The validation process revealed at least five logs of linear range for each viral target, as well as low CV values (range 0.500-1.855%) for within-run precision and moderate CV values (range 2.8%-28.9%) for between-run precision depending on the target. In addition, the assay appears to perform well in stool matrices having varying degrees of inhibition. After validation, the assays were evaluated at five international field sites each using one of three different real-time PCR platforms including the BioRad CFX96, Corbett/Qiagen RotorGene, and the Applied Biosystems ViiA 7. The field evaluations were based upon measures of linearity, limit of detection, within- and between-run precision ("repeatability" and "reproducibility", respectively), as well as accuracy and carry-over studies. Similar to the results from the validation, the field performance evaluation revealed low CV values (range 0.08-7.52%) for within-run precision and moderate CV values (range 2.36-11.06%) for between-run precision analyses. While there were site-to-site and target-to-target differences, overall, the assays performed similarly over the five field sites and similarly between the three real-time PCR platforms.

1381

SEVERE HEMORRHAGIC FEVER IN STRAIN 13/N GUINEA PIGS INFECTED WITH LUJO VIRUS

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Lujo virus (LUJV) is a novel member of the *Arenaviridae* family that was first identified in 2008 after an outbreak of severe hemorrhagic fever (HF). In what was a small but rapidly progressing outbreak, this previously unknown virus was transmitted from the critically ill index patient to 4 attending healthcare workers. Four persons died during the outbreak, for a total case fatality of 80% (4/5). The suspected rodent source of the initial exposure to LUJV remains a mystery. Because of the ease of transmission, high case fatality, and novel nature of LUJV, we sought to establish an animal model of LUJV HF. Initial attempts in mice failed, but infection of inbred strain 13/N guinea pigs resulted in lethal disease. A total of 41 adult strain 13/N guinea pigs were infected with either wild-type LUJV or a full-length recombinant LUJV. Results demonstrated that strain 13/N guinea pigs provide an excellent model of severe and lethal LUJV HF that closely resembles what is known of the human disease. All infected animals experienced consistent weight loss (3-5% per day) and clinical illness characterized by ocular discharge, ruffled fur, hunched posture, and lethargy. Uniform lethality occurred by 11-16 days post-infection. All animals developed disseminated LUJV infection in various organs (liver, spleen, lung, and kidney), and leukopenia, lymphopenia, thrombocytopenia, coagulopathy, and elevated transaminase levels. Serial euthanasia studies revealed a temporal pattern of virus dissemination and increasing severity of disease, primarily targeting the liver, spleen, lungs, and lower gastrointestinal tract. Establishing an animal LUJV model is an important first step towards understanding the high pathogenicity of LUJV and developing vaccines and antiviral therapeutic drugs for this highly transmissible and lethal emerging pathogen.

1382

ORAL ROTAVIRUS IMMUNIZATION PROTECTS UNDERNOURISHED WEANLING MICE AGAINST INFECTION DESPITE REDUCED VACCINE SHEDDING AND MODULATED ANTIBODY RESPONSES

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Oral rotavirus vaccines protect against the most common cause of severe childhood diarrhea worldwide, but are less effective in low-income countries. A higher prevalence of undernutrition, reducing both innate and adaptive immunity, may partially explain this disparity. Therefore, we designed mouse experiments to test the hypothesis that undernutrition impairs immune responses to rotavirus vaccine and infection. Wild type BALB/c dams with 10-day-old sucklings were randomized to a standard diet or an isocaloric, multideficient "regional basic diet" (RBD) we have previously shown produces moderate malnutrition and phenocopies key features of human environmental enteropathy, including villous blunting and reduced gut integrity (Ueno et al. *AJP* 2011). We immunized RBD mice and controls at 6 weeks of age with a live oral rotavirus vaccine (RRV) or a vehicle control. We then challenged immunized mice and unimmunized controls with murine rotavirus (EDIM 10^4 SD_{50}) 4 weeks later. Stool and blood were collected after RRV or EDIM challenges to determine viral shedding and antibody responses. RRV shedding in stool following immunization was decreased by 50% in RBD mice vs. controls (15.1 ng/mL vs. 30.8 ng/ml, $P<0.03$), however protection against EDIM was undiminished. Following immunization, RBD mice had 2-fold higher anti-rotavirus serum IgA levels vs. controls. Following infection, unimmunized RBD mice produced 50% lower levels of anti-rotavirus IgG vs. well-nourished controls ($P=0.16$). This was not significant after correcting for marked decreases in total IgG levels in RBD mice). In conclusion, weanling undernutrition alters host immune responses to rotavirus vaccination and infection, but does not mitigate vaccine efficacy. Further research defining the role of malnutrition and other host factors is needed to improve vaccination outcomes in children who bear the greatest risk of disease.

1383

IRES-BEARING VENEZUELAN EQUINE ENCEPHALITIS VIRUSES ARE POTENTIAL VACCINE CANDIDATES

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Venezuelan equine encephalitis virus (VEEV) is an arbovirus associated with morbidity and mortality in equines and humans across Central and South America. Despite its success in curbing the severity and range of epidemics in the past, the current VEE vaccine, TC-83 is a suboptimal vaccine for the following reasons: (i) unstable attenuation; (ii) high reactogenicity and poor long-term immunogenicity (in humans); and (iii) transmissibility in nature. Previously, we reported that when the EMCV IRES was placed in lieu of the virus' subgenomic promoter, the resulting virus exhibited an attenuated phenotype and inability to replicate in mosquito cells. Here, we describe the use of IRES-based attenuation strategy for VEEV vaccine (subtypes ID and IE) construction and characterization. *In vitro*, VEEV/IRES vaccines produce small plaques and replicate to lower titers than the parental 68U201 (IE) or ZPC738 (ID) viruses. Mice injected subcutaneously with 1x10^5 pfu of VEEV/IRES show no signs of illness or changes in weight, produce neutralizing antibodies and are fully protected against their respective wild-type lethal challenge. Moreover, VEEV/IRES viruses are unable to propagate in C6/36 cells, implying that these viruses would be unable to be transmitted by mosquitoes in nature. These results, as well as those obtained from studies on chikungunya and eastern equine encephalitis viruses, demonstrate that the IRES-based method of alphavirus vaccine generation provides a predictable method for alphavirus attenuation while maintaining a host-restricted range of replication.

1384

HANTAVIRUS-DAF/CD55 ENGAGEMENT INITIATES RHOGTPASE ACTIVITY AND PARACELLULAR PERMEABILITY IN EPITHELIAL CELLS

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Pathogenic Hantaviruses cause hemorrhagic fever with renal syndrome (HFRS) and hantavirus cardiopulmonary syndrome (HCPS). Hantaviruses have profound tropism for microvascular endothelial cells, and capillary leak at sites of infection is important in pathogenesis. Hantaviruses are hypothesized to target the decay accelerating factor (DAF/CD55), a ubiquitous molecule expressed on the apical surface of polarized epithelia, as a co-receptor for accessing $\alpha_v\beta_3$ integrin, the entry receptor, expressed basolaterally. Initial engagement of DAF by UV-killed Sin Nombre virus results in upregulation of GTP-bound Rho GTPases (Rac1, Cdc42, RhoA), which is measured by confocal microscopy and G-LISA assays. The Rac1 inhibitor, NSC23766 and a novel Cdc42 inhibitor, ML 141 are used to establish the role of signal cross-talk among Rac1, Cdc42, and RhoA, during the induction of paracellular permeability in epithelial cells. These results are important for understanding the pathogenesis of hantavirus disease.

1385

EVIDENCE OF OUTDOOR BLOOD FEEDING IN THE HIGHLAND *ANOPHELES* OF WESTERN KENYA: A NEW CHALLENGE FOR MALARIA CONTROL?

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Indoor residual spraying (IRS) and long-lasting insecticide treated nets (LLINs) are core elements in most malaria control programmes. The effectiveness of these methods relies on the assumption that the majority of *Anopheles* vectors feed indoors and at times when people would be under a net. It is therefore vital to establish whether this assumption is true and determine the time and location that these mosquitoes bite. In addition to divergent behaviors, vector control methods may be undermined by development of insecticide resistance which will affect the success of both IRS and LLINs and may lead to the persistence of malaria transmission. This study aimed to compare the proportion and age of vector species biting at different hours of the night inside and outside houses in an area of seasonal low malaria transmission in Rachuonyo South district, Nyanza Province, western Kenya. This study also aimed to calculate the risk of infected bites with respect to the time that local residents entered their houses and used LLINs. The study took place between June 2011 and July 2012. Collections occurred each month for 6 nights, in 24 houses per night which were randomly allocated to indoor or outdoor trapping. CDC light-traps were hung adjacent to occupied LLINs, located either within houses or in rain shelters set outdoors. Hourly collections were made between 17:30 and 22:30 and then 05:30-06:30 the next morning. Questionnaires were used to capture the time that the local population entered houses and used LLINs. Seasonal fluctuations in vector species were recorded and more *Anopheles* were caught in outdoor traps. The morphological identification of vectors was confirmed by rDNA and mtDNA sequencing and exposure to each vector is discussed. There was evidence that the local population were at risk of infected bites both outdoors and indoors at a time when LLINs were not protecting a sizeable proportion of the population. Exposure to such bites may be responsible for maintaining transmission in the area. This work provides data on vector dynamics that can inform future malaria control programmes.

1386

EVALUATION OF AN ATTRACTIVE LETHAL OVITRAP (ALOT) AGAINST *AEDES AEGYPTI* FOR DENGUE CONTROL IN IQUITOS, PERU

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Dengue, the most important mosquito-borne virus world-wide, is primarily transmitted by the container-inhabiting mosquito *Aedes aegypti*. Without a vaccine to prevent new human infections, vector control remains the only method of preventing transmission. Female mosquitoes acquire the virus by taking blood from an infected human, then use a variety of cues to identify potential oviposition sites, where they must deposit eggs before taking another bloodmeal. We hypothesize that by specifically targeting older gravid females, we can most efficiently reduce dengue virus transmission. Toward that end, we have worked to develop an effective lethal ovitrap (Attractive Lethal OviTrap = ALOT) for *Ae. aegypti* control, with concentration on identification of oviposition attractants and stimulants. Starting in June 2011, we tested the ALOT in a large scale field trial in Iquitos, Peru, where dengue is endemic. The study design was a prospective nonrandomized controlled trial in two cohorts of Iquitos residents from two comparable city neighborhoods each of 2500 houses selected as either intervention or control zones. Traps were placed in houses at a density of ~3 per residence, with ~85% participation in the intervention area. Local ministry of health fumigation to control adult mosquitoes was ongoing in both areas during the study. Entomological indices were monitored in participating households at 3 month intervals, and individuals were monitored serologically, both through a longitudinal survey (at months 0, 12, 18) and through 3X weekly febrile surveillance. Nine months into the trial, dengue incidence as measured by febrile surveillance was 78% lower (0.3% vs. 1.34%) in the intervention area compared to the control area ($p<0.0001$). Confirmation of these results through separate longitudinal surveillance is pending. We also observed a difference in adult mosquito indices of approximately 50% (e.g. 65 to 30 females/100 houses) between the two areas. These preliminary results suggest that area-wide application with the ALOT could significantly lower dengue transmission.

1387

CHANGING BEHAVIORAL PATTERNS OF ARBOVIRAL VECTOR *AEDES AFRICANUS*: A CONCERN FOR EMERGING AND REEMERGING DISEASES

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Aedes (Stegomyia) africanus Theobald is a key arboviral vector to humans. It is a highly competent vector for several viruses that cause hemorrhagic fevers including; Yellow fever (YF), Rift Valley fever, dengue fever, and other arboviral vectors, most of which are widely distributed in Africa. There are no vaccines for these arboviral diseases except YF which has a safe and effective vaccine, yet YF outbreaks are still reported in Africa. Vector control is therefore crucial yet little is known about the biology of Ae africanus to enable effective vector targeted control and management of hemorrhagic fevers. Aedes africanus has specific behavioral preferences, some of which facilitate their spread within the rapidly changing landscape of Africa. *Ae. africanus* is reportedly confined to forests. However, it was implicated in the peridomestic transmission of YF to humans during the 1987 Nigerian YF epidemic. This study aims to understand the behavior of *Ae. africanus*. Bamboo pots containing water were placed on a 120 feet steel tower in Zika forest, Entebbe. The pots were placed at ground level, platforms at 20, 40, 60, 80, 100 feet above the ground and at shaded spots of the encroached forest buffer zone. Immature samples were collected weekly, reared to maturity and morphologically identified. After 32 weeks, a total of 734 mosquitoes were collected inside the forest, 89% of which were collected at 60 feet and below. A total of 642 Ae. africanus mosquitoes were collected at 200 and 400 feet from the forest boundary. These results indicate that *Ae. africanus* prefers to oviposit at levels below the tree canopy. These are shaded areas in the forest and heights at which the host, primates, are found. There is also a tendency for a change from a sylvatic (forest confinement) to a peridomestic behavior. This is probably due to the increased human activities in the forest buffer zone. Encroachment on the forest buffer zone must be strongly discouraged given previous isolations of several arboviruses from the forest, most of which have been isolated in *Ae. africanus*.

1388

SUMILARV 0.5G, A PROMISING INSECTICIDE FOR THE CONTROL OF *ANOPHELES GAMBIAE* S.L.?

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Malaria vector control with long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) has resulted in a significant decline of malaria in Africa. However, recent reports have shown both behavioural avoidance and physiological resistance to insecticides by vectors. Thus, the need to explore and integrate other tools for malaria vector control. Larval source management is a proven tool for malaria vector control. Difficulties with larviciding are (1) interventions are based at targeting all aquatic habitats and (2) current larvicides have short residual activity requiring weekly application which necessitate large labour and large amounts of larvicides which is not cost effective especially in Africa. Thus the need to evaluate persistent larvicides and study sound application strategies of the persistent larvicides. Sumilarv is a persistent larvicide with great potential for mosquito control. Bioassays showed that Sumilarv was ten times more effective that microbials for *Anopheles* control. Sumilarv application at 0.05 ppm a.i. resulted in 80% emergence inhibition for up to 6 weeks under standardized field conditions. Evaluations of the optimum dose of Sumilarv identified in the standardized field tests are on-going in an area of focal malaria transmission in Western Kenya. Preliminary results from the first three months of field testing indicate that the three week application results on average in 71% emergence inhibition of malaria vectors from treated sites. The use of residual larvicides has a risk of vector production from untreated habitats created or filled with water after larvicide application. Intensive monitoring and sampling of sites is ongoing to estimate this risk in the dry and rainy seasons.

1389

DECLINE IN FREQUENCY OF THE 2LA CHROMOSOMAL INVERSION IN AN *ANOPHELES GAMBIAE* S.S. POPULATION WITH INCREASING USE OF INSECTICIDE TREATED BED NETS IN WESTERN KENYA

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The 2La chromosomal inversion is polymorphic in populations of *Anopheles gambiae* s.s. and has been positively associated with indoor resting behavior. Although the genotype/phenotype relationship is not precisely known, it is likely adaptive for microclimatic conditions related to humidity. Specifically, reports from West Africa indicate that the 2La arrangement is more common in mosquitoes found resting indoors where a nocturnal saturation deficit exists. Ownership of insecticide treated bed nets (ITNs) has risen rapidly in western Kenya in the last decade, with subsequent declines in malaria transmission and malaria-related mortality and altered vector population genetic structure, with an increase to fixation of the East African kdr allele in *An. gambiae* s.s. Our study focused on the frequency of the 2La chromosomal inversion of *An. gambiae* s.s. in that setting. Adult *An. gambiae* mosquitoes were sampled from 1996 to 2011 in Asembo, an area with high ITN coverage since 1999, and from the adjacent community of Seme, where ITN ownership was <5% in 1999 but increased to over 60% by 2006. The 2La analysis was done using a PCR assay with primers designed for 2La and 2La+ proximal breakpoints and visualization of amplicons by electrophoresis on agarose gels. In Asembo, the frequency of the 2La chromosomal inversion declined from 93% in 1996 to 15% in 2005 and remained low through 2011(21%). Similarly in Seme, the frequency declined from 55% in 2000 to 19% in 2005 and remained low in 2008 (17%). These results suggest that high coverage of ITNs may have selected for the 2La+ chromosomal arrangement in *An. gambiae* s.s, a genotype not associated with indoor resting. A possible explanation is that ITNs are effective against indoor resting *An. gambiae* s.s., which are more likely to have the 2La inversion karyotype. Further studies are proposed to determine if populations with the 2La+ karyotype successfully avoid ITNs, and are responsible for maintaining residual malaria transmission in areas with high ITN coverage.

1390

AN EXAMPLE FROM ZAMBIA OF USING NOVEL APPROACHES TO MONITORING AND MANAGE INSECTICIDE RESISTANCE FOR EFFECTIVE VECTOR CONTROL

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Increased coverage with insecticide treated nets (ITNs) and indoor residual spraying (IRS) with DDT and pyrethroids, have led to impressive decreases in malaria transmission in Zambia. However, the detection of high levels of insecticide resistance in both *Anopheles gambiae* and *An. funestus* is a serious risk to vector control efforts. In 2011 an Insecticide Resistance Management Technical Working Group was established to develop a plan to sustain current control levels and conserve insecticides for malaria control in the country. Here we report on results from bioassays and molecular analysis of resistance mechanisms for two regions, the Copperbelt and Eastern Provinces of Zambia. A high prevalence of resistance to deltamethrin (27% Mortality), permethrin (34%M), etofenprox (5%M) and DDT (6%M) was detected in *An. gambiae* from the Copperbelt; the same populations were susceptible to bendiocarb and malathion. Resistance to the pyrethroids and DDT was due to kdr (the 1014F mutation is fixed in this population) and over expression of several p450's including *CYP6Z3* and *CYP6M3*. *An. funestus* from Eastern Province also exhibited resistance to diagnostic doses of deltamethrin (45% M), permethrin (81.5% M), etofenprox (18%M) and bendiocarb (77% M), but was susceptible to DDT. This population has elevated *CYP6P9a, CYP6Z1* and *CYP6M3*. A more susceptible population of *An.funestus* was found in the Copperbelt and only had elevated *CYP6M3*. As well as a different resistance profile in these regions the collections indicated very different malaria vector species abundance patterns that will impact vector control decisions. The impact of this information has allowed the Zambian malaria control programme to move away from ineffective insecticides used in the Copperbelt (DDT) and Eastern (etofenprox) to effective insecticides and to put an insecticide resistance management programme in place with the aim of prolonging the successes already gained. We examine the entomological M&E in Zambia and how lessons learnt here can be applied to other vector control programmes in the region.

1391

A LONG-LASTING *BACILLUS SPHAERICUS* (BS) AND *BACILLUS THURIGIENSIS VAR ISRAELENSIS* (BTI) FOR CONTROLLING MALARIA VECTORS: TRIALS FROM KENYA

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Bio-larvicides are alternatives for larval mosquito control as they are benign to the environment instead of synthetic insecticides. However, the currently available bio-larvicide formulations have a short effective duration, and consequently larval control incurs a high operation expense due to requirement for frequent re-treatment of larval habitats. Therefore, formulation of biological larvicides that has long-lasting effects is highly desired. A recently developed fourStarTM Single Brood Granules (SBG)

of Bacillus thuringiensis israelenis (Bti) was evaluated under semi-natural and natural conditions to test its effectiveness in reducing mosquito population in western Kenya. This formulation is designed to be effective against mosquito larvae for up to 6 months. In semi-natural habitats containing soil and rain water, second-instar larvae of *Anopheles gambiae* were introduced and FourStarTM Bti granules dissolved in rain water with appropriate concentrations were added. The number of pupae produced from the larvae was recorded daily as the outcome. Formulation was also tested in natural productive habitats. Formulation was then tested for its efficiency to reduce mosquito population during the transmission season, when it is applied earlier in sentinel sites. Larval control was undertaken in field trials in three sites and with three other sites taken as control. We found 100% mortality rate within 48 hrs after introduction of 2nd instars larvae in semi-natural habitats. The Bs/Bti formulation killed larval mosquitoes for 6months. Formulation killed larvae for 5 months in natural habitats despite the effects of rain. In larval control field trials the formulation reduced density of mosquitoes in houses from between 60-80% in the intervention sites during the transmission season. Larval control has the potential to reduce the population of malaria mosquitoes. The Bs/Bti briquets present a promising biological formulation to use for larval control. This formulation is recommended to the National Malaria Control programme.

1392

RE-ASSESSMENT OF DENGUE NEUTRALIZING ANTIBODY AND VIREMIA TITERS IN DENGUE PATIENTS USING FCΓR-EXPRESSING CELLS

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One of the major obstacles in dengue vaccine development is the potential infection-enhancement activity induced by vaccination. Sub-neutralizing levels of antibody against dengue virus (DENV) is speculated to enhance infection, and play a central role in the pathogenesis of severe and life-threatening illness, dengue hemorrhagic fever (DHF). General understanding on the biological properties of antibody in protection against dengue infection is based on the titers determined by the use of FcγR-negative cells in conventional neutralizing antibody. Additionally, conventional viremia titration assays do not consider infectious immune complex which may be infectious only through FcγR. Using FcγR-expressing BHK cells and FcγR-negative BHK cells, we examined the infection-enhancement activity and neutralizing activity in serum samples from patients with secondary and primary DENV infection. Serum samples with low neutralizing activity demonstrated infection-enhancing activity and those with high neutralizing activity demonstrated low or no infection-enhancement activity in FcγR-expressing cells. Additionally, neutralizing activity to the infecting DENV serotype detected by using FcγR-negative was absent in FcγR-expressing cells. Higher levels of viremia were detected using FcγR-expressing cells as compared to FcγR-negative cells in serum samples obtained from patients and a dengue non-human primate (NHP) model during secondary dengue infection. The results suggest that DENV-antibody complexes which are incapable of infecting FcγR-negative cells retain infectivity in FcγR-expressing cells due to infection mechanism through FcγR. Our findings suggest that in comparison to FcγR-negative cells, FcγR-expressing cells may better reflect the biological properties of antibodies *in vivo*. In summary, we established an assay which possesses the ability to detect the sum of infection-enhancement and neutralizing activities. The newly developed assay provides a platform to define dengue virus infectivity and viremia titers in the presence of neutralizing and enhancing antibody activities and offer insights into the role of antibodies in protection in natural infection and vaccination.

1393

DISSECTING HUMAN ANTIBODY RESPONSES TO SILENT AND CLINICALLY-APPARENT DENGUE VIRUS INFECTION

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Dengue is the most significant vector-borne viral disease of humans. The dengue virus (DENV) complex consists of 4 serotypes. Following primary DENV infection people develop immunity to the infecting serotype, but remain susceptible to a second infection with a new serotype. Secondary DENV infections are more likely to result in severe disease than primary infections. Antibody dependent enhancement is proposed to explain this phenomenon. Using prospectively collected samples from a cohort of children from Colombo, Sri Lanka, we explored the quantity and quality of pre-infection antibodies in children who experienced secondary silent and apparent DENV infections. Quantity of DENV-specific IgG was determined by ELISA, and antibody quality was determined by performing virus neutralization or enhancement assays. Children who acquired secondary silent and apparent DENV infections had similar pre-existing DENV-specific IgG levels. However, children who acquired secondary silent DENV infections had pre-existing antibodies that were more broadly neutralizing than children who acquired secondary apparent DENV infections. In this presentation, we will also discuss the ability of pre-infection antibodies from silent and apparent cases to enhance DENV infection of Fc-receptor bearing cell lines and primary human cells. Together, our findings demonstrate how neutralization capacity and enhancement ability of pre-existing antibodies influences disease presentation in secondary dengue infections.

1394

LONGITUDINAL ANALYSIS OF THE LEVELS OF CROSS-REACTIVE ANTIBODIES RECOGNIZING THE FUSION LOOP OF DENGUE VIRUS AND CORRELATION WITH NEUTRALIZING ANTIBODY TITERS IN NICARAGUAN DENGUE CASES

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The envelope (E) protein of dengue virus (DENV) is the major target of neutralizing antibodies (Abs). Previous studies of human convalescent sera after DENV infection revealed that a significant proportion of anti-E Abs recognized the highly conserved fusion loop (FL) of domain II of E protein (FL Abs), whereas a minor proportion recognized domain III. The role of FL Abs in dengue pathogenesis remains unclear. In this study, we tested the hypothesis that cross-reactive FL Abs, though not contributing to the monotypic neutralization profile after primary DENV infection, may play a role in protection against heterologous serotypes after secondary DENV infection. A quantitative virion-capture ELISA was established by using known concentrations of a human anti-E monoclonal Ab as a standard to measure the concentration of anti-E Abs, [anti-E Abs], in sera of dengue patients from Nicaragua. The proportion of FL Abs was determined by a previously described capture ELISA using virus-like particles, and the concentrations of FL Abs, [FL Abs], were calculated. Neutralization titers were determined using a flow cytometry-based neutralization assay with reporter viral particles of the different DENV serotypes. Analysis of sequentially collected serum samples (3M, 6M, 12M and 18M) from 10 cases of primary or secondary DENV infection revealed that [anti-E Abs] and [FL Abs] stabilized at 12 M after infection and were higher in secondary DENV infection cases than in primary infection cases. The [FL

Abs], while not correlated with neutralization titers in primary infection cases, increased as the neutralization titers against heterologous serotypes increased in secondary infection cases. These findings are being verified in sera from 26 additional secondary DENV infection cases. Our results demonstrate the kinetics of FL Abs over time after DENV infection and suggest that FL Abs might play a protective role against heterologous serotypes after secondary DENV infection.

1395

CORRELATION BETWEEN DENGUE VIRUS-SPECIFIC NEUTRALIZATION, SERUM AVIDITY AND ANTIBODY TITERS IN PRIMARY AND SECONDARY DENV-3 NATURAL HUMAN INFECTIONS

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The 4 serotypes of dengue virus (DENV1-4) infect ~100 million people annually. While heterotypic secondary (2) DENV infection has been associated with severe disease, the vast majority of 2 infections are mild or asymptomatic, suggesting protective cross-reactive immunity in addition to long-lasting homotypic immunity. The mechanism of antibody (Ab)-mediated protection is not well defined. We are analyzing DENV-specific neutralization titer, IgG avidity and Ab titer in well-characterized serum samples from a pediatric dengue hospital-based study in Managua, Nicaragua. In 2010, 130 DENV-positive cases were enrolled (primary (1) n=75; 2 n=55), with DENV3 as the dominant serotype (83.1%). The 50% neutralization titer (NT_{50}) was measured by flow cytometry. Avidity and Ab titer were measured by a modified ELISA with urea washes and by Inhibition ELISA, respectively. We observed a significant increase in avidity vs. DENV3 between the convalescent and 3-month (3m) timepoints (% IgG bound = 45.8 vs. 82.6, p<0.0001) in 1 infections, reflecting affinity maturation. We also noted a significant increase in avidity between the acute and convalescent phase in 2 infections (69.2% vs 79.7, p=0.0015), without further increase over time (3-6m), attributable to newly formed Ab against the current infecting serotype. The NT_{50} peaked at convalescence in both 1 and 2 cases, with significantly higher titer detected in 2 cases (5284 $\pm$ 683 vs. 11476 $\pm$ 1183, p<0.0001). In the convalescent phase and 3m after 1 infection, neither DENV3-specific avidity nor DENV-specific Ab titer correlated with DENV3-specific NT_{50}, implying that either innate immune and/or naïve T cell responses and/or low-avidity Abs control 1 infections. In acute 2 infections, we observed a correlation between avidity and NT_{50} vs. DENV3 (Spearman r=0.50, p=0.002) and a correlation with DENV-specific Ab titers (Spearman r=0.61, p<0.0001), most likely reflecting an expansion of cross-reactive DENV-specific memory B cells formed during the previous infection. We are currently processing these samples against DENV2, the most likely 1 infecting serotype, to confirm this hypothesis. Lastly, we find that at the 3m timepoint, DENV3-specific avidity correlates positively with DENV3-specific NT_{50} (Spearman r=0.49, p=0.0015). A better understanding of the protective immune response in natural infections is critical for the development of safe and effective vaccines.

1396

MAPPING ENHANCING ANTIBODIES PRODUCED BY THE HUMAN IMMUNE RESPONSE AFTER PRIMARY DENGUE VIRUS INFECTIONS

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Dengue virus (DENV) is a mosquito-borne flavivirus of global significance. DENV exists as four serotypes, named DENV1 through DENV4. Following a primary infection, individuals produce a mixture of type-specific and cross-reactive antibodies (Abs). Pre-existing immunity is sufficient to protect against re-infection with the same serotype, but may enhance infection and increase disease severity during a secondary infection with one of the other three DENV serotypes. A leading theory to explain the higher frequency of severe disease is the antibody-enhancement (ADE) theory, where a fraction of pre-existing DENV-specific Abs are thought to bind viral particles and aid infection of host cells through Fcγ receptors. Due to the complexity of the human humoral immune response, the enhancing anti-DENV Abs within human polyclonal sera have not been well characterized. Previously, Abs in DENV-immune human sera were fractionated using DENV virions, and the role of specific antibody populations in DENV enhancement was investigated in cell culture and in the AG129 mouse model of DENV infection and disease. We demonstrated that people exposed to primary DENV infections have serotype-specific and serotype cross-reactive populations of circulating Abs. The serotype-specific Abs were responsible for neutralization of the homologous serotype, whereas the serotype cross-reactive Abs were responsible for ADE of heterologous serotypes. The ability of the serotype cross-reactive Abs to enhance DENV was observed both *in vitro* and *in vivo*. Further studies were then performed to identify the antigens and epitopes engaged by enhancing Abs in human serum by fractionating DENV-immune sera using recombinant viral proteins and assaying the depleted sera in *in vitro* ADE assays and in the AG129 mouse model. Our studies demonstrate that enhancing Abs in DENV-immune sera recognize epitopes on E protein as well as prM. Further studies are in progress to quantify the relative contribution of Abs against different antigens to ADE and to map specific epitopes responsible for ADE.

1397

ANTIBODY RESPONSES TO THE DENGUE VIRUS PROTEOME DURING SEASONAL OUTBREAKS OF INFECTION

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Dengue is a mosquito-borne infection caused by four distinct serotypes of dengue virus, each appearing cyclically in the tropics and subtropics along the equator. The viral proteome is comprised of capsid, membrane, envelope and the non-structural (NS) proteins NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5. Though each of these proteins synthesized during infection are potential targets of host defenses, current knowledge of the immune response to the DENV proteome is limited. Here we describe a protein microarray approach for measuring antibody responses to the complete viral proteome of dengue virus serotypes 1-4. Using this microarray, we examined humoral immunity to dengue occurring during seasonal outbreaks in Puerto Rico, and identified unique immunological profiles resulting from pediatric and adult infections. Our results demonstrate discriminating details concerning the nature of antibody responses to dengue virus at the proteomic level and suggest the usefulness of this information for vaccine development.

1398

CONSIDERING THE ROLE OF ANTIBODY IN DENGUE VIRUS CLEARANCE: DATA ANALYSIS AND MODELLING

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Antibodies in dengue infection are thought to play a critical role in controlling infection, but also may enhance viral replication in secondary infection via the phenomenon of antibody-dependent enhancement. Here, we consider mainly the former role, using sequentially sampled measurements of virus and antibody titres (IgM, IgG, anti-E IgG and anti-D3 IgG) from patients hospitalised with dengue infection in Vietnam. Analysis of such data is not straightforward, due to differences in timing of measurements relative to virus peak and symptoms onset, however using multiple sequential measurements from throughout natural infections is an excellent way to consider the interaction between virus and the immune response. In addition to descriptive statistical analyses, we fitted a mechanistic mathematical model of dengue pathogenesis within the human host to these data to investigate whether the observed kinetics were compatible with antibody playing the dominant role in controlling viral replication. A model variant which assumes clearance of virus or infected cells is proportional to overall IgM titres is able to fit data from both primary and secondary infections, and the same model with clearance proportional to overall IgG titres is able to fit data from secondary infections. However, this fit relies on variation in how much measured antibody is useful, and in some individuals there are issues in timing of virus peaks and antibody increase. A model for secondary infections in which viral clearance rates were proportional to anti-E IgG titres gives, in most cases, the best model fit, overcoming some of these issues. We will also present extensions to this work to include mechanistically the interactions between the different antibody measures. Interestingly, for all model fits, we estimate that the level of antibody required to control viral replication is low, and over an order of magnitude below the peak titres reached by the time infection is cleared. In our presentation we will consider the implications of this result for measurement of antibody kinetics.

1399

INNOVATIVE IMMUNOLOGICAL ASSAYS FOR DIAGNOSIS OF *SCHISTOSOMA MANSONI* FOR CLINICAL ACUTE AND/OR CHRONIC FORMS

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Control constraints of schistosomiasis include the lack of diagnostic methods with high sensitivity. We initiated a prospective study in southeast Brazil in order to develop sensitive diagnostic methods for *Schistosoma mansoni* infection. Residents on 3 endemic areas in Minas Gerais state, together with 84 travelers infected in a freshwater pool on a country house located on a new focus of the disease, participate of this survey. Sera samples from all those patients are used for the standardization of innovative methods for schistosomiasis mansoni. Comparisons are performed with the presence of eggs in faecal samples, IgG antibody titers, presence of eggs in liver after biopsy, encephalomyelitis by magnetic resonance imaging, and/or clinical symptoms. The first assay using schistosomula antigen is capable of properly diagnosing all the 84 travelers with clinical acute form as soon as 10 days post-infection, including patients with severe hepatic form and encephalomyelitis. Two other assays using egg and adult worm antigens are capable of detecting more than 95% of positive cases from chronic and low parasite load patients (1-36epg). A forth method called Immunomagnetic separation (IMS) was developed in order to concentrate sera samples. We used several antigens, in separate, for IgG titers detection, as purified glycoprotein Circulating Cathodic Antigen (CCA), CCA recombinant protein (CCAr), and five different types of peptides (10 amino acids each) of CCA. Data showed that IMS is superior to ELISA ($p=0.001$) since it is capable of detecting a higher number of positive patients. The purified CCA was not a good candidate due to its susceptibility for cross reaction. On the contrary, peptides and especially CCAr, are excellent tools for the differential diagnosis with 100% of sensitivity. Furthermore, IMS method was standardized for a direct detection of CCA in sera. For that matter, monoclonal antibodies against the protein portion of the native CCA (MAb-CCA) were produced. Using only 0.05ml of concentrated sera, we were able to detect 100% of chronic patients and 98% of patients with acute form of the disease. Finally, a last methodology were developed, a qualitative method using magnetic beads and CCA-MAb conjugated to Alexa Fluor for the direct visualization of fluorescent CCA in sera samples. A double-blinded study showed that 3 slides of each sample are sufficient to achieve a sensitivity of 98% and a specificity of 95%.

1400

DIAGNOSTIC APPROACHES FOR PEDIATRIC TUBERCULOSIS AMONG HIV-INFECTED AND HIV-UNINFECTED CHILDREN IN PERU

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Children with pulmonary tuberculosis (PTB) usually present with paucibacillary disease and without sputum, and HIV co-infection may further complicate diagnostic testing. We evaluated HIV-infected Peruvian children with suspected PTB with a series of culture and PCR-based techniques, and compared results from these subjects with similar results from HIV-infected controls and from HIV-negative cases and controls. TB culture and a heminested IS 6110 polymerase chain reaction (PCR) assay were performed on specimens from children with symptoms of PTB and well controls. Two specimens of each type (gastric aspirates [GA], nasopharyngeal aspirates [NPA], and stools) from each case were examined by 1) auramine smear, 2) broth culture by Microscopic-Observation Drug-Susceptibility (MODS) technique, 3) standard culture on Lowenstein Jensen (LJ) agar, and 4) PCR. Specimens from controls included one NPA and 2 stools. The study included 209 HIV-negative cases, 81 HIV-positive cases, 200 HIV-negative controls, and 35 HIV-positive controls. Overall, 22 HIV-negative case subjects (10%) had at least one positive TB culture. In contrast, TB was only isolated from one HIV-positive case (1.2%), from both GA specimens only ($p<0.01$). In contrast to the difference in TB isolation between HIV-positive and HIV-negative cases, the proportions of subjects in these groups with at least one positive PCR result were similar, and both case groups had more positive PCR results than the HIV-negative controls ($p<0.001$). Rates of PCR positive specimens were similar for HIV-positive cases and controls. In contrast to reports from Africa, TB recovery from HIV-positive patients with suspected PTB in our Peruvian pediatric population is less common. In spite of the differences in culture-based MTB recovery, HIV-positive cases had similar rates of PCR-positive specimens as compared to HIV-negative case subjects. These PCR-positive, culture-negative specimens may reflect paucibacillary disease, or in HIV-positive controls they may indicate latent or subclinical infection.

1401

PEPTIDE YY, GHRELIN, LEPTIN AND IL-10 AS MEDIATORS OF APPETITE AND RESPONSE TO TREATMENT IN PERUVIAN ADULTS WITH TUBERCULOSIS

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Cachexia is one of the sentinel symptoms of pulmonary tuberculosis (TB). TB causes an inflammatory response that leads to alterations of appetite hormones affecting appetite and satiety. Yet the relationship of TB disease severity and appetite hormone levels has not been well studied, despite its potential utility as an indication of treatment failure. 23 adult patients with sputum positive TB were evaluated on days 0, 14, and 28 days of treatment by Simplified Nutritional Appetite Questionnaire (SNAQ), body mass index (BMI), and appetite and inflammatory markers. Peptide YY, ghrelin, leptin, and IL-10 levels were determined using Luminex and ELISA kits. We also administered a questionnaire to qualitatively determine appetite. Appetite questionnaire results trended towards a gain in appetite with treatment, displaying a significant difference between day 0 vs.14 (ρ=0.004) and day 28 (ρ=0.0095). Peptide YY levels dropped 14.6% by Day 14 of treatment (not significant), while ghrelin levels dropped 54% by Day 14 (ρ<0.05). Leptin levels increased 67.36% by day 28 of treatment (ρ<0.05), and the anti-inflammatory cytokine IL-10 decreased 12.5% by Day 14 (not significant). Subjective appetite improved with treatment as early as day 14, while BMI was slower to respond and still had not increased significantly by day 30. Delayed recovery of weight gain suggests that the increase in leptin is secondary to TB infection. Wasting in TB patients may partly be mediated by upregulation of anorexigenic PYY with resulting appetite suppression. Decrease in IL-10 levels may indicate intact immunity with normal response to treatment. Deviation from improving appetite status, clinical factors and appetite hormone levels may be used to detect treatment failure in cases such as multi-drug-resistant TB. While loss of appetite is a well-known symptom of TB, little work has been done in utilizing measurements of appetite in the characterization of the disease, and this work suggests that it may be a useful indicator of treatment success.

1402

BRUCELLOSIS AMONG HOSPITALIZED FEBRILE PATIENTS IN NORTHERN TANZANIA

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Brucellosis is an important cause of zoonotic disease worldwide. However, non-specific clinical features, low clinical suspicion, and lack of access to adequate diagnostic services result in brucellosis being underdiagnosed and untreated in many low-resource countries. Human clinical data are scarce in sub-Saharan Africa. Acute and convalescent serum samples were collected from febrile inpatients admitted to two hospitals in Moshi, Tanzania serving a catchment area dominated by smallholder farming communities. Confirmed brucellosis was defined as a positive blood culture for *Brucella* spp or a ≥4-fold increase in microagglutination test (MAT) titer, and probable brucellosis was defined as a single reciprocal titer ≥160. A total of 870 patients were enrolled in the study, 403 (46.3%) adults and adolescents and 467 (53.7%) infants and children. Of 455 participants with paired sera tested for brucellosis, 16 (3.5%) met criteria for confirmed brucellosis. Of 830 participants with ≥1 serum sample, 4 (0.5%) met criteria for probable brucellosis. Five (31.3%) of the participants with confirmed brucellosis were female. The median (range) age of participants with confirmed brucellosis was 28.4 (1.1, 68.5) years. Brucellosis was associated with increased median age (p = 0.024), leukopenia (odds ratio [OR] 7.8, p = 0.005), thrombocytopenia (OR 3.9, p = 0.018), and evidence of other zoonoses (OR 3.2, p = 0.026). There was no association between brucellosis and rural residence, hepato- or splenomegaly, lymphadenopathy, anemia, pleural effusion, or HIV. Brucellosis was never diagnosed clinically. Although all participants with brucellosis received antibacterials or antimalarials in the hospital, none received standard brucellosis treatment. Brucellosis is an underdiagnosed and untreated cause of febrile disease among hospitalized adult and pediatric patients in northern Tanzania. Increased clinician awareness, access to reliable diagnostic tests, and additional research on risk factors are needed to identify, appropriately manage, and prevent brucellosis in this area.

1403

INFECTIOUS DISEASES ARE A LARGER CONTRIBUTOR THAN OBSTETRIC CAUSES TO MATERNAL MORTALITY IN RURAL WESTERN KENYA

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Improving maternal health is a high priority for the United Nations' development agenda where it is targeted as the fifth Millennium Development Goal. In Kenya, the maternal mortality ratio remains high, at 488 per 100,000 live births per the 2008/09 Demographic Health Survey.

It is commonly assumed that maternal deaths are primarily a result of direct obstetric complications that occur around the time of childbirth. We conducted descriptive analyses of data from a Health and Demographic Surveillance System encompassing a population of approximately 220,000 individuals in rural western Kenya, an area that bears a disproportionate share of infectious diseases. Standard WHO methodology for verbal autopsy (VA) was implemented to determine contributors to maternal mortality (defined as the death of a woman while pregnant or within 42 days of the termination of pregnancy). The maternal mortality ratio for the six year period between 2003 and 2008 was 740 per 100,000 live births, with no evidence for a linear trend over time. Of 249 maternal deaths, one-third (n=85) were due to directly ascribed causes, predominantly by postpartum hemorrhage (n=22), complications from abortion/miscarriage (n=14), and puerperal sepsis (n=13). However, the majority of maternal deaths (n=164) were classified through VA as deaths from infectious diseases, predominantly from HIV (n=74), malaria (n=22) and TB (n=16). While the impact of HIV on maternal mortality has been previously recognized, in this area with high levels of malaria transmission, malaria was also a significant factor among deaths of pregnant or recently delivered women (65 maternal deaths associated with malaria per 100,000 live births). This was equal to the number of directly attributed obstetrical deaths due to documented postpartum hemorrhage. These data add to our awareness of the relationship between infectious diseases and poor maternal outcomes in Africa. Our data suggest that improved access to, and increased uptake of, emergency obstetric care, as well as preventive measures against HIV, malaria and TB among all women of childbearing age, will result in measurable impact on maternal health outcomes.

1404

SCABIES COMMUNITY PREVALENCE AND MASS TREATMENT IN TWO FIJIAN VILLAGES

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Scabies is a major public health problem with complications caused by bacterial secondary infection. A community mass treatment study in two Fijian villages was undertaken to compare the efficacy and tolerability of topical benzyl benzoate and oral ivermectin. Two research sites with approximately 600 inhabitants each were chosen, and study participants enrolled, completed questionnaires and examined to assess for scabies. In one village participants received benzyl benzoate and in the other either oral ivermectin or, for children under 2 years, permethrin cream. At follow-up, participants were re-examined and possible adverse events documented. Pre and post-treatment questionnaires included questions regarding history, itch, adverse events and satisfaction with treatment. Although ethnic and age demographics were similar in the two villages, scabies prevalence rates differed significantly, 38% and 24%. Prevalences in both villages were particularly high in children, with superinfection of scabies lesions common. Only 43% of those treated returned for follow-up overall. The scabies prevalence rate in those who returned for follow-up dropped from 37.9% to 19.9% after treatment with benzyl benzoate, compared to 23.7% and 9.5% following ivermectin treatment. Thus scabies prevalence was reduced by 53% following therapy with benzyl benzoate, and by 52% in those who received ivermectin. People treated with benzyl benzoate more commonly reported initial worsening of itch and of pre-existing dermatologic conditions after application than those treated with ivermectin. No serious side effects occurred with either treatment, and patient satisfaction did not differ between the treatments. In conclusion, mass treatment with oral or topical therapy in a village setting with high prevalence of scabies is feasible. Despite the difficulties in assessing ongoing active scabies infestation when the papules persist, a reduction in scabies prevalence of 53% and 52% was recorded.

1405

FACTORS INFLUENCING ATTENDANCE AT TREATMENT AND PREVENTION CLINICS BY PATIENTS WITH PODOCONIOSIS IN SOUTHERN ETHIOPIA: A QUALITATIVE STUDY

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Podoconiosis is a lymphoedema of non-infectious cause which results in long-term ill health in affected individuals. Simple, effective treatment is available in certain parts of Ethiopia, but anecdotally, not all patients continue collecting treatment supplies from clinic sites once started. We used qualitative techniques to explore factors affecting continued collection of treatment supplies from outreach clinics of a non-government organization in southern Ethiopia. A cross-sectional qualitative study was conducted in four clinic sites through unstructured in-depth individual interviews, key informant interviews and focus group discussions with the involvement of 88 study subjects.Sub-optimal continuation with clinic visits is common among podoconiosis patients. The reasons were: remoteness from the clinic sites, unrealistic expectation of 'special' aid, worry about increasing stigma, illness, misconceptions about treatment, and being too busy.Several of these factors are remediable through community and individual information and education. Appropriate routes to deliver this information must be identified. Certain factors (such as distance to clinic sites and stigma) require substantial expansion of services or liaison with village-level government health services.

1406

PREVENTION OF TUNGIASIS AND TUNGIASIS-ASSOCIATED MORBIDITY USING A HERBAL REPELLENT: A RANDOMIZED CONTROLLED FIELD STUDY IN RURAL MADAGASCAR

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Tungiasis (sand flea disease) is a neglected tropical disease. It is endemic in many resource poor populations in South America, the Caribbean and in sub-Saharan Africa and is associated with significant morbidity. Since there is no effective drug treatment, prophylaxis is the only means to prevent sand flea disease. In a randomized, controlled field study in rural Madagascar, two preventive measures were compared: the twice-daily application of Zanzarin (a repellent based on coconut oil) on the feet and the free distribution of closed shoes. A control group was left without any intervention. Over a period of 10 weeks, study participants were examined every two weeks and the number of newly penetrated sand fleas, the total number of lesions, the proportion of different developmental stages, and tungiasis-associated morbidity were determined quantitatively. Compared to the control group, the total number of penetrated sand fleas decreased only by 5% after the distribution of shoes. The regular application of Zanzarin reduced the parasite load by 85%. In the shoe group, the median attack rate fell by 22%, in the Zanzarin group by 95%. The distribution of shoes reduced tungiasis-associated morbidity only marginally. The protective effect of shoes was related to the regularity with which shoes were worn. After 10 weeks of application of the repellent tungiasis-associated morbidity had disappeared almost completely. The study shows that twice-daily application of a repellent based on coconut oil provided an excellent protection against the development of sand flea disease. The free distribution of shoes had only a minimal protective effect, mainly because shoes were not worn regularly.

1407

IMPACT OF INTRODUCTION OF THE *HAEMOPHILUS INFLUENZAE* TYPE B CONJUGATE VACCINE INTO CHILDHOOD IMMUNIZATION ON MENINGITIS IN BANGLADESHI INFANTS

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Some Asian countries have been reluctant to adopt Hib vaccination because of uncertainty over disease burden. We assessed the impact of introduction of Hib conjugate vaccine into the Expanded Program on Immunization (EPI) in Bangladesh on purulent and laboratory confirmed *Haemophilus influenzae* meningitis. Within a well-defined catchment area around two surveillance hospitals in Dhaka, Bangladesh, we compared the incidence of Hib meningitis confirmed by culture, latex agglutination and polymerase chain reaction (PCR) assay among infants one year before and one year after introduction of Hib vaccine. We adjusted the incidence rate for the proportion of children who sought care at the surveillance hospitals. Among infants, the incidence of confirmed Hib meningitis decreased from 92 to 16 cases per 100 thousand within 1 year of vaccine introduction [Vaccine preventable incidence (VPI) =76; 95% CI: 18, 135/ 100 thousand]. The incidence of purulent meningitis decreased from 1659 to 1159 per 100 thousand [VPI=500; 95% CI: 203, 799/ 100 thousand]. During the same time period, there was no significant difference in the incidence of meningitis due to *Streptococcus pneumoniae*. Introduction of conjugate Hib vaccine into Bangladesh EPI markedly reduced the burden of Hib and purulent meningitis.

1408

IMMUNOGENICITY, SAFETY, DOSE AND SCHEDULE RESPONSE OF A MENINGOCOCCAL GROUP A CONJUGATE VACCINE IN INFANCY: A HOPE FOR ROUTINE IMMUNIZATION IN THE AFRICAN MENINGITIS BELT

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Meningitis epidemics remain a major plague in countries in the African meningitis belt, with group A meningococcus being the predominant causal agent. An affordable meningococcal group A conjugate vaccine was developed through the Meningitis Vaccine Project, and introduced at public health scale in 2010-11, using single dose mass campaigns among 1 to 29 year-olds in 6 out of 26 target countries of the meningitis belt, with extremely promising results. Roll-out in all countries is ongoing. To maintain population immunity level after initial campaigns, protection of new birth cohorts should be achieved early in life. We conducted a dose ranging study of the newly developed MenA conjugate vaccine in infants to evaluate the safety and immunogenicity of three different doses administered in a two dose schedule at 14 weeks and 9 months, or in one dose schedules at 9 or 12 months concomitantly with the EPI vaccines. Starting in 2008, 1198 infants were recruited in the Kassena Nankana districts of Northern Ghana and followed up till 2012. Results confirmed noninferiority of the alternate dosages to the licensed dosage. No significant interferences with co-administered EPI vaccines were found. The proportions of subjects with seroconversion at Day 28 post 9 months vaccination were high and similar in all MenA vaccine groups (1 or 2 doses regimens), but the magnitude of the responses was higher in subjects previously primed with MenA vaccine (2 doses regimens *vs.* 1 dose regimen), nonetheless administration of a single dose at 9 months of age induced a high immune response. No significant safety concerns were identified. The majority of adverse events were due to infections consistent with background morbidity in the area. Sustainable protection from MenA disease among new birth cohorts could be achieved through immunization starting in late infancy at 9 months. This could be a powerful strategy for sub-Saharan countries, leveraging on vaccine herd protection effect, preventing overcrowding early infancy schedules, and allowing paired administration of the MenA with that of the measles vaccine.

1409

PERSISTENT, WIDESPREAD OUTBREAK OF TYPHOID FEVER ASSOCIATED WITH INTESTINAL PERFORATIONS - BUNDIBUGYO AND KASESE DISTRICTS, UGANDA, 2009-2011

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Salmonella enterica serovar Typhi causes approximately 22 million typhoid fever infections worldwide each year; among these, 1-3% of patients develop intestinal perforation (IP). In 2008, an outbreak of typhoid fever with a high rate of IP was reported in Kasese, a rural district in western Uganda. A 2009 investigation of this outbreak identified 577 cases through July 15, 2009; 249 had IP. A high rate of IP was sustained in Kasese through 2011 and the neighboring district of Bundibugyo reported a typhoid fever outbreak in August 2011. We gathered information about cases through enhanced surveillance and hospital and district health office (DHO) records. A suspected typhoid case was defined as diagnosis of IP or symptoms of fever, abdominal pain, and one or more of the following: vomiting, diarrhea, constipation, joint pain, headache, general body weakness, clinical suspicion of IP, or failure to respond to antimalarials in a Kasese resident from July 16, 2009-December 31, 2011 or in a Bundibugyo resident in 2011. Among Kasese residents, 658 suspected cases were identified; 519 were diagnosed with IP. Among Bundibugyo residents, 330 suspected cases were identified and 56 were diagnosed with IP. Laboratory surveillance from October -December 2011 isolated *Salmonella* Typhi by blood or stool culture from 9 Kasese and 15 Bundibugyo patients. Among 19 isolates tested for antimicrobial sensitivity, 1 had intermediate susceptibility to ciprofloxacin, 15 were multidrug resistant but sensitive to ciprofloxacin, and 3 were pan-susceptible to all antimicrobials tested. Several pulsed field gel electrophoresis patterns were shared by isolates from both districts, suggesting that the outbreak spread from Kasese to Bundibugyo. Untreated drinking water was suspected as the chief transmission route. Drinking water sources in areas of high typhoid incidence in both districts yielded *Escherichia coli*, an indicator of fecal contamination. Recommended control measures included emergency point-of-use water treatment interventions and community education about sanitation and hygiene.

1410

IDENTIFICATION OF ANTI-*SALMONELLA ENTERICA SEROVAR TYPHI* IMMUNE RESPONSES IN CHRONIC CARRIERS OF S. *TYPHI* IN KATHMANDU, NEPAL

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Salmonella enterica serotype Typhi can colonize and persist in the gallbladder of infected individuals. This can result in an asymptomatic chronic carrier state and chronic carriers can act as persistent reservoir of infection within a community. Unfortunately, little is known about host-pathogen interactions in the biliary tract of chronic carriers, and there is currently no reliable diagnostic assay to identify asymptomatic S. Typhi carriage. To address this, we applied an immunoscreening technique, *in vivo*-induced antigen technology (IVIAT), to identify potential biomarkers unique to S. Typhi chronic carriers. IVIAT identifies humorally immunogenic antigens expressed uniquely *in vivo*, and we hypothesized that S. Typhi surviving in the biliary tract of humans may express a unique proteomic profile. In brief, we generated a 120,000 clone genomic inducible expression library of S. Typhi CT18 (500-1500 bp fragments) in *E. coli* BL21DE3 and screened the library against pooled sera of patients (pre-adsorbed with *in vitro* grown S. Typhi and *E. coli* BL21DE3) who had bile cultures positive for S. Typhi at the time of elective cholecystectomy in Kathmandu. We identified 268 genes of interest from our primary screen, and subsequently sub-cloned each identified gene. Thus far, we have identified 50 proteins that are immunoreactive in S. Typhi carriers; these include a number of putative membrane proteins, lipoproteins, and hemolysin-related proteins. We are comparing these responses to those in patients with acute S. Typhi infection (typhoid fever) and patients from S. Typhi endemic zones with bile cultures negative for S. Typhi to identify uniquely immunoreactive antigens in Typhi carriers.

1411

IMPACT ASSESSMENT OF A MASS TYPHOID FEVER VACCINATION CAMPAIGN - FIJI, 2011

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Typhoid fever, a life-threatening disease, is endemic in Fiji. During June-December 2010, a mass typhoid vaccination campaign was conducted in Fiji targeting 65,000 persons ≥2 years old in cyclone and outbreak-affected areas. Considering limited use of typhoid vaccine in post-disaster or outbreak settings, we evaluated the campaign impact. We calculated confirmed typhoid incidence rates for 2008-11 using Fiji's national laboratory surveillance data. For all reporting subdivisions, we calculated risk ratios (RR) and 95% confidence intervals (CIs) for incidence in years post- (2011) versus pre-campaign (2008-9 annual average). The percentage of the population vaccinated was determined from campaign doses administered and medical area census populations; subdivision populations >20% vaccinated were called "vaccinated." In subdivisions with high pre-campaign incidence (≥100/100,000/year), we used log-binomial regression to estimate RRs and 95% CIs for the proportion of positive blood cultures in the high season months (January-August) post-(2011) versus pre-campaign (2008-10). Nationwide, 7% of the population was vaccinated, and confirmed typhoid was unchanged at 44/100,000/year between 2008-9 and 2011. In 11 unvaccinated subdivisions, post-campaign incidence was either unchanged, or significantly increased in 6 subdivisions (individual RRs ranged 2.2-7.8). In the 3 vaccinated subdivisions, post-campaign incidence was significantly decreased (individual RRs ranged 0.2-0.6). In the 2 high-incidence, unvaccinated subdivisions, the post-campaign proportion of positive-cultures increased (RR=1.8, CI=1.2-2.7; RR=1.6, CI=1.1-2.2). In three high-incidence, vaccinated subdivisions, the post-campaign proportion of positive-cultures decreased (RR=0.3, CI=0.1-0.6; RR=0.5, CI=0.3-0.9) or was unchanged (RR=1.4, CI=0.9-2.0). Post-campaign, confirmed TF cases in Fiji decreased in vaccinated areas and increased in unvaccinated areas. Typhoid vaccination can be considered in other high-incidence areas in Fiji and similar settings along with comprehensive typhoid control measures.

1412

ORIENTIA TSUTSUGAMUSHI, RICKETTSIA AND *LEPTOSPIRA* SPECIES AS CAUSES OF MENINGOENCEPHALITIS IN LAOS

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Rickettsial and leptospiral diseases have been recorded as rare causes of meningoencephalitis. We see such patients in the Lao PDR (Laos) where *Leptospira* spp., *Orientia tsutsugamushi* (scrub typhus), *R. typhi* (murine typhus), *Rickettsia* spp. (Spotted Fever Group) are important causes of fevers. There have been no prospective studies to determine the clinical importance and the epidemiology of rickettsial and leptospiral CNS infections in endemic countries using modern techniques. We therefore investigated the incidence of Leptospira, *Rickettsia* spp and *O. tsutusgamushi* among patients presenting with CNS infections to Mahosot Hospital in Vientiane, between 2003 and 2011. We performed paired MAT serology for anti-IgM/G Leptospira, paired IFA anti-IgM serology for rickettsial pathogens and cereberospinal fluid (CSF) and blood PCR for *Leptospira* spp., *Rickettsia* spp. and *O. tsutusgamushi*, by qPCR using 47kDa, 17kDa and rrs targets, respectively. We found evidence, using CSF PCR assays, for *O. tsutsugamushi*, *Leptospira* and *Rickettsia* spp. in 17/1030 (1.7%), 16/994 (1.6%) and 14/ 975 (1.4%) consecutive patients, respectively. In comparison to these 47 positive patients, CSF PCR for *S. pneumonia, N. meningitis* and *H. influenzae* b identified 38 patients in the same series with 'conventional' meningitis pathogens in CSF. These data suggest that scrub typhus, leptospirosis and murine typhus are important causes of CNS disease in Laos. The data underline the need for timely testing of patients with meningoencepahlitis for these 'atypical' pathogens. Such tests would be clinically important as rickettsial CNS disease would not be expected to respond to third generation cephalosporins that are commonly used for the empirical therapy of meningitis.

1413

LEPTOSPIROSIS IN MAMMALIAN RESERVOIRS AND SURFACE WATER IN ALTO MAYO VALLEY, SAN MARTIN, PERU

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Leptospirosis is caused by bacterial spirochetes of the genus *Leptospira.* All mammals can chronically shed *Leptospira* in their urine and humans

can become infected following contact with contaminated water or soil. In 2010, the Institute of Tropical Medicine of the Universidad Nacional Mayor de San Marcos found that 64.75% (CI 95%: 58.76-70.74) of rice field workers (n=261) in Alto Mayo Valley, Peru were seropositive for pathogenic leptospirosis by microagglutination test (MAT). The present study aimed to identify mammalian reservoirs and water sources of pathogenic leptospirosis in this region. In October 2011, at the start of the rainy season, serum and urine samples were collected from 179 domestic animals, including 57 dogs, 56 cows, 49 pigs, and 17 sheep from three rural settlements in the Alto Mayo Valley. In addition, 217 rodents, primarily *Mus musculas,* were trapped from rice fields and houses, and serum and kidneys were collected. Water samples were collected from 146 locations including rice fields (n=28), canals (n=47), standing water sources (n=45), and tap water (n=26). Epidemiological surveys were conducted (n=114) to identify risk factors associated with leptospirosis-positive households. MAT analysis of domestic animal and rodent sera is currently underway, as well as PCR of urine samples. To date, 14.29% (31/217) of kidney samples and one water sample (1/146) were positive by PCR for *Leptospira spp.* Genetic sequencing revealed that 2 of 31 rodent kidney samples were colonized by pathogenic *Leptospira interrogans*, while the remaining were colonized by the non-pathogenic species *Leptospira biflexia.* Water sources did not appear to be a significant source of leptospirosis prior to the rainy season. Preliminary results indicate that *Mus musculas* in rice fields may be a significant reservoir for leptospirosis in this region. Upon completion of all sample processing, this data will complement our understanding of the site-specific epidemiology of this disease and will provide information necessary for public health interventions in the Alto Mayo Valley.

1414

IMPACT OF A RURAL BANGLADESH SCHOOL WATER SANITATION AND HYGIENE INTERVENTION WITH AND WITHOUT ADDING HARDWARE

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To improve hygienic practices in Bangladesh, a countrywide school-based behavior change communication (BCC) intervention was implemented for 18 months that included hygiene promotion sessions taught by trained teachers; formation of student brigades engaged in maintaining clean school compound; and quarterly hand hygiene demonstration through street shows, fairs and rallies. In a subset of these schools the intervention added to or improved existing water, sanitation and hygiene (WASH) facilities along with the BCC. We evaluated whether BCC alone was sufficient, or if provision of WASH facilities combined with BCC was necessary to improve hygiene practices. We selected 800 intervention schools, 200 of which received combined interventions, and 600 control schools, each from 50 similar clusters, where the probability of selection was proportional to the size of the cluster. We interviewed 1400 headmasters and 5600 students. We calculated the risk difference (RD) adjusted for clustering for facilities and practices between combined and control schools and also between schools receiving only BCC and controls. We calculated difference in difference to estimate the effect of WASH facilities in addition to BCC. Fifty-six percent of combined intervention schools had clean water points with proper drainage compared with 42% of BCC only schools (p=0.004) and 35% of control schools (RD= 20; 95% CI= 11, 29). Of combined intervention schools, 64% had soap available inside/ near the toilet compared with 62% of BCC only schools (p=0.62) and 49% of control schools (RD= 16; 95% CI= 7, 25). Of combined intervention schools, 66% had clean toilets compared with 65% of BCC only schools (p=0.80) and 56% of control schools (RD=10; 95% CI=0.3, 20). When we asked students to demonstrate how they usually washed their hands, 52% of students from combined intervention schools washed both hands with soap compared with 54% of students from BCC only schools (p=0.32) and 42% of students from control schools (RD= 10; 95% CI=8, 18). Levels of hygiene practice and WASH facilities among all intervention schools were significantly better than the schools that did not receive any intervention. Behavior was no better in schools that received combined interventions compared with those that received only behavioral communication interventions. Behavioral communication messages may be a particularly cost effective approach to improving hand washing in schools.

1415

THE IMPACT OF IMPROVED SCHOOL WATER, SANITATION AND HYGIENE ACCESS ON PUPIL DIARRHEA: A CLUSTER-RANDOMIZED TRIAL

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Numerous studies have assessed the impact of improved access to water, sanitation, and hygiene (WASH) at the household level in reducing diarrheal disease, but few have rigorously assessed the impact of WASH in the school setting. Lack of access to improved WASH facilities and behaviors at school may increased risk of diseases due to the vulnerable age of children, increased opportunity for transmission of infectious agents, and lack of an immune response to organisms circulating in the public domain. We conducted a cluster-randomized trial to assess the impact of a school-based WASH intervention on diarrheal disease among primary school pupils. The study was carried out among 4,665 pupils in 185 public primary schools in Nyanza Province, Kenya. Two study populations were used: schools with a dry season water source within 1KM and those without. Schools with water nearby were randomly assigned to receive hygiene promotion and water treatment (HP&WT), HP&WT + sanitation, or no intervention (control). Schools without a nearby water source were randomly assigned to receive HP&WT, sanitation, and water supply improvements or no intervention (control). Our primary outcome was pupil-reported seven-day recall of diarrheal symptoms. At endline, pupils in schools with nearby dry-season water sources that received improvements in HP&WT and sanitation demonstrated similar measures of diarrhea period prevalence (RR 0.88, 95% CI 0.60-1.28) and diarrhea illness duration (IRR 0.85, 95% CI 0.57-1.24) compared to pupils attending associated control schools. Similar results were noted for pupils attending schools with HP&WT interventions only. Pupils attending schools without a water source in the dry season that received a water supply improvement followed by HP&WT and sanitation showed a 66% reduction in diarrheal disease (RR 0.34, 95% CI 0.17-0.64) and 70% reduction in days of illness (IRR 0.30, 95% CI 0.15-0.60) compared to associated controls. In settings with no water supplies in the dry season, an integrated school-based intervention to improve water supply, water quality, sanitation, and handwashing can reduce diarrheal illness among pupils. Since many schools in low-income settings function without year-round water supplies, these should be a priority for implementing WASH interventions.

1416

EVALUATION OF EDUCATION THROUGH LISTENING, A COMMUNITY ENGAGEMENT METHODOLOGY, TO PROMOTE THE ADOPTION OF SAFE HOUSEHOLD WATER TREATMENT BEHAVIORS IN COMMUNITIES IN WESTERN KENYA

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Household water treatment has been shown to reduce diarrhea risk by nearly 40%, but relatively low rates of adoption of these interventions have limited the scale at which they are being used. New behavior change approaches are needed to accelerate adoption. In 2010, we evaluated the impact of Education through Listening (ETL), a behavior change methodology, on the adoption of household chlorination. ETL is a community engagement technique that is a person-centered way of communicating and giving feedback to promote behavior change. We randomized 12 villages in Vihiga District, Kenya into an intervention group in which ETL was used to motivate home water chlorination and a comparison group that used the standard village-based social marketing approach promoted by the Safe Water and Aids Project, a local Kenyan non-governmental organization. Over a 6-month period, during biweekly home visits mothers were interviewed about reported water treatment and diarrheal disease in children <2yo; water treatment was confirmed by testing stored water for residual chlorine. A higher percentage of households in ETL villages than comparison households had reported (14% versus 11%, Pearson's chi-square, p = 0.03) and confirmed (7.5% versus 3.6%, Pearson's chi-square, p <0.0001) household water treatment with chlorine products. There was no difference in the proportion of children <2yo reported to have diarrheal disease between the intervention (6%) and comparison (6%) groups. However, the percentage of children with reported diarrheal disease was significantly lower in households that reported treating drinking water by any method than non-treating households (4% vs 7%, Pearson's chi-square, p=0.027). Although use of ETL appeared to increase the reported and observed use of chlorine products, adoption was modest. Further study of barriers to water treatment is needed.

1417

SOAPY WATER: A LOW-COST SOLUTION FOR HAND WASHING PROMOTION

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Cost, theft and difficulty in sharing are barriers to keeping soap at a hand washing station that hinder regular hand washing in low income communities. Soapy water, a solution of water and locally available detergent, contained in plastic soda bottles is a low cost alternative to bar soap. We piloted soapy water in rural communities and measured uptake. We enrolled rural Bangladeshi households with children age <3 years in 12 villages, for four study arms: promotion only (n=148), promotion plus handwashing stations with soapy water bottles (n=118), promotion plus handwashing stations with bottles plus detergent refills (n=107) and control (no products no promotion; n=73). Our hand washing stations (wholesale cost per unit USD 6.5) included a bucket fitted with a tap, a stool, a basin and a soapy water bottle. Health workers promoted hand washing, the convenience of having soap and water together and the utility of making and using soapy water in all study arms except the control. We collected data on handwashing resources and practices through observations and survey questions 3-4 months after commencement of the intervention. Soapy water or soap together with water was observed in 6% of (4/72) control households, 23% (26/116) of households with promotion only, 63% (65/103) of households with handwashing station plus bottles, and 75% (68/90) of households with station, bottles plus detergent. Intervention arms had significantly higher proportions of handwashing stations stocked with soap or soapy water compared to controls (p=<0.001 in all three arms). Additional intervention components were associated with significant increase in uptake: 40% (p<0.001) higher with stations plus bottles versus promotion only and 12% (p<0.04) higher with stations, bottles and detergent compared with stations plus bottles. Soapy water was an acceptable low cost hand washing agent alternative to bar soap in rural low income communities. Providing hand washing stations increased uptake of soapy water, but even in the absence of project provided detergent and hardware, households prepared this easily and kept it at the handwashing station. Soapy water may increase habitual handwashing by addressing key barriers such as cost, sharing and availability near water sources. This uptake should be further evaluated to assess its longer term impact on habits and health.

1418

MICROBIOLOGICAL EVALUATION OF THE EFFICACY OF SOAPY WATER TO CLEAN HANDS

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The high cost of bar soap relative to household income is an important barrier to handwashing in low-income communities. Soapy water made from powdered detergent is a low-cost alternative that could overcome these barriers. Among low-income households in Dhaka, Bangladesh, we compared the efficacy of handwashing with soapy water to washing with bar soap or water alone for removal of fecal indicator organisms from hands. We enrolled 84 mothers with at least one child < 5 and randomly assigned 28 mothers to each of three handwashing agents: water alone, bar soap and soapy water (30g of powdered detergent mixed with 1.5 liters of water). For each mother, field workers randomly selected the right or left hand to collect a hand rinse sample before handwashing and then collected a hand rinse sample from the opposite hand after washing. An unwashed hand rinse sample and a washed hand rinse sample were collected in each of 5 different visits: two after 15 seconds of washing with soapy water, two after washing with bar soap at two rubbing times (15s and 30s), and one after 15s rinsing with water alone. We assessed the concentration of thermotolerant coliforms in hand rinse samples (log CFU per hand) by membrane filtration, and used paired t-tests to compare these concentrations before and after handwashing with each agent. We collected 168 hand rinses each for soapy water and bar soap, and 84 hand rinses for water alone. Soapy water and bar soap removed thermotolerant coliforms effectively after 15s of rubbing (log mean reduction=0.66, p<0.001 for soapy water; and 0.58, p=0.001 for bar soap). Increasing rubbing time from 15s to 30s did not significantly alter the microbiological efficacy of soapy water or bar soap (log mean reduction of 15s minus log mean reduction of 30s =0.04, p=0.48 for soapy water; and 0.08, p=0.53 for bar soap). Washing hands with water alone also reduced thermotolerant coliforms (log mean difference=0.30, p=0.029). Washing hands with soapy water was more effective than washing hands with water alone in reducing thermotolerant coliforms (difference in log mean reduction = 0.35, p=0.048).Soapy water is more effective than water alone and as effective as bar soap in removing indicator organisms from hands. Washing for 15s is sufficient to remove bacteria from hands with bar soap and soapy water. In low-income communities, washing hands with soapy water can be promoted as an effective, low-cost alternative to bar soap.

1419

CHRYSOMYA PUTORIA, A PUTATIVE VECTOR OF DIARRHEAL DISEASES

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Chrysomya spp are common blowflies in Africa, Asia and parts of South America and some species are generated in prodigious numbers from pit latrines. Because of their strong association with human faeces and their synanthropic nature, we examined whether these flies are likely to be vectors of diarrhoeal pathogens. Flies were sampled using exit traps placed over the drop hole of latrines in Gambian villages. A median of 12.5 flies/latrine/day (IQR=0.0-86.0) was collected, of which 95% were *Chrysomya* spp, nearly all *C. putoria*. Odour-baited traps were used to determine the relative attractiveness of different breeding media and foods to these flies. More flies were collected from traps with faeces from young children (median=2.5, IQR=1.0-8.5) and dogs (median=1.0, IQR=0.0-12.0) than from herbivores (median=0.0, IQR=0.0-0.0; calf, cow, goat and horse; p<0.001). Flies were strongly attracted to raw meat (median=44.5, IQR=26.2-143.0) and fish (median=0.0, IQR=0.0-19.8) compared with cooked and uncooked rice, and mangoes (median=0.0, IQR=0.0-0.0; p<0.001). The presence of bacteria in wild caught flies was confirmed by culture and bacterial DNA was identified using PCR. *Escherichia coli* were cultured from the surface of 21% of *Chrysomya* and 10% were enterotoxigenic (ETEC). Enteroaggregative *E. coli* (EAEC) were identified by PCR in 2% of homogenized *Chrysomya* spp, *Shigella* spp in 1.4% and *Salmonella* spp in 0.6% of samples. The large numbers of *Chrysomya* that can be produced from pit latrines, the presence of enteric pathogens on flies, and their strong attraction to raw meat and fish suggests these flies may be important vectors of diarrhoeal diseases in Africa.

1420

QUANTITATIVE PCR-BASED DETECTION OF PATHOGENIC *LEPTOSPIRA* IN SLUM WATER

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Leptospirosis has emerged as a major public health problem in urban slum settlements worldwide. Environmental surface water is an important reservoir for disease transmission in this setting. Pathogenic *Leptospira* have been detected in surface water from slum communities. However the ecological factors which influence the spatial and temporal dynamics of leptospires in this reservoir remain poorly understood. We performed a one-year longitudinal survey of leptospires in environmental surface water in an urban slum community, which was situated in a valley of 0.1 km^2 in the city of Salvador, Brazil. Pooled water and sewage samples were systematically collected from study households during a two-week period in the months of July, October and January. A lipL32-based qPCR assay was used to determine genome equivalents of leptospires in DNA extracted from 50ml samples. We detected leptospires in 12% (61) from 498 surface water samples collected during two survey periods of July and October. The proportion of qPCR positive samples (18% vs. 9%, P<0.05) and leptospiral concentration (10.1 vs. 6.6/ml, P<0.05) were significantly increased for the month where rainfall was greater (October vs. July; 230 vs. 81mm). Samples collected in the morning were significantly more frequently positive (17% vs. 6%) and had higher leptospiral concentration (9.3 vs. 5.8/ml) than those collected in the afternoon samples. The proportion of qPCR positive samples and leptospiral concentrations were also significantly higher in sewage (18%; 8.8/ml) than pooled water (6%; 6.2/ml). These findings indicate that the diurnal and seasonal variations influence the dynamics of leptospires in the environment. Furthermore they also suggest sewage may be a key transmission source in slum communities, and interventions targeting this reservoir will be necessary for effective prevention.

1421

APPLICATION OF NANOTRING™ TECHNOLOGY TO MEASURE CHANGES IN GENE EXPRESSION IN *PLASMODIUM FALCIPARUM*

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Experiments that investigate differential gene expression have traditionally taken a gene-by gene approach (using quantitative real-time PCR) or a genome-scale approach (using microarrays). Nanotring™ technology is based on direct multiplex measurement of gene expression, effectively "counting" transcripts using barcoded probes and single molecule imaging. This approach offers a middle-level throughput to assay hundreds of transcripts simultaneously, using much less material than a microarray. We sought to apply Nanostring™ to measure gene expression in *Plasmodium falciparum* culture-adapted parasites and patient samples. We designed a custom codeset of 328 genes that distinguish between stages of the malaria asexual life cycle as well as between distinct transcriptional profiles previously observed in parasites isolated directly from infected patients. Using this subset of genes we were able to distinguish between asexual life cycle stages using small volumes (10uL parasitized red blood cells) of cell lysate. Life cycle stage correlations between Nanostring™ and microarray data were maintained with as few as 10,000 parasites. Direct patient samples, containing an abundance of human RNA, showed good correlation with microarray data gathered from the same samples. Even at parasitemia levels relevant to human infection, life cycle correlations were very strong. Whole genome imputation from the codeset for direct patient samples was also performed. Overall, Nanostring™ performs well with very small amounts of both cell lysate and extracted RNA, and constitutes a highly sensitive, enzyme-free approach to measuring gene expression in the malaria parasite. This tool could be ideal for screening patient samples prior to performing in-depth RNA sequencing, or as independent data for studies of parasite physiology during drug treatment or other experiments.

1422

COPY NUMBER VARIATION WITHIN A NATURAL POPULATION OF *PLASMODIUM FALCIPARUM*

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Copy number variation is a key evolutionary mechanism for gene evolution and diversification. In *Plasmodium falciparum*, it is known to play important roles in virulence and drug resistance. Copy number variants (CNVs) have been extensively studied in culture-adapted laboratory strains. However, the genome-wide extent of CNVs in natural populations is not well understood. In order to address this we have analyzed over 30 short-term cultured field isolates from Senegal. Using whole-genome next-generation sequencing, using our novel correction algorithms for

sequencing biases and a mean shift approach to delineate CNVs allows the precise demarkation of CNVs, often to the base pair resolution. We find that on average the number of CNVs > 1kb in a strain was 158 of which 38 were duplications and 122 deletions. The vast majority of CNVs fall within the virulence genome compartment (e.g. var, rifin and stevor gene families and subtelomeric regions) highlighting their important role in host evasion. The higher proportion of deletion CNVs is mainly due to inadequate remapping of highly polymorphic var genes and as such do not strictly represent a deletion at the given var nor a reduced var complement. The core genome is relatively invariant compared to the virulence regions. It also appears less variant relative to culture-adapted strains suggesting variation may be selected for or more tolerated in such settings. Many of the core CNVs detected are shared within the Senegalese population, indicating either regional selection or the 3D7 reference genome being the rare variant. Interestingly several isolates demonstrated extensive and markedly-elevated read depth within the subtelomeric var regions - over three times the CNV content - suggesting that the virulence compartment may vary more extensively then previously appreciated. Together with our ongoing experimental validation we will present a detailed picture of the pattern and nature of copy number variation within this important pathogen.

1423

PLASMODIUM COATNEYI CAUSES SEVERE ANEMIA AND INFLAMMATION IN BONE MARROW AND OTHER ORGANS OF RHESUS MACAQUES

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Plasmodium coatneyi infection in rhesus macaques has been considered a relevant animal model of *P. falciparum* infection in humans, due to its tertian periodicity, tissue sequestration features, and severe disease outcomes. In particular, *P. coatneyi* infection in rhesus macaques has been proposed for studies of cerebral malaria, with sequestration in the brains of infected rhesus macaques and associated clinical sequelae. However, the clinical features of this infection, including neurological syndromes, have not been recently evaluated for their similarity to severe human malaria disease. We conducted a pilot infection of six rhesus macaques with blood-stage *P. coatneyi* (frozen stabilate obtained at 7.05% parasitemia; strain deposited in MR4 by Dr. W. Collins) and obtained clinical, immunological, pathological, and parasitological data longitudinally. The infection was allowed to progress in individual monkeys until pre-determined clinical endpoints appeared, including any features of severe malaria. All monkeys developed severe anemia with a >60% drop from baseline hematocrit (final hemoglobin levels 3.2 - 5.6 g/dL) twelve to fourteen days post-infection and displayed peak parasitemias between 6.5 and 12.45%. Animals demonstrated lassitude and withdrawal at higher parasitemias, but not convulsions, unresponsiveness, or focal neurologic signs. Phagocytic cells and red blood cells containing pigment were observed in several organs without prominent parenchymal changes, including cerebral vessels without evidence of ischemia. T cell activation and proliferation, together with pigment-laden macrophages, were evident during in peripheral blood and tissues, including bone marrow. *P. coatneyi* infection in rhesus macaques routinely causes acute severe anemia, which may be useful for future mechanistic studies of this common severe malaria manifestation in humans.

1424

IMPAIRED SKELETAL MUSCLE MICROVASCULAR FUNCTION AND INCREASED SKELETAL MUSCLE OXYGEN CONSUMPTION IN SEVERE *FALCIPARUM* MALARIA

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Organ dysfunction in severe *falciparum* malaria (SM) is associated with tissue hypoxia, which results from an imbalance between oxygen supply and demand. In SM, microvascular obstruction from parasite sequestration results in impaired oxygen delivery. However, microvascular function (capacity to increase oxygen delivery in response to ischemia) and oxygen consumption have not been assessed in host tissue. We used near-infrared resonance spectroscopy (NIRS) to measure tissue oxygen saturation (StO_2), combined with an ischemic stress to compare microvascular function (StO_2recov) and oxygen consumption (VO_2) in thenar muscles among adults in Papua, Indonesia with SM (n=36), moderately-severe malaria (MSM; n=33), severe sepsis (n=24) and healthy controls (HC; n=36). Mean StO_2recov (skeletal muscle reoxygenation rates) was 16% and 22% lower in SM (2.7%/s) compared to MSM (3.1%/s) and HC (3.5%/s) ($p<0.001$), and comparable to severe sepsis (2.5%/s). In SM, StO_2recov inversely correlated with venous lactate ($r=-0.63$; $p<0.001$) after adjustment for disease severity. StO_2recov was a significant predictor of death (ROC: 0.71[95%CI: 0.51-0.92]), with each percentage decrease associated with an increased risk of death (OR 2.49 (95%CI 1.05-6.2). In contrast, VO_2 was increased in SM by 8% compared to MSM and 18% with HC and sepsis ($p<0.001$), and associated with parasite biomass (plasma HRP2); $r=0.49$, $p=0.04$. Microvascular function is impaired in SM and associated with increased mortality, while oxygen consumption is increased. Tissue hypoxia and organ dysfunction may arise not only from parasite sequestration and heterogeneous microvascular obstruction, but also from impaired functional ability of the microvasculature to match oxygen delivery to increased oxygen demand.

1425

USING LABORATORY AND SEASONAL DIFFERENCES IN RETINOPATHY NEGATIVE VERSUS POSITIVE CEREBRAL MALARIA TO IMPROVE UNDERSTANDING OF DISEASE PATHOPHYSIOLOGY

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Children with cerebral malaria (CM) can be categorized by the presence or absence of malaria retinopathy. We compared admission laboratory, demographic, and seasonal data between children admitted with retinopathy positive versus negative and used these comparisons to gain insight into the underlying pathophysiology of retinopathy negative CM. We retrospectively reviewed admission laboratory and clinical parameters and the seasonal pattern of disease presentation in patients admitted with CM in Blantyre, Malawi from 1997--2010 and compared these data across retinopathy status. Patients with retinopathy negative CM had higher glucose concentrations, hematocrits, platelet counts, and lower lactate concentrations and peripheral parasite counts than those with retinopathy positive CM. Children with retinopathy negative CM were more likely to be in deeper coma upon admission than those with malaria retinopathy. The seasonal pattern of disease presentation also varied by retinopathy

status. Taken together, these findings support the hypothesis that these conditions have different underlying etiologies. Acute *Plasmodium falciparum* infection is likely not sufficient to produce the retinopathy negative CM syndrome.

1426

MOLECULAR PATHOLOGICAL INVESTIGATIONS OF FATAL *PLASMODIUM FALCIPARUM* MALARIA

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To investigate the pathophysiology of fatal human malaria using molecular pathology techniques, we are conducting an autopsy study in Beira, Mozambique, examining malaria and control deaths in children and adults. Full clinical and autopsy based clinicopathological correlation determined the spectrum of clinical complications of severe malaria and cause of death. Tissues from different organs were used to extract total mRNA and microRNA. Whole genome and miRNA expression profiles were generated using the Illumina Human-12 V4 BeadChip array and Affymetrix GeneChip miRNA array v2 respectively. An initial screening analysed total mRNA and microRNA transcriptomes from the brain of patients dying of fatal malaria and non-malaria control deaths (both n=3, 3 separate brain regions, samples = 9 per group). Clustering analysis showed no significant differences between three brain regions. A total of 223 mRNAs and 54 miRNAs were significantly differentially expressed in malaria (using cutoffs of fold difference x1.5, and $p<0.05$). The network, functional and canonical pathway analyses were generated using Ingenuity software pathway analysis. Integration of the putative mRNA targets of differentially regulated miRNAs with mRNA expression data from the same specimens revealed a wide number of enriched functions and pathways, mainly associated with host immune responses, cellular morphological changes and cell death regulation. Gene families which were significantly upregulated included pathways of cell signalling and transmigration, inflammation and cellular homeostasis. Transcripts encoding markers of neuronal damage, such as S100 and APP, were highly increased, as were the chemokine MCP-1, cytokines Ang-4, IL-6 and IL-17 (but not TNF or IFN-γ). Hypoxic inducible molecules such as C7orf68 were increased, and mediators of cerebral oedema formation, such as aquaporin 4 and fibrinogen. There was downregulation of several genes stimulating cell death and neuronal apoptosis. Neurotransmitters and proteins involved in synaptic function and stabilization or microtubule formation were downregulated, such as PENK (proenkephalin precursor A). This study, the first integrated analysis of miRNA and mRNA expression profiling in fatal P.*falciparum* malaria, represents a proof of concept for using molecular techniquea on autopsy tissues to understand the pathology and pathogenesis of human malaria.

1427

SURROGATE MRI MEASURE (SAMKAM RATIO) PREDICTS OUTCOME IN PEDIATRIC CEREBRAL MALARIA

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Pediatric cerebral malaria (CM) increases both brain volume and intracranial pressure (ICP). The mortality from CM is 15-25% even with highly attentive care. Massive increases in brain volume, assessed by magnetic resonance imaging (MRI) and interpreted by radiologists, are strongly associated with fatal outcomes in Malawian children with cerebral malaria. We developed a surrogate measure of brain volume for use by non-radiologists, and evaluated its utility, using images generated by 0.35T GE Signa Ovation scanner . The SamKam ratio is calculated using the height of the right parietal lobe on the first coronal T2 slice behind the splenium divided by the peri-brainstem CSF. The latter is the sum of the measurements of the CSF anterior and posterior to the brainstem at the level of the 4th ventricle apex, measured on the midsagittal section (T1). Three independent observers calculated the ratio on the same 20 scans. Pearsons correlation coefficients were calculated and were greater than 0.86 for all combinations. When SamKam ratio is used to predict severe brain swelling as measured by two independent radiologists the AUROC is 0.75. During the 2009 and 2010 seasons, 120 Malawian children with retinopathy-positive CM underwent brain MRI scanning on admission and daily thereafter until death or discharge(Age 9-168mo, mean 48mo; 45.8% male). There were twenty fatalities, and in 85%, the admission SamKam ratio was > 6.5. SamKam ratios declined over time in survivors with serial scans (n=*8).* The SamKam ratio may be used to identify CM patients increased brain volumes when radiologists are not available.

1428

USE OF GEOSPATIAL MAPPING MODELS TO ACCURATELY PREDICT *SCHISTOSOMA MANSONI* PREVALENCE IN NYANZA PROVINCE, KENYA

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Schistosomiasis, a parasitic disease that affects over 200 million people, can lead to significant morbidity and mortality; distribution of single dose preventative chemotherapy significantly reduces disease burden. Implementation of control programs is dictated by disease prevalence rates. For *Schistosoma mansoni,* infection prevalence is determined by costly and labor intensive screening of stool samples. Because ecological and human factors are known to contribute to the focal distribution of schistosomiasis, we sought to determine if specific environmental and socioeconomic factors could be used to accurately predict *S. mansoni* prevalence. We designed a mixed model to assess associations with *S. mansoni* rates in schools and controlled for spatial autocorrelation. Data on *S. mansoni* prevalence, school name, and GPS location of the school were obtained from 457 primary schools in Nyanza province in western Kenya. *S. mansoni* rates were calculated through examination of stool samples from children in the selected school; the median number of children tested per school was 42 (range 9-80). Geographic layers for environmental and population features, such as water source proximity, poverty rates, land elevation, and soil type, were obtained from publicly available sources. Mapping models were constructed using ArcGIS 10 and R 2.13.0. Higher *S .mansoni* rates were associated with closer distance (km) to Lake Victoria (prevalence ratio = 0.75, 95% CI = 0.73-0.77), increasing soil pH (0.83, 0.79-0.86), and increasing monthly rainfall (mm; 0.991, 0.989-0.993). Distance to health facility, human influence index, poverty rate, and agricultural land use were not significantly associated with *S. mansoni* rate. Our mapping model suggests that easily assessable geographic data can be used by schistosomiasis control programs to accurately predict schistosomiasis prevalence. Development and use of these prevalence maps will allow control programs to plan and prioritize efficient control campaigns to decrease schistosomiasis burden.

1429

EVALUATION OF A NOVEL RAPID DIAGNOSTIC TEST FOR SCHISTOSOMIASIS HAEMATOBIUM (RDT-SH) BASED ON THE DETECTION OF HUMAN IMMUNOGLOBULINS BOUND TO FILTERED *SCHISTOSOMA HAEMATOBIUM* EGGS

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Schistosomiasis haematobium is a major cause of morbidity in Africa and the Middle East. A rapid diagnostic test for *Schistosoma haematobium* is needed to facilitate diagnosis and treatment, assist with disease surveillance and guide public health interventions. We evaluated a rapid diagnostic test for *S. haematobium* (RDT-Sh), a novel method for diagnosing *S. haematobium* infection. *S. haematobium* eggs are highly immunogenic and excreted into the urine coated in human IgG. We filtered 160 urine samples from children in the Kwale distract of Kenya to isolate eggs and used anti-human IgG antibody conjugated to horseradish peroxidase to bind to the human IgG attached to the eggs. We then added 3,3'5,5'-tetramethylbenzidine base (TMB) _ which turns blue in the presence of horseradish peroxidase _ to detect the presence of *S. haematobium* eggs. The RDT-Sh was compared in a double-blinded manner to the gold-standard method of diagnosing infection by urine microscopy. The RDT-Sh was positive in 89% of urine samples containing >1 egg/10mL (58/65 samples) and 97% of urine samples containing >11 eggs/10mL urine (35/36 samples) seen by microscopy. The RDT-Sh was negative 79% of the time when no eggs were seen on urine microscopy, but because up to three times more urine was used for the RDT-Sh, there were likely cases in which eggs were on the RDT-Sh filter but not detected by microscopy. We used latent class analysis incorporating urine microscopy, hematuria, proteinuria, and RDT-Sh results to determine an overall 97% sensitivity and 78% specificity for RDT-Sh, 96% and 81% for urine microscopy, 71% and 98% for microscopic hematuria, and 46% and 89% for proteinuria, respectively. The RDT-Sh is quick, inexpensive and easy to perform in the field for the diagnosis of *S. haematobium*. The RDT-Sh is able to detect all but the lightest of *S. haematobium* infections with a high degree of accuracy.

1430

URINE FOAM AS A MARKER FOR INFECTION WITH SCHISTOSOMIASIS HAEMATOBIUM

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The Baddorf-Sheele (BS) shake test measures urine foam to help diagnose Schistosomiasis haematobium in the field. The BS-shake test was performed by shaking 20mL of urine in a 50mL test tube as vigorously as possible by hand for approximately five seconds. Immediately after shaking, the height of the foam inside the test tube was recorded and these results compared to urine microscopy counts of *Schistosoma haematobium* eggs. The average height of the urine foam for study subjects with 17 to >1000 eggs/10mL urine was at the 36.4mL (SD 1.35) mark on the test tube, and for subjects with 0 eggs/10mL urine was at the 32.2mL (SD 2.36) mark. The sensitivity and specificity of the BS-shake test (positive when foam was measured at or above 34mL) was 74% and 72%, for microscopic hematuria 61% and 97%, and for proteinuria 43% and 83%, respectively, compared to microscopy. When >17 eggs/10mL urine were present, the BS-shake test, microscopic hematuria, and proteinuria were positive in 100%, 90%, and 80% of cases, respectively. Combining hematuria and the BS-shake test results detected 87% of samples with eggs seen on microscopy. The current gold standard test requires slide preparation, a trained technician, access to a microscope, and significant time and resource costs. A more easily performed and cost effective, though still reliable test is needed, especially for field studies and large public health screenings. The BS-shake test is an inexpensive, quick, and easy way to diagnose *S. haematobium* in endemic areas.

1431

TOWARDS THE DEVELOPMENT OF A RAPID DIAGNOSTIC TEST (RDT) FOR DETECTION OF ANTI-SCHISTOSOME ANTIBODIES

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Mass drug administration (MDA) of praziquantel is widely used in control programmes for both *Schistosoma mansoni* and *S. haematobium* infections. Different MDA strategies are used depending on how prevalent infection is in a given area. The Kato-Katz method is used for mapping *S. mansoni* infections, and questionnaires, detection of macrohaematuria and/or urine filtration methods for *S. haematobium*. Parasitological methods of diagnosis are however relatively insensitive, often misdiagnosing the infected as uninfected, and so prevalence is often underestimated. This can lead to the implementation of an inappropriate treatment strategy for a given community. The problem of underestimating prevalence is likely to become exacerbated in areas where praziquantel treatment has taken place, since the number of lighter infections which parasitology cannot detect is likely to increase. The need for more sensitive diagnostic assays is therefore greater than ever and it is envisaged that antibody-detection methods are likely to become increasingly useful. Indeed, they are already widely used in travellers' medicine clinics and have been integrated into the Chinese national control programme for *S. japonicum*. To be useful in schistosome-endemic areas however, a diagnostic test needs to meet the ASSURED criteria (particularly with regard to Affordability and User-friendliness), and so we are developing a rapid diagnostic test (RDT) that works by detection of anti-schistosome antibodies in human blood. Preliminary results indicate that this RDT could be useful for diagnosis of both *S. mansoni* and *S. haematobium* infections, and its low cost could make it useful not only for mapping purposes but also for diagnosis at the individual patient level.

1432

EVALUATION OF POINT-OF-CONTACT CIRCULATING CATHODIC ANTIGEN ASSAYS FOR THE DETECTION OF *SCHISTOSOMA MANSONI* INFECTION IN LOW AND MODERATE PREVALENCE SCHOOLS IN WESTERN KENYA

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Increased attention to schistosomiasis control efforts has highlighted the need for improved field diagnostics where rapid screening and mapping of *Schistosoma mansoni* infection guide control efforts. A urine point-of-contact circulating cathodic antigen (POC/CCA) assay manufactured by Rapid Medical Diagnostics (Pretoria, South Africa) has shown promise in areas where prevalence of schistosomiasis is high, but the assay has not been evaluated extensively in areas where prevalence is low. To evaluate the performance of the POC/CCA assay in areas of low to moderate prevalence, we tested primary school children for schistosomiasis in the Asembo region of western Kenya, using two versions of the POC/CCA assay_one commercially available and one experimental formulation_as well as duplicate Kato-Katz stool examinations and an anti-schistosome IgG ELISA. Latent class analysis was used to estimate sensitivities and specificities of the individual tests at each of three school prevalence levels: <10%, 10-25%, and >25%. Respective sensitivities and specificities of the POC/CCA assays among all participants (n=1798) were 93.9% and 57.8% for the commercial test and 76.9% and 89.8% for the experimental test.

The commercial POC/CCA assay was found to be most sensitive overall, but the experimental POC/CCA assay offered the best combination of sensitivity and specificity (82.0% and 91.4%, respectively) in the lowest prevalence zone. Both POC/CCA assays demonstrated positive correlation with infection intensity (as measured by egg count). The commercial POC/CCA assay was also evaluated for consistency and for measurement of treatment outcome, demonstrating substantial agreement across three daily administrations and reductions in POC/CCA band intensity one week after treatment. As intervention programs move toward sustained control and elimination, a diagnostic assay's abilities to perform in areas of low prevalence becomes paramount. Our findings suggest that the experimental POC/CCA assay may be a field-friendly alternative to the Kato-Katz exam in low prevalence settings.

1433

COMPARING HIGH-THROUGHPUT QUANTITATIVE DETECTION OF *SCHISTOSOMA*-DNA USING REAL-TIME PCR AND EXTENSIVE MICROSCOPY IN URINE SAMPLES FROM PRIMARY SCHOOL GIRLS IN COASTAL KWAZULU NATAL

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Of 300 million women and girls in Africa at risk of schistosomiasis, those most vulnerable to infection are pre-school and primary school children, adolescent girls and women of childbearing age. Genital schistosomiasis is reported as a complication among children. Since the diagnosis of female genital schistosomiasis (FGS) is difficult among children, diagnosing urinary schistosomiasis may help identify endemic areas for mass treatment where young girls are at risk for FGS. The diagnosis of schistosomiasis from urine may be challenging since haematuria and egg excretion are variable particularly in adults and in children with light infections with low levels of egg excretion. The aim was to compare two diagnostic tests using urine samples: real-time PCR for detection of *Schistosoma*-genus DNA and extensive microscopy as a practical tool for multiple exploration. Urine samples were collected on three consecutive days from 688 girls, aged 10-12 years, during a cross-sectional study in 18 primary schools. Quantification of *Schistosoma*-specific DNA was performed on a 200 µL aliquot of the first day urine, using a custom-made automated handling system for high through-put DNA isolation and PCR set-up. Full microscopy (3 days 2x10mL urines) was done on 621 (90.3%) and day 1 microscopy (2x10 mL urines) was done on 677 (98.4%) of the participants. Only 250 (36.3%) showed eggs in all 10 mL examinations collected over 3 days, while 210 (30.5%) were positive in only one out of six screenings collected on day 1. In addition, the number of eggs counted varied highly from day-to-day. *Schistosoma* DNA was detected using 200 µl of urine in 197 (28.6%) urine samples and DNA loads corresponded significantly with the average intensity of infection determined by microscopy. Also at school level, PCR determined *Schistosoma* infection reflected the focal distribution of disease transmission seen after extensive microscopy. The automated system facilitated high throughput quantification of *Schistosoma*-specific DNA loads in urine. In addition only 200 µl urine samples were required to achieve a sensitivity similar to extensive and labour intensive microscopy on consecutively collected large volume samples. The described PCR set-up could be used as a relatively straightforward laboratory-based procedure to assess the distribution of schistosomiasis in one urine only for large study populations, identifying communities at risk.

1434

COMPARISON OF DIAGNOSTIC METHODS AGAINST PCR FOR THE DETECTION OF *SCHISTOSOMA MANSONI* AMONG SCHOOL CHILDREN IN WESTERN KENYA

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The most widely used tools for detection of *Schistosoma mansoni* infection, stool examination and serology, are limited by low sensitivity or inability to distinguish between current from former infections, respectively. As a result, there is not an accepted "gold standard" for *S. mansoni* diagnosis for evaluation of new diagnostic assays. However, recent development of a semi-quantitative PCR that detects schistosome DNA in stool provides a tool for this purpose. We utilized the PCR method to evaluate a point of contact (POC) test designed to detect circulating cathodic antigen (CCA) in urine of persons infected with *S. mansoni*. School children (n = 1898) aged between 8-12 years from villages in western Kenya provided 3 stool and 3 urine samples on consecutive days for testing by Kato-Katz and a commercially available POC-CCA cassette. A portion of the first day's stool was preserved in ethanol and subsequently tested for presence of schistosome DNA by PCR(n=950). In addition, serum from a single blood sample was tested by ELISA for adult worm antigen-specific IgG. Children who were infected with *S. mansoni* were treated using praziquantel. A single urine sample was collected 1 week later for post-treatment POC-CCA testing. Compared to PCR, the single day POC-CCA urine test had an average sensitivity of 81.7% and an average specificity of 54.4%. Average single day Kato-Katz sensitivity was 58.2% and average specificity was 91.5%. When testing from the 3 days was combined, the POC-CCA sensitivity was 92.1%, and the Kato-Katz sensitivity was 70.4%; 3 day specificities of these tests were 68.7% and 92.1%, respectively. The ELISA was 52.6% sensitive and 85.1% specific. There were 675 children that were initially positive by POC-CCA and provided a urine sample 1 week after treatment with praziquantel; 461 (68.3%) of these children demonstrated decreased POC-CCA band intensity following treatment. Comparison of *S. mansoni* diagnostic tools demonstrates attributes and limitations of each test. Further development to optimize detection methods for *S. mansoni* is needed.

1435

MALARIA PREVENTION IN PREGNANCY IS ASSOCIATED WITH REDUCTIONS IN LOW BIRTH WEIGHT AND NEONATAL MORTALITY: A META-ANALYSIS OF 32 NATIONAL CROSS-SECTIONAL DATASETS IN AFRICA

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Low birth weight (LBW) is a significant risk factor for neonatal death. A prominent cause of LBW is *Plasmodium falciparum* infection during pregnancy. Intermittent preventive therapy (IPTp) and insecticide-treated mosquito nets (ITNs) have been shown by randomized trials to significantly reduce the risk of LBW in areas of stable transmission. We created a retrospective birth cohort from 32 national cross-sectional datasets in 25 African countries from 2000-2010 to examine the association of malaria prevention in pregnancy (IPTp and/or ITNs) with LBW and neonatal mortality under routine program conditions. An important innovation in this meta-analysis is the substantial effort made to limit potential selection

bias through exact matching on confounding factors associated with both exposure to malaria prevention in pregnancy and birth outcomes. A logistic regression model was used for assessing the association of malaria prevention in pregnancy on LBW, while a Poisson model was used for the outcome of neonatal mortality. Both models incorporated the matched strata as a random effect, while accounting for additional confounding factors with fixed effect covariates. Exposure of women in their first or second pregnancy to malaria prevention with IPTp and/or ITNs was significantly associated with decreased risk of neonatal mortality [Incident rate ratio = 0.820; 95% Confidence interval (CI): 0.698-0.962], compared to women with no protection. Compared to no protection, exposure of pregnant women during their first 2 pregnancies to malaria prevention in pregnancy through IPTp and/or ITNs was significantly associated with reduced odds of LBW, as measured by a combination of weight and perceived birth size [adjusted odds ratio = 0.792; 95% CI: 0.732-0.857). These data show malaria prevention in pregnancy to be associated with substantial reductions in neonatal mortality and LBW under routine malaria control program conditions, and for the most part are consistent with the efficacy results from controlled trials.

1436

A TRIAL OF INTERMITTENT SCREENING AND TREATMENT AS AN ALTERNATIVE TO INTERMITTENT PREVENTIVE TREATMENT WITH SULFADOXINE-PYRIMETHAMINE FOR THE CONTROL OF MALARIA IN PREGNANCY

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The incidence of malaria, including the incidence in pregnant women, is declining in some African countries, and resistance to sulfadoxine-pyrimethamine (SP) is widespread. Thus, intermittent preventive treatment in pregnancy with SP (SP-IPTp) may no longer be appropriate in certain situations, and alternative strategies are needed. A randomised, multi-centre controlled trial has been undertaken in four west African countries, including 5000 pregnant women who slept under an insecticide treated bed net. The standard SP-IPTp regimen (two to three courses of SP in the second and third trimester) will be compared to intermittent screening and treatment (IST) of parasitaemia using a rapid diagnostic test at scheduled antenatal clinic visits in the second and third trimester. The primary end points of the trial are prevalence of low birth weight (LBW), mean maternal haemoglobin at 38 ±2 weeks of gestation and prevalence of placental malaria. Other outcomes affecting mothers (anaemia, parasitaemia, clinical malaria) and children (still births, perinatal mortality) will also be analysed.The study was powered to show non-inferiority of IST compared to SP-IPTp with respect to prevalence of LBW. Recruitment of study participants is complete. Analyses will be finalised in the third quarter of 2012 and available to present at ASTMH in November. The study will provide information to national malaria control programmes in countries whether there are alternative, safe and effective methods to the WHO recommended SP-IPTp regimen for managing malaria in pregnancy. This could have particular important implications for the control of malaria in pregnancy in areas with high levels of SP resistance.

1437

EFFECTIVENESS OF INTERMITTENT PREVENTIVE TREATMENT WITH SULFADOXINE-PYRIMETHAMINE IN PREGNANT WOMEN IN WESTERN KENYA: RESULTS OF AN OBSERVATIONAL STUDY

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Intermittent preventive treatment with sulfadoxine pyrimethamine (IPTp) remains a key strategy for malaria prevention in pregnant women living in malaria endemic regions. However, increasing SP resistance threatens IPTp effectiveness. We assessed IPTp effectiveness in an area of western Kenya where *Plasmodium falciparum* malaria transmission is intense and resistance to SP is high. From August 2008 to June 2009, women delivering at two district hospitals were enrolled in a cross-sectional survey. We collected information on obstetric history, IPTp use (self-report or as recorded in the antenatal card), insecticide treated net use, and antimalarial treatment during pregnancy. At delivery, we measured the prevalence of maternal anemia (Hb< 8g/dL), peripheral parasitemia, placental parasitemia (impression smear) and low birth weight (LBW) (multivariate analysis pending). Overall, 977 HIV-negative women were enrolled and included in this analysis. Of these, 637 were gravida 1 or 2 and 340 were gravida 3+. Among women who were gravida 1 or 2, anemia prevalence by number of IPTp doses received was 14%, 11%, 7% and 2% for 0, 1, 2, and 3+ IPTp doses respectively (p<0.01); peripheral parasitemia prevalence was 19%, 12%, 12% and 7% for 0, 1, 2, and 3+ IPTp doses received respectively (p=0.07); placental parasitemia prevalence was 22%, 12%, 13% and 8% for 0, 1, 2, and 3+ IPTp doses received respectively (p=0.04); and LBW prevalence was 5%, 11%, 9% and 9% for 0, 1, 2, and 3+ IPTp doses received respectively (p=0.73). Among multigravidae, we found no significant reduction in the prevalence of anemia, or peripheral or placental parasitemia with increased number of IPTp doses; LBW prevalence was 11%, 6%, 3% and 0% for 0, 1, 2, and 3+ IPTp doses received respectively (p=0.02). Among gravida 1 or 2, IPTp was associated with a reduction in maternal anemia and placental parasitemia. In multigravidae, IPTp was associated with a reduction in LBW. During this time period, IPTp remained beneficial in this area of western Kenya, despite high SP resistance.

1438

ORIGIN OF PLACENTAL MALARIA INFECTION AND RESPONSE TO TREATMENT DURING PREGNANCY

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Placental malaria is a significant cause of maternal anemia and infant low birth weight. Little is known about the characteristics of peripheral malaria infections during pregnancy that lead to placental infections. We sought to determine when during pregnancy peripheral infection leads to placental sequestration and whether sulfadoxine-pyrimethamine intermittent preventive treatment (SP-IPT) or lumefantrine-artemether (LA) treatment clear the parasites sequestered in the placenta. We screened 325 placentas from women enrolled in an observational study of malaria during pregnancy. We used 6 neutral microsatellite markers to genotype placental and peripheral parasites. Placental parasites from 17 women

were fully genotyped. Mean gestational age (GA) at enrollment was 18 weeks (range 13-24). Of 39 visits with any peripheral parastemia, 25 (65%) were sub-microscopic. Four of 17 women with molecular evidence of placental malaria did not experience any peripheral parasitemia during follow-up. Among the 13 women with peripheral parasitemia during follow-up, 6 (46%) had peripheral genotypes matching placental genotypes. Matching genotypes occurred later in pregnancy than did non-matching (34 weeks vs. 25 weeks). SP-IPT cleared peripheral parasitemia in 4 of 8 (50%) cases and LA cleared peripheral parasitemia in 7 of 7 cases. Recrudescence after treatment with LA occurred after 3 of 7 doses at 25, 45, and 78 days after treatment. These data suggest the majority of women experience the peripheral infection leading to placental infection prior to 18 weeks GA and the majority of peripheral infections experienced by women who went on to have placental malaria were submicroscopic infections. LA, but not SP-IPT, cleared all peripheral infections. Both SP-IPT and LA treatment allowed for recrudescence of parasites during pregnancy, which likely reflects their failure to eliminate parasites sequestered in the placenta.

1439

ASSESSING MALARIA SURVEILLANCE DATA QUALITY: EXPERIENCE FROM BENIN, ETHIOPIA AND UGANDA

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Health facility-based malaria surveillance in Africa presents challenges due to reporting based on clinical diagnosis but lacking diagnostic confirmation. However, the scale up of rapid diagnostic tests and shifting national policies to universal testing may alleviate many of these challenges. We evaluated three models of malaria sentinel surveillance in Benin, Ethiopia, and Uganda to identify the unique attributes of each system and evaluate standard metrics of data quality. Compared to routine health facility data, Benin's system provided complete and comprehensive malaria data and filled an important data gap for the national program. In Ethiopia, a multi-tiered reporting system leveraged an existing network of health extension workers for monitoring malaria cases in the community. This system provided epidemic detection for entire health facility catchment areas. In Uganda, ongoing supervision provided by the implementing partner resulted in strengthened malaria diagnostic capacity and a high testing proportion of suspect cases. In all three countries, key performance indicators were high: completeness of malaria indicators was >95%; accuracy was >75%; and the average proportion of suspect cases tested was >75%. Timeliness of monthly reporting was satisfactory for all systems but epidemic detection would be strengthened by more frequent reporting. Results of analyses in all three countries showed that system performance improved with frequent supervision, clear standard operating procedures, a laboratory quality control system, and simple data collection tools. Data use by health workers resulted in greater compliance to reporting procedures and better data quality. In all countries sentinel surveillance data was of superior quality compared to routine system data. Our results suggest that near universal testing and improved data quality exemplified by these three surveillance systems with distinctly different implementation have improved the usefulness and public health impact of malaria surveillance data.

1440

RELIABILITY OF SCHOOL SURVEYS IN ESTIMATING GEOGRAPHIC VARIATION IN MALARIA TRANSMISSION IN THE WESTERN KENYAN HIGHLANDS

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To evaluate the effectiveness of control interventions against malaria, reliable estimates of malaria transmission within the community are essential. Cross-sectional surveys can be logistically demanding and prohibitively expensive for control programs if required repeatedly. Health facility data, whilst less expensive and logistically simpler, often rely on clinically diagnosed malaria so are therefore likely to miss asymptomatics and will be affected by health-seeking behavior. An alternative approach is to use school surveys, which are increasingly being used for estimating disease prevalence and may act as a focal point for rolling out interventions. Here we carried out surveys in primary schools in Rachuonyo South district in the highlands of western Kenya in July 2010 at the same time as cross-sectional surveys within the immediate community to compare prevalence of malaria by rapid diagnostic tests (RDT) and antibody responses to the *P.falciparum* merozoite antigen MSP-1_{19} and AMA. All results obtained at the school were geolocated by following up children to their homes. Crude RDT prevalence from school data was 24% whilst that recorded from community surveys was 16%. Comparing RDT prevalences between school-level data and community surveys, resulted in a correlation coefficient of 0.74, with 42% of the community results being significantly different to those obtained at the school. This increased to a correlation coefficient of 0.81 when data within the community was restricted to school-age children. For this subset of data, only 13% of the paired school and community prevalence estimates were significantly different. Factors determining these differences focusing on altitude, distance of pupil households from the school and use of malaria control interventions will be presented. This data will be supplemented with age specific sero-prevalences and estimates of the sero-conversion rates. The utility of school-based sampling using RDT results and serology to discriminate areas of high and low transmission will be discussed.

1441

ASSESSMENT OF MALARIA CONTROL PROGRESS OVER A TWO-YEAR PERIOD USING A CONTINUOUS 'ROLLING' MALARIA INDICATOR SURVEY ACROSS AGE GROUPS IN CHIKHWAWA DISTRICT, MALAWI

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Low cost, district-level monitoring and evaluation (M&E) tools that can provide real-time malaria control progress are urgently needed to guide and optimize control efforts and impact. From May 2011 we have conducted a continuous 'rolling' Malaria Indicator Survey (rMIS) in children aged 6-59 months in 51 villages within Chikhwawa district, Southern Malawi. In 2011, district wide indoor-residual spraying and the use of Rapid DiagnosticTests were added to facility-based ACT case-management and the distribution of insecticide treated bednets. Monthly collection of standard malaria intervention coverage and burden indicators were conducted by a small team of 2 nurses and 2 field workers, sampling all villages twice a year, using PDAs for data capture. Findings from the first

year identified substantial temporal and spatial variation in intervention coverage and malaria transmission within the area. The continuous rMIS approach provided real-time feedback on coverage gaps and burden hotspots, suggesting that this type of M&E surveys would become an intervention in itself if could trigger specific local focused control action, and could strengthen our current arsenal of interventions. With the increasing focus on universal coverage and transmission reduction, the rMIS was expanded to include older children and adults in the second year (June 2011-May2012). Preliminary results of this second year rMIS will be presented, with a focus on the added value of including older age groups in MIS surveys and control progress over both years.

1442

BILHARZIA IN THE INFORMAL URBAN SETTLEMENTS OF WESTERN KENYA: PREVALENCE, DISTRIBUTION AND EVALUATION OF COMMUNITY AND SCHOOL-BASED APPROACHES FOR CONTROL

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Urban areas present unique challenges for primary health care, which have remained poorly researched, and urban bilharzia remains a neglected area when prioritizing intervention strategies. Control of schistosomiasis and soil-transmitted helminthiasis is hampered by poverty, inadequate clean water, occupational hazards and poor sanitation. The cross-sectional study determined the prevalence and distribution of schistosome and soil-transmitted helminth (STH) infections, among 1,308 children in 34 primary schools and in intermediate snail vectors in 8 informal urban settlements in Kisumu City. Schools, water bodies and snails were mapped and fecal contamination (presence of Escherichia coli) of public water sources determined. Community health workers, village elders, and teachers were sensitized on Bilharzia and trained on mass drug administration (praziquantel) to community members or school going children respectively. Prevalence of Bilharzia was 36% in one of the informal settlement areas (Nyalenda B) and over 10% in all other informal settlements. The overall prevalence for STHs was 16%. Of the snails collected, 1.8% shed schistosome cercariae and 95% of water sources sampled were contaminated with fecal matter. In the MDAs, about 60% of the target population was treated by CHWs in the community while the school-based treatment achieved over 90% coverage.This study observed that schistosomiasis and STH are important health priorities among schools in informal settlements of Kisumu City. The study confirmed that besides L. Victoria, schistosomiasis transmission exists within the informal settlements of Kisumu City. Snail control, treatment of public water sources and improvements in local sanitation and public health awareness are advocated for in such settings.

1443

PREDICTIVE VALUE OF SCHOOL AGE CHILDREN'S SCHISTOSOMIASIS PREVALENCE FOR PREVALENCE IN OTHER AGE GROUPS AND THE EFFECT OF ONE ROUND OF SCHOOL-BASED OR COMMUNITY-WIDE TREATMENT IN WESTERN KENYA - THE SCORE PROJECT

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With increased global commitment to schistosomiasis control, mass drug administration (MDA) programs are being implemented in several settings. Depending on the prevalence of infection in school age children within a given community, WHO recommends that either school-based or community-wide MDA be employed. To test the assumption that school prevalence reflects the underlying community prevalence, we evaluated how well infection prevalence and intensity in 9-12 year old school pupils correlated with infection levels in other children and adults within the same community. Cross-sectional surveys of pre-adolescents (9-12 years old) were compared to those of first year students (7-8 years old), adolescents (13-14 years old) and adults (20-55 years old) in 150 villages along the shores of Lake Victoria. Written informed consent was obtained from adults and both consent and assent were obtained for children. A single stool sample was collected from 50 adults, 50 adolescents and 100 first year students and three stools were collected from 100 pre-adolescents in each village. Two slides per stool were screened for *Schistosoma mansoni* using the Kato Katz method. Data were analyzed using Spearman's nonparametric correlation analysis; p values < 0.05 were considered significant. We surveyed 3900 first year students, 12037 pre-adolescents, 5417 adolescents and 7566 adults. Of these, 1098 (28.2%) first year students, 7390 (61.4%) pre-adolescents, 2207 (40.7%) adolescents, and 3185 (42.1%) adults were positive for *S. mansoni* infection. Initial evaluation suggested that a village's schistosomiasis prevalence for 9-12 years olds significantly correlated with prevalence for all other age groups, suggesting that this age group is in fact a good predictor. Preliminary analysis of infection levels in the 9-12 year old age group one year following MDA suggests that children in villages randomized to the school-based treatment arms had lower prevalence and intensity of infection than children in villages randomized to the community-wide treatment arms.

1444

COMPARING THE COST OF SCHOOL-BASED VERSUS COMMUNITY-WIDE PRAZIQUANTEL MASS DRUG ADMINISTRATION IN KENYA

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In addition to impact on burden of schistosomiasis, cost can be an important factor in mass drug administration (MDA) program design. The Schistosomiasis Consortium for Operational Research and Evaluation (SCORE) is conducting studies in several African countries that analyze the benefits and costs of implementing either school-based or community-wide treatments over a 4-year intervention period. As part of the cost benefit analysis, cost estimates of the second year of MDA in SCORE projects in Kenya were calculated. Twenty-four out of 175 villages were selected for the study based on distance, school size, district, and initial schistosomiasis prevalence, including 8 villages with 10-25% initial prevalence and 16 with > 25% initial prevalence. Information about various inputs and resources used to conduct MDA were collected from the transportation department, human resources department, study coordinators, field coordinators, and project associates. Costs that varied across villages were identified and costs that were consistent across villages were determined a priori. Each cost was associated with an MDA activity such as advocacy, mobilization, drug distribution, coverage or feedback. Preliminary data analysis suggests that school based MDA costs less than community wide treatment. The major drivers of cost associated with community wide MDA were transportation and personnel costs. In contrast school based distribution of treatment was centralized and five days of salary for community distributors was not required. The final Kenya MDA cost effectiveness analysis will include impact of treatment, either school-based or community-wide, on disease prevalence after 4 years of intervention in order to describe the relationship between the cost of alternative MDA approaches and the benefits achieved in terms of decreases in prevalence and intensity of schistosomiasis.

1445

EVALUATION OF THE HEALTH-RELATED QUALITY OF LIFE (HRQOL) OF CHILDREN IN A *SCHISTOSOMA HAEMATOBIUM*-ENDEMIC AREA IN KENYA

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Schistosomiasis remains a public health challenge; 93% of the estimated 237 million infections occur in sub-Saharan Africa. Though rarely fatal, its recurring nature makes it a lifetime chronic disorder with significant health burden. Much of its negative health impact is due to subtle conditions such as anemia, undernutrition, pain, exercise intolerance, poor school performance, and decreased work capacity. This makes it difficult to estimate the disease burden specific to schistosomiasis using the current DALY metric. In our study, we used Pediatric Quality of Life Inventory (PedsQL), a modular instrument available for a wide range of ages (2-18 y), to assess Health-related Quality-of-Life (HrQoL) in children living in a S. haematobium-endemic area in coastal Kenya. The PedsQL questionnaires were administered by interview to children aged 5-18 y (and their parents) in 5 villages spread across three districts. HrQoL (total score) was significantly lower in villages with high prevalence of S. haematobium (-4.0 + 0.8%, $p<0.001$) and among the lower socioeconomic quintiles (-2.0 + 0.8%, $p<0.01$) after adjustment for age, sex, and undernutrition. A greater effect was seen in the psychosocial scales as compared to physical function scale. Individual S. haematobium egg output was not associated with PedsQL score within the subset of three high-prevalence villages, whereas, in low prevalence villages, detection of any eggs in the urine were associated with a significant -2.1 + 0.9% ($p=0.025$) reduction in total score. The PedsQL reliabilities were high (Cronbach alphas generally ≥0.70), floor effects were acceptable, and identification of children from low socioeconomic status was valid. We conclude that urogenital schistosomiasis is specifically associated with at least a 2-4% reduction in HrQoL. Further research is needed on reproducibility and responsiveness properties of QoL testing in relation to schistosomiasis; we expect that a case definition based on more sensitive diagnosis will better define the immediate and long-term QoL impact of S. haematobium infection.

1446

COMMUNITY PERCEPTIONS OF SCHISTOSOMIASIS RISK AMONG SCHOOL CHILDREN IN ZANZIBAR

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School-aged children on Unguja and Pemba Islands (Zanzibar) are at particular risk of infection by *Schistosoma* haematobium, a schistosome species that causes urinary schistosomiasis, a neglected tropical disease common throughout much of Africa. Despite the high prevalence of schistosomiasis (locally called Kichocho) in some communities, little is known about the community's perspectives on the disease among school children. In 2011, as part of a larger study aiming for schistosomiasis elimination, qualitative data were collected in Zanzibar from 39 groups of children, 45 community leaders, 21 teachers and 16 parents to better understand their knowledge, perceptions and practices associated with preventing, controlling, and treating Kichocho in children. Using a grounded theory approach, we transcribed, coded, and analyzed the data. Kichocho was not seen as a disease of females. People typically acquired their knowledge through informal social networks and characterized the disease as one of young boys spending time in a dirty pond or stream. Identification of the parasite and mode of transmission was lacking. People often failed to seek treatment for children due to anticipated costs and home treated with plant-based teas and water. Schools lacked Kichocho education curriculums. People recognized the need for prevention and suggested organizing educational trainings for public and religious schools and the community; developing interactive teaching tools; partnering with student clubs to educate students; working with the community to build latrines, urinals, wells, and washing platforms near the river and at home; building play areas and offering play opportunities for children; and providing free local drugs. Our findings illuminated major gaps in local knowledge as well as practical, structural, educational, cultural and medical issues to consider when preparing for mass drug distribution and school-based interventions as well as the need to collaborate with the community on future prevention efforts.

1447

MEFLOQUINE-PRAZIQUANTEL FOR THE TREATMENT OF *SCHISTOSOMA* HAEMATOBIUM INFECTIONS IN SCHOOL-AGED CHILDREN IN CÔTE D'IVOIRE

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The global strategy for schistosomiasis control is morbidity control, relying on a single drug, praziquantel. Although no clinically relevant resistance to praziquantel has been described to date, development of drug resistance is of growing concern as control efforts are going to scale. We have recently shown that mefloquine possesses promising antischistosomal properties *in vitro*, *in vivo*, and in proof-of concept clinical trials. In contrast to praziquantel, high worm burden reductions were observed following mefloquine treatment in the juvenile *Schistosoma* mansoni infection mouse model. Additionally, synergistic interactions were observed *in vitro* and in the S. mansoni-mouse model, when praziquantel was combined with mefloquine. We present results from the first exploratory randomized trial in school-aged children in southern Côte d'Ivoire evaluating the efficacy and safety of mefloquine (25 mg/kg) combined with praziquantel (40 mg/kg), and mefloquine/artesunate (3 x (100 mg artesunate + 250 mg mefloquine) combined with praziquantel (40 mg/kg) compared to standard praziquantel treatment (40 mg/kg) against S. haematobium. In the absence of prior drug interaction studies, drugs were administered on subsequent days. Two urine samples were collected before and on days 21-22 and 78-79 after the first dosing. Sixty children were present on all examination time points. No significant difference in efficacy was observed between the three treatment groups on the first treatment follow-up (mefloquine-praziquantel: cure rate (CR), 32%, egg reduction rate (ERR), 95%; mefloquine-artesunate-praziquantel: CR, 32%, ERR 95%; praziquantel: CR, 30%, ERR, 93%) and on days 78-79 posttreament (mefloquine-praziquantel: CR, 32%, ERR, 94%; mefloquine-artesunate-praziquantel: CR, 33%, ERR, 92%; praziquantel: CR, 19%, ERR, 93%). Adverse events were mostly mild in all treatment groups. In conclusion, the addition of mefloquine or mefloquine-artesunate does not enhance the efficacy of praziquantel in the treatment of S. haematobium.

1448

PREDICTIVE MAPPING VS. EMPIRIC ASSESSMENT OF SCHISTOSOMIASIS: IMPLICATIONS FOR TREATMENT PROJECTIONS IN GHANA

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Mapping the distribution of schistosomiasis is essential to determine where control programs should operate, but because it is impractical to assess infection prevalence in every potentially endemic community, model-based geostatistics (MBG) is increasingly being used to predict prevalence and determine intervention strategies. To assess the accuracy of MBG predictions for *Schistosoma hematobium* infection in Ghana, school surveys were evaluated at 79 sites to yield empiric prevalence values that could be compared with values derived from recently published MBG predictions. Based on these findings schools were categorized according to WHO guidelines so that practical implications of any differences could be determined. Using the predicted values alone, 21 of the 25 empirically determined 'high-risk' schools requiring yearly praziquantel would have been *undertreated* and almost 20% of the remaining schools would have been treated despite empirically-determined absence of infection - translating into 28% of the children in the 79 schools being undertreated and 12% receiving treatment in the absence of any demonstrated need. Using the current predictive map for Ghana by aggregating prevalence estimates to the district level was clearly not adequate for guiding the national program, but the alternative of assessing each school in potentially endemic areas of Ghana or elsewhere is not at all feasible; modelling must be a tool complementary to empiric assessments. We conclude that for practical usefulness, predictive risk mapping should not be thought of as a one-time exercise but must, as in the current study, be an iterative process that incorporates empiric testing and model refining to create updated versions with increasingly accurate predictions.

1449

INTERVENTIONS TO STABILIZE ENDOTHELIUM IMPROVE SURVIVAL IN EXPERIMENTAL CEREBRAL MALARIA

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Cerebral malaria (CM) pathogenesis is associated with endothelial activation and perturbation of the blood brain barrier (BBB). Endothelial specific signalling pathways, including the Angiopoietin (Ang)-Tie-2 and Slit/ Roundabout (Robo)-4 systems, are key regulators of endothelial integrity and vascular leakage. Our lab and others have reported that adult and pediatric CM is associated with increased circulating levels of biomarkers of endothelial activation and dysfunction (e.g. Ang-2, sTie-2, sICAM-1). We hypothesize that interventions to promote endothelial stability will prevent deleterious alterations to the BBB and improve outcome following *Plasmodium* infection. Using the murine model of *Plasmodium berghei* ANKA (PbA)-induced experimental CM (ECM), we show that alterations in protein and mRNA levels of angiopoietins are associated with disease severity in the ECM, similar to observations in human populations. Time course experiments established a temporal relationship where PbA-associated alterations in endothelial regulators directly precede the loss of BBB integrity and the onset of neurological symptoms of ECM, such as seizures and paralysis. Pro-Ang-1 treatment strategies (e.g. Adenoviral mediated expression of Ang-1) significantly improved survival in PbA-infected ECM-susceptible C57Bl/6 mice compared to empty adenoviral vector and vehicle controls ($p=0.001$). Pharmacological activation of the Slit-Robo pathway, using therapeutic administration of recombinant Slit2N, also significantly prolonged survival in PbA-infected C57Bl/6 mice compared to untreated controls ($p=0.0007$). This benefit was further increased when Slit2N was used as adjunctive therapy in combination with a sub-curative dose of artesunate. To establish direct experimental evidence for a causal role of angiopoietins in ECM, the effect of Ang-1 genetic deletion on disease outcome is currently under investigation using a conditional Cre/loxP system. In summary, we show that adjunctive treatment strategies based on promoting endothelial quiescence and BBB integrity improve survival in ECM.

1450

POSITRON EMISSION TOMOGRAPHY - AN *IN VIVO* IMAGING SYSTEM FOR FOLLOW UP ENCEPHALIC METABOLISM IN CEREBRAL MALARIA

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Cerebral malaria (CM) is a neurological manifestation of *Plasmodium falciparum* infection which accounts for over 1 million deaths per year worldwide. About 25% of CM patients develop neurocognitive deficits, including memory loss and speech and learning impediments. As proper brain metabolism is critical to neurocognition, it may be altered in CM, but its role is poorly characterized. In addition, human CM studies are restricted to postmortem observations thus limiting our ability to characterize brain metabolic activity during disease. Non-invasive *in vivo* diagnostic tools are therefore needed to monitor the progression of CM. To investigate cerebral metabolic alterations in murine CM, we used positron emission tomography (PET) to monitor radioactive concentrations of fluorodeoxyglucose (FDG), a glucose analogue which reflects tissular metabolic activity. We examined encephalic metabolic activity in uninfected C57BL/6 mice and mice infected with *Plasmodium berghei* ANKA (PbA), a mouse malarial strain which causes CM. Throughout the course of disease, glucose uptake was decreased in several brain regions in PbA-infected mice compared to controls, including the cerebral cortex, olfactory bulb, brainstem and cerebellum. There was also a significant effect of infection and time on mean expression of FDG in the eyes, indicating an ocular decrease of metabolism, which might be correlated to the known retinopathy of the disease. More importantly, decreased glucose uptake correlated temporally with increased CM pathology, thereby establishing a new tool to study disease. With FDG-PET, we have come up with a novel imaging tool to non-invasively study brain metabolism during CM. FDG-PET-CT will serve as an unprecedented translational technique to understand the brain metabolism in human CM patients.

1451

SCHISTOSOMA MANSONI POLO-LIKE KINASES : KEY REGULATORS OF REPRODUCTIVE ORGAN DEVELOPMENT

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Polo-like kinases (Plks) constitute a family of conserved serine/threonine protein kinases known as important regulators of cell cycle progression and mitosis. Yeasts have only one Plk whereas vertebrate species possess five Plks (Plk1-5). Plk1, homolog to the *Drosophila* kinase Polo, is the best characterized member of the Plk family. Plk1 plays a major role in cell cycle progression by triggering G2/M transition and since it is overexpressed in various cancers, Plk1 constitutes a valuable target for anti-cancer therapy. Plk4/Sak (Snk akin kinase) is a divergent member of the family, structurally distinct from other Plk members, with essential functions in centriole duplication. The trematode parasite *Schistosoma mansoni*, responsible for schistosomiasis, has only, like *Drosophila*, two Plks, SmPlk1 and SmSak. Transmission and pathogenesis of schistosomiasis is due to the exceptional fecundity of schistosomes, for which Plks has been shown to play a decisive role. Both transcripts for schistosome Plks have been localized specifically in reproductive organs of female and male worms, with a majority of *SmSak* in ovary. Moreover, the treatment of worms with BI2536 (the anti-cancer drug inhibiting specifically Plk1 and SmPlk1) has shown a key role of SmPlk1 in gametogenesis and parasite reproduction, emphasizing its potential use as a novel therapeutic target against schistosomiasis. Studies in *Xenopus* oocyte, used as a protein expression system, have shown that the respective role of SmPlk1 and SmSak in G2/M transition triggering and centriole duplication during the cell cycle progression. Moreover, in these experiments, an unexpected interaction was demonstrated between SmPlk1 and SmSak, that could lead to Plk activation and spontaneous meiosis resumption in Plx1-depleted oocytes. These results suggest that Plk1 and Plk4 proteins are susceptible to interact and cross-activate in cells and thus attribute for the first time a potential role of Plk4 proteins in meiosis/mitosis entry. In addition to SmPlk1, this unexpected role of SmSak in meiosis could be relevant to further consider the function of this novel Plk in schistosome reproduction.

1452

UBIQUITIN FOLD MODIFIER (UFM-1) PROTEIN IS AN *L. MEXICANA* VIRULENCE FACTOR WHICH CONTRIBUTES TO PATHOGENESIS IN CL

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In this study, we generated *Leishmania mexicana* (Lm) lacking ubiquitin fold modifier (Ufm-1) gene, and examined *in vitro* parasite survival in dendritic cells and virulence *in vivo* using a murine model of CL. We found efficient internalization of both WT and Ufm-1-/- parasites by bone marrow derived dendritic cells (DCs), although Ufm-1-/- parasites were cleared significantly faster than WT parasites by DCs. We also found that Ufm-1-/- *Lm*-infected DCs produce significantly less IL-10 compared to WT *Lm*-infected DCs upon LPS stimulation *in vitro*. BALB/c mice infected with WT *L. mexicana* developed large non-healing lesions while Ufm-1-/- *Lm*-infected BALB/c mice had delayed lesion growth and developed smaller lesions by week 10 post-infection. Analysis of *Leishmania*-specific serum antibodies revealed that WT infected mice produced significantly higher titers of *Lm*-specific Th2-associated IgG1 than Ufm-1-/- *Lm*-infected mice, although *Lm*-specific IgG2a production was undetectable in both groups. Upon *in vitro* stimulation with *Lm* antigen, draining lymph node cells from WT *Lm*-infected mice produced significantly more IL-4 compared to similarly stimulated cells from Ufm-1-/- *Lm*-infected mice although IFN-γ production was comparable between the two groups. Taken together, our findings show that Ufm-1 is a *L. mexicana* virulence factor which contributes to establishment of infection and pathogenesis in CL. Furthermore, we demonstrate that Ufm-1 is not essential for parasite survival.

1453

THE EXISTENCE OF A G1 CELL CYCLE CHECKPOINT IN *P. FALCIPARUM* MEDIATED BY THE CYCLIN-DEPENDENT PROTEIN KINASE PFMRK; IMPLICATIONS FOR COMPOUND SELECTION AND INHIBITORY GROWTH ASSAY DEVELOPMENT

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Rapid growth and multiplication of *Plasmodium falciparum* during erythrocytic schizogony result in clinical symptoms and disease progression of malaria. Parasite growth is controlled by an unknown cell cycle regulatory mechanism, but believed to be similar to that of mammalian cells. However, there are many features of parasite schizogony that are unique. The ring stage of *P. falciparum* is representative of the G1 phase, while trophozoite and schizont stages are equivalent to S and M phases respectively. The regulation of how the parasite transits through these cell cycle phases and whether cell cycle checkpoints exist are unknown. Cyclin-dependent protein kinases (CDKs) are essential regulators for sequential growth and proliferation. Pfmrk, a sequence homologue of human CDK7 is suggested to play a role in both cell cycle control and DNA replication in *P. falciparum*. Transgenic parasites that over-express functional Pfmrk (HPG), non-functional Pfmrk (HKG), or control (empty vector control), revealed that HKG parasites exhibited a delay in the completion of the intraerythrocytic development cycle. To investigate the role of cell cycle regulators in *Plasmodium* growth and development, we assessed 41 mammalian cell cycle inhibitors, such as CDK inhibitors, DNA synthesis inhibitors and mitotic inhibitors, for growth inhibition. Of these compounds, 8 that significantly inhibited parasite growth (IC_{50} < 10 μM) were shortlisted for further studies. FACS analysis demonstrated that control parasites treated with kenpaullone, a G1/S mammalian cell cycle inhibitor that inhibits Pfmrk kinase activity, "arrested" at trophozoite stages, whereas HPG parasites treated with the same inhibitor transitioned sooner from trophozoites to schizonts. In stage-specific growth inhibition studies, HPG parasites treated at trophozoite-stage were less sensitive to the growth inhibitory effects compared to early ring-staged treatment. Moreover, HPG parasites treated at early ring-stage development indicated a delay in the initiation of the next growth cycle by approximately five hours. The results suggest Pfmrk functions at the ring-trophozoite transition, reminiscent of a G1 checkpoint. The existence of a checkpoint would have a profound effect on the selectivity of compounds and warrant consideration for how and when compounds are tested in growth inhibition assays.

1454

INVASION GENE HAPLOTYPES ASSOCIATE WITH PARASITEMIA IN HUMANS REPORTING WITH *PLASMODIUM KNOWLESI* MALARIA IN MALAYSIAN BORNEO

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Human infections with *Plasmodium* knowlesi, a parasite of long and pig-tailed macaques, continue to be reported in most countries within Southeast Asia. Parasite invasion occurs daily in P. knowlesi infections and parasitemia is associated with disease severity. In this study we test the hypothesis that parasitemia is associated with particular alleles of two genes, P. knowlesi normocyte binding protein xa and xb (Pknbpxa and Pknbpxb), encoding invasion proteins on the merozoite apex. In the first instance a fragment 8500bp beginning at exon II of the Pknbpxa gene and a fragment 3500bp beginning at Exon I of the Pknbpxb gene were cloned and sequenced to high stringency using 5 reference isolates collected at geographically distinct locations. Sequence alignment indicated that most diversity occurred at the 5' region of exon II for both genes. Fragments with 37 non-synomymous and 7 synonymous substitutions with nucleotide diversity (ϖ) = 0.024 of Pknbpxa and 14 non-synonymous and 2 synonymous substitutions, ϖ=0.0056 of Pknbpxb were chosen to haplotype 147 P. knowlesi isolates from clinically well-characterised patients. Pknbpxa haplotypes were obtained for 138 isolates, 7 failed to amplify and 2 failed to sequence. Pknbpxb haplotypes were obtained for 134 isolates, 3 patients had multiple genotype infections and were excluded and 10 isolates failed to amplify. Within the Pknbpxa haplotyping fragment there were 82 polymorphic sites (56 non-synonymous, 26 synonymous substitutions) ϖ = 0.02269 and 47 polymorphic sites (28 non-synonymous and 19 synonymous substitutions) ϖ = 0.00642 within the Pknbpxb fragment. There were 75 Pknbpxa and 51 Pknbpxb haplotypes in the study population with haplotype diversity (h) of 0.9729 and 0.9216 respectively, suggesting high polymorphism among the isolates. Non-synonymous single nucleotide polymorphisms(SNPs), where the minor allele was represented in >10% of the isolates, were analysed for association with parasitemia. Preliminary analyses found significant associations between two Pknbpxa and one Pknbpxb SNPs and parasitemia suggesting that particular alleles may influence erythrocyte invasion efficiency in human infections. The results of this study will be presented within the context of parasitemia and disease severity in P. knowlesi malaria.

1454

INVASION GENE HAPLOTYPES ASSOCIATE WITH PARASITEMIA IN HUMANS REPORTING WITH *PLASMODIUM KNOWLESI* MALARIA IN MALAYSIAN BORNEO

Mohammed Atique Ahmed[1], Balbir Singh[1], Ing Tien Wong[2], Chan Woon Lu[3], Ramlah bt Zainudin[1], Paul C.S. Divis[1], David J. Conway[4], Sanjeev Krishna[5], Janet Cox-Singh[5]

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Human infections with *Plasmodium* knowlesi, a parasite of long and pig-tailed macaques, continue to be reported in most countries within Southeast Asia. Parasite invasion occurs daily in P. knowlesi infections and parasitemia is associated with disease severity. In this study we test the hypothesis that parasitemia is associated with particular alleles of two genes, P. knowlesi normocyte binding protein xa and xb (Pknbpxa and Pknbpxb), encoding invasion proteins on the merozoite apex. In the first instance a fragment 8500bp beginning at exon II of the Pknbpxa gene and a fragment 3500bp beginning at Exon I of the Pknbpxb gene were cloned and sequenced to high stringency using 5 reference isolates collected at geographically distinct locations. Sequence alignment indicated that most diversity occurred at the 5' region of exon II for both genes. Fragments with 37 non-synomymous and 7 synonymous substitutions with nucleotide diversity (ϖ) = 0.024 of Pknbpxa and 14 non-synonymous and 2 synonymous substitutions, ϖ=0.0056 of Pknbpxb were chosen to haplotype 147 P. knowlesi isolates from clinically well-characterised patients. Pknbpxa haplotypes were obtained for 138 isolates, 7 failed to amplify and 2 failed to sequence. Pknbpxb haplotypes were obtained for 134 isolates, 3 patients had multiple genotype infections and were excluded and 10 isolates failed to amplify. Within the Pknbpxa haplotyping fragment there were 82 polymorphic sites (56 non-synonymous, 26 synonymous substitutions) ϖ = 0.02269 and 47 polymorphic sites (28 non-synonymous and 19 synonymous substitutions) ϖ = 0.00642 within the Pknbpxb fragment. There were 75 Pknbpxa and 51 Pknbpxb haplotypes in the study population with haplotype diversity (h) of 0.9729 and 0.9216 respectively, suggesting high polymorphism among the isolates. Non-synonymous single nucleotide polymorphisms(SNPs), where the minor allele was represented in >10% of the isolates, were analysed for association with parasitemia. Preliminary analyses found significant associations between two Pknbpxa and one Pknbpxb SNPs and parasitemia suggesting that particular alleles may influence erythrocyte invasion efficiency in human infections. The results of this study will be presented within the context of parasitemia and disease severity in P. knowlesi malaria.

1455

PHASE I TRIAL OF PFS25-EPA/ALHYDROGEL® A TRANSMISSION BLOCKING VACCINE AGAINST *FALCIPARUM* MALARIA IN HEALTHY MALARIA-NAÏVE ADULTS

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We describe the results of a phase I dose-escalating clinical trial to assess the safety and immunogenicity of the transmission blocking vaccine Pfs25-EPA/Alhydrogel®. Pfs25 has previously been shown to induce antibody which inhibits parasite development in a standard membrane feeding assay (SMFA), but an immunogenic formulation safe for human use has been lacking. EPA as a conjugate has been shown to enhance immunogenicity and to be safe in humans. Pfs25 and EPA were chemically conjugated and adjuvanted with Alhydrogel. 30 subjects have received up to three doses of 8, 16 (at 0 and 2 months) or 47-µg of Pfs25 at 0, 2 and 4 months. Vaccinations were generally well tolerated. The majority of solicited adverse events were mild in severity. No vaccine related serious adverse events occurred. The most common solicited adverse event was pain at the injection site, and the frequency of adverse events decreased with each successive dose of vaccine. A decrease in hemoglobin was seen in 8 of 30 subjects after the first vaccination, in 11 of 26 subjects after the second and 2 of 15 after the third vaccination. The majority of these were mild in nature, a few were moderate, and most were in subjects who had a previous history of low hemoglobins or anemia and were judged to be unrelated to vaccination. The vaccine was more immunogenic with each successive dose. Geometric mean antibody levels in the 47 µg dose group

were 92 EU (95% CI 55, 155) and 228 EU (95% CI 151, 344) after the second and third vaccinations respectively. Sixteen of 17 subjects in the 47 µg dose group had detectable antibody response after 2 vaccinations; 15/15 had responses after 3 vaccinations. Transmission blocking activity correlates with antibody titer, as demonstrated by SMFA. The data to date demonstrate that Pfs25-EPA/Alhydrogel® is well tolerated, increasingly immunogenic with each dose, and induces antibodies which inhibit parasite development in the mosquito.

1456

COMPARATIVE ASSESSMENT OF TRANSMISSION BLOCKING MALARIA VACCINE CANDIDATE ANTIGENS USING AN ADENOVIRUS-MVA PRIME-BOOST REGIME

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Transmission blocking vaccines (TBVs) target *Plasmodium falciparum* sexual stages, aiming to block development within the mosquito. Different delivery systems, mainly protein-in-adjuvant formulations, have been previously employed giving varied transmission blocking activity (TBA). However, leading TBV candidate antigens have not been comparatively assessed to determine a rank order of their TBA. Simian adenovirus (ChAd63) and Modified Vaccinia Ankara (MVA) in a prime-boost regime were used to induce antibodies against five candidate antigens and assessed their TBA. Antigen sequences were codon optimised and cloned into ChAd63 and MVA to generate recombinant viral vectored vaccines. These were used to vaccinate Balb/c mice in a 70 day regimen comprising of a day 0 ChAd63 prime and MVA boost at day 56. Antibody responses were measured days 14, 55 post-prime and day 70 post-boost by ELISA. TBA against *P. falciparum* NF54 strain and African field isolates was assessed by SMFA using purified IgG from sera taken at day 70. Antibody responses measured provided evidence that the antigens were immunogenic with ChAd63 priming responses boosted following MVA vaccination. TBA exhibited against *P. falciparum* NF54 in *Anopheles* stephensi ranged between 16-100% giving a rank order of the antigen-specific antibody's ability to inhibit oocyst intensity. This rank order was replicated against field *P. falciparum* isolates from gametocyte carriers in *Anopheles gambiae*. Hence the antigens with the highest TBA (90-100%) were further tested at varying IgG concentrations giving 39-100% efficacy. Two out of the five antigens consistently showed 99-100% with 0-5% infectivity to the mosquito. Antibodies induced by viral vectors showed partial to complete blockade depending on the target antigen. This antigen delivery system provides a robust vaccine platform for inducing antibodies against target antigens and has enabled a head-to-head comparison of TBV candidates. This comparative analysis is essential to guide and inform future assessment of candidates for clinical development.

1457

FUNCTIONAL COMPARISON OF LEADING *PLASMODIUM FALCIPARUM* TRANSMISSION BLOCKING VACCINE CANDIDATES BY STANDARD MEMBRANE FEEDING ASSAY

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Recently, there has been a renewed interest in the development of vaccines against the sexual stages of *P. falciparum* malaria. While several potential transmission blocking vaccine (TBV) candidates have been reported, studies directly comparing them in a functional assay are limited. To this end, recombinant proteins of 5 leading TBV candidates, Pfs25, Pfs48/45, Pfs230, PfHAP2, AnAPN1, and GST (as a control) were expressed in the wheat germ cell-free expression system. CD-1 outbred mice (n=10 per group) were immunized twice with the antigens adjuvanted with Montanide ISA720. Two weeks after the boost, antibody levels were measured by ELISA and the functionality of antibodies was assessed by a standard membrane feeding assay (SMFA) using cultured *P. falciparum* NF54 gametocytes and *Anopheles* stephensi mosquitoes. The levels of antibodies for all antigens were relatively similar (33,000 to 88,000 ELISA units as a median). For the functional analysis we prepared a pool of serum from each group and isolated IgGs from each by Protein G purification. The purified IgGs were tested at 0.75 mg/ml (the concentration at which mouse IgGs have shown minimum non-specific inhibition) by SMFA. Anti-Pfs25, anti-Pfs230 and anti-PfHAP2 antibodies showed 97-100% inhibition in oocyst density compared to anti-GST antibody, and these inhibitions were all statistically significant ($p<0.01$, Kruskal-Wallis test followed by Dunn's multiple comparison test). We confirmed the inhibitory activity of these three antibodies in an independent assay (93-100% inhibition in the second test), and the inhibition was dose-dependent. Alternatively, anti-Pfs48/45 (-48% inhibition) and anti-AnAPN1 (-11% inhibition) antibodies did not show any inhibition at 0.75 mg/ml. Of these 5 antigens expressed in the wheat germ cell-free expression system, antibodies to Pfs25, Pfs230 and PfHAP2 proteins showed superior functional activity in this study. Further studies of these 3 products are in progress and the current SMFA results support future TBV development of the candidates produced in this system.

1458

EFFICACY OF TRANSMISSION BLOCKING VACCINE CANDIDATES IN BURKINA FASO

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Malaria parasite transmission from humans to mosquitoes requires the ingestion of gametocytes that circulate in the human blood by *Anopheles* mosquito vector followed by several steps of parasite development in the mosquito. Transmission-blocking vaccines aim at impeding parasite development in the vectors and are nowadays viewed as a promising strategy for breaking this transmission and an important component for achieving malaria elimination and eradication. Antibodies specific for the vaccine candidate antigens Pfs25 and Pfs230 showed efficacy to limit *Plasmodium falciparum* transmission to mosquitoes in laboratory conditions. In the present study we aimed at assessing their efficacy against field isolates of parasites from Burkina Faso in semi natural conditions of transmission. Standard Membrane Feeding Assays (SMFA)

were carried out by exposing An. gambiae s.s females to gametocyte-infected blood from naturally infected patients. Pfs25 and Pfs230 antibodies, produced by mice immunization using recombinant viral vectors, were tested at different concentrations added to the blood, using different gametocytes densities. In parallel, an entomological study was performed in order to assess the natural parasite load in local mosquito vectors. SMFA revealed 100% transmission blocking activity (TBA) for both antibodies at titer from 62.5 to 500µg/ml, depending on infection intensity in the control mosquito group. Field collections showed that among the 2,293 wild mosquitoes dissected, 275 carried oocysts with an average of 8 oocysts per infected mosquito. For such a parasite load, we observed that a concentration of 250µg/ml of either Pfs25 or Pfs230 antibodies has a complete TBA activity. Our results demonstrated that Pfs25 and Pfs230 antibodies strongly limit human to mosquito *P. falciparum* transmission, suggesting that these antigens are valuable candidates for transmission blocking strategies against malaria if the required antibody titer can be obtained by immunization in human.

1459

PASSIVELY TRANSFERRED *P. FALCIPARUM* MSP1P42-SPECIFIC ANTIBODIES MEDIATE PROTECTION AGAINST CHALLENGE WITH BLOOD STAGES OF *PF*MSP1P19-TRANSGENIC *P. BERGHEI* PARASITES

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MSP1 is the major surface protein on merozoites and a prime candidate for a blood stage malaria vaccine. Preclinical and seroepidemiological studies implicate a role for anti-MSP1 antibodies in protection against malaria. These antibodies interfere with invasion or affect the growth of intra-erythrocytic parasites *in vitro*, depending on parasite strain. However, the biological activity of MSP1-specific antibodies is not fully captured by *in vitro* growth or invasion inhibition assays (GIA), which are frequently used to predict vaccine efficacy. GIA fail to address the potential role of cellular receptors that interact with antibodies and mediate anti-parasite activity through diverse antibody-dependent cellular mechanisms. Currently, this potentially cell-mediated functional activity of MSP1-specific antibodies can only be determined *in vivo*. Thus, we employed a PfMSP1p19-transgenic *P.berghei* parasite to test the ability of MSP1-specific antibodies to control parasitemia after challenge with infected erythrocytes. Various immune IgG preparations were tested in this model: a) IgG purified from rabbits immunized with MSP1p42 (FVO) using either complete Freund's adjuvant or an Adjuvant System, $AS01_8$ and b) Human IgG isolated from either high or low titer serum pools of malaria-naïve subjects immunized with MSP1p42 (FVO) adjuvanted with $AS01_8$. Purified IgG was injected intraperitoneally thrice (Day -1, 0, 1) and blood parasitemia was measured daily by qRT-PCR (Day 1-5) and by flow cytometry (Day 5-10). Lack of parasitemia was confirmed by qRT-PCR at the end of the study. Anti-MSP1p42- rabbit IgG conferred 40-50% sterile protection. Human anti-MSP1p42 IgG derived from the low titer pool protected40% of mice, while IgG derived from the high titer pool protected 80% of mice from developing parasitemia. These data suggest that the transgenic *P. berghei* mouse model could be useful in selection of candidate vaccines for future clinical studies.

1460

PARTICLE DELIVERY OF MALARIAL PROTEINS USING AN ATTENUATED STRAIN OF *SHIGELLA FLEXNERI*

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Particle-presentation of malarial antigens can significantly improve vaccine efficacy as show-cased by the RTS,S vaccine in which the circumsporozoite protein (CSP) is expressed as a fusion with hepatitis B surface antigen. While soluble CSP antigen plus adjuvant has not induced sterile protection, the RTS,S vaccine sterilely protects about 50% of malaria-naïve individuals. The advantages of particle delivery are that they can directly target antigen-presenting cells, contain immunostimulatory signals and provide increased epitope density thus assuring a potent immune stimulation. Our previous work using *E. coli* as particulate delivery platform has demonstrated that expression of malarial antigens at different cellular localizations (i.e., periplasmic space and outer membrane) modulates the type of immune response and can induce sterile protection against sporozoite challenge in murine models. In the present study, we use *Shigella*, a gram-negative bacterium, for particle presentations of malaria antigens and in the process potentially develop a dual-disease vaccination approach. Two malarial antigens were expressed in different compartments of strain 15G, an attenuated strain of *Shigella flexneri* 2a, to evaluate their immune responses in mice. The cell-traversal protein for ookinetes and sporozoites (CelTOS) was fused with the maltose binding protein, targeting it to the periplasmic space and the CSP was fused with the peptidoglycan associated lipoprotein in the outer membrane. We will report the results of bacterial dose selection, immunogenicity (humoral and cellular responses) and the protective efficacy against sporozoite challenge with either *P. berghei* or *Pf*CSP transgenic *P. berghei* sporozoites.

1461

A NOVEL GLYCOLIPID ADJUVANT STRONGLY ENHANCING THE CELLULAR IMMUNOGENICITY OF ADENOVIRUS-BASED MALARIA VACCINES IS ATTRIBUTABLE TO ITS LOCALIZED BIO-DISTRIBUTION

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A key strategy to a successful vaccine against malaria is to identify and develop new adjuvants that can enhance T cell responses elicited by a malaria vaccine. α-galactocylceramide (α-GalCer), a glycolipid that has been extensively investigated, is known to display a significant biological activity, including an adjuvant effect, by binding CD1d molecules and stimulating invariant NKT (*i*NKT) cells. Recently, we identified a novel synthetic α-GalCer analog, 7DW8-5, which can display a stronger adjuvant effect on the immunogenicity and efficacy of malaria vaccines in mice. Most recently, we have co-injected increasing doses of 7DW8-5 intramuscularly (i.m.) to rhesus macaques with an AdPfCA vaccine that consists of two Ad5-based vaccines each expressing the CS or AMA-1 antigen of *Plasmodium falciparum*, and found that 7DW8-5 could significantly enhance the level of malaria antigen-specific T cell responses without showing a significant side effect. Very surprisingly, we discovered that upon i.m. injection, α-GalCer, but not 7DW8-5, induced a systemic

production of cytokines including IFN-γ and IL-12 in the sera, whereas both glycolipids induced a similar level of systemic cytokine production upon their intravenous (i.v.) administration. Using labeled glycolipids with fluorophores, we found that the two glycolipids exhibited a distinctly different bio-distribution upon i.m. but not i.v. administration, resulting in only 7DW8-5 got trapped by DCs residing in the draining lymph nodes. The localized 7DW8-5 seems to facilitate the activation and maturation of lymph node DCs, thus improving the capability of DCs to prime malaria antigen-specific T cells and ultimately leading to its super adjuvant activity. Taken together, our study demonstrates a uniquely localized bio-distribution of our novel *i*NKT-activating glycolipid, 7DW8-5, upon its i.m. injection, which could lead to a potent adjuvant effect on the cellular immunogenicity of an adenovirus-based malaria vaccine not only in rodents but also in non-human primates.

1462

THE ROLE OF THE PROTEIN KINASE C SUPERFAMILY IN THE INNATE IMMUNE RESPONSE OF ANOPHELINE MOSQUITOES

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Anopheline mosquitoes are the primary vectors of medically important parasites in the genus *Plasmodium,* the causative agents of malaria. Malaria parasites undergo a series of complicated developmental transformations upon ingestion by *Anopheles* mosquitoes and during this process innate immune defenses can reduce parasite numbers significantly. While some mosquito anti-parasite effectors have been well characterized, the regulatory factors that control the timing and magnitude of these responses are poorly understood. The protein kinase C (PKC) superfamily consists of serine/threonine kinases that serve as central signaling molecules and regulators of a broad spectrum of cellular processes including growth, reproduction, and immunity. PKCs are highly conserved, ranging from seven isoforms in *Drosophila* to 16 isoforms in mammals, yet none have been identified in mosquitoes. Additionally, PKC-dependent signaling is central to the regulation of mammalian immunity and has been targeted aggressively for drug development. Despite conservation of the PKC superfamily and their potential as targets for transmission-blocking strategies for malaria, no direct connections between PKCs and the mosquito immune response exist. Here, we present the identification and characterization of six PKC superfamily members – PKCβ, PKCδ, PKCε, PKCζ, PKD, PKN – in *Anopheles gambiae* and *Anopheles stephensi*. Phylogenetic analysis of the anopheline PKCs confirmed subfamily assignments. All six PKCs are expressed in the midguts of *A. gambiae* and *A. stephensi*, indicating availability for signaling in a tissue that is critical for malaria parasite development. Inhibition of PKC enzymatic activity *in vitro* decreased NF-κB-regulated anti-microbial peptide expression in response to bacterial and parasitic specific factors. Further, PKC inhibition significantly decreased development of *P. falciparum* oocysts in *A. stephensi*, suggesting that PKC-dependent signaling is a positive regulator of the mosquito immune response and a potential target for transmission-blocking strategies.

1462

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1463

ANTI-ADHESION MOLECULES INHIBIT *PLASMODIUM* INFECTION IN *ANOPHELES* MOSQUITOES

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Plasmodium gametocytes ingested by mosquitoes with blood meal undergo gametocytogenesis and fertilization in mosquito midgut and develop to ookinetes. Ookinetes penetrate through mosquito gut wall to reach the gut epithelia where they attach themselves to the underlying basal lamina and develop to oocysts. Oocysts mature, develop thousands of sporozoites, and eventually rupture and release sporozoites into the mosquito haemolymph. Sporozoites then invade mosquito salivary glands. All these steps are governed by a series of adhesion phenomena made possible by interaction between receptors on the parasites and ligands expressed on mosquito tissues. Sporozoite invasion of salivary glands is also controlled by receptor-ligand interaction. Here we investigated the effect of several disintegrins and a c-lectin, which are proteins that interfere with adhesion phenomena mediated by integrins, on the *P. berghei* development in *Anopheles stephensi.* After mosquitoes were infected with *P. berghei*, they were fed daily with either 1 ug/ml of seven different disintegrins, c-lectin, or sugar (control). The mosquitoes were then examined for oocyst infection at Day 11 post infection and for sporozoite infection at Day 16. Mosquitoes treated with echistatin and VP12 showed decreased numbers of oocysts averaging 20/msq as opposed to averaged 50/msq in the controls. Only 30-40 % of mosquitoes treated with echistatin or VP12 showed at least 10 sporozoites in their salivary glands, while 90 % of mosquitoes fed on sugar (control) did. The results show that these disintegrins interfered with the adhesion phenomena leading to a decrease in oocyst attachment to mosquito midgut and sporozoite invasion of salivary glands.

1464

SELECTION FOR CHLOROQUINE-SENSITIVE *PLASMODIUM FALCIPARUM* BY *ANOPHELES ARABIENSIS* IN SOUTHERN ZAMBIA

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The emergence of *Plasmodium falciparum* drug resistance poses a major obstacle for malaria control and elimination. Public health strategies are needed to delay or minimize escalation. Field observations point to a link between mosquito control and the prevalence of *P. falciparum* drug resistance, the origin of which has remained unclear. Here we show field evidence for natural selection of wild type chloroquine-sensitive malaria parasites by *An. arabiensis* in southern Zambia. We screened 753 *An. arabiensis* by PCR, of which 8% and 10% were positive for salivary gland and mid-gut *P. falciparum* infections, respectively. We typed *P. falciparum* in humans and mosquitoes at the chloroquine resistance conferring amino acid codon 76 of the *Pf*CRT gene. Our data showed that despite being acquired from humans within a few weeks, *P. falciparum* infections in mosquitoes were up to 10X more likely to bear wild type *Pf*CRT K76 than in humans (OR [95%CI]: 10 [4.3 - 25.3], $p < 0.001$, n = 370). We concluded that a sporogonic selection occurs against mutated *Pf*CRT 76T-bearing *P. falciparum* in mosquitoes, presumably owing to altered biological fitness. This strong selection would seem to explain the association seen in the field between mosquito control and prevalence of drug resistance. We hypothesize that through this sporogonic selection, mosquitoes contribute to restoration of chloroquine-sensitive K76 parasites after suspension of drug use in humans. Understanding the nature and direction of the sporogonic selection could be instrumental in rational curtailment of drug resistance in integrated malaria control or elimination programmes.

1465

DEVELOPMENT OF A NEW BIOMARKER OF EXPOSURE TO *ANOPHELES* BITES BASED ON HUMAN ANTIBODY RESPONSES TO SALIVARY PROTEINS: FROM THE CONCEPT TO THE APPLICATIONS

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The study of human-vector immune relationships could allow several applications for the control of vector-borne diseases. Indeed, some salivary proteins from blood-feeding arthropods could induced a specific immune responses in human populations exposed to arthropod vectors bites. One hypothesis is that human immune response and especially antibody (Ab) response to whole saliva of mosquito could be an epidemiological biomarker of human exposure to vector bites. In the objective to increase the specificity to vector exposure, the second step was to identify salivary proteins i) specific to *Anopheles* genus and ii) antigenic in individuals exposed to malaria. First, the identification of antigenic salivary proteins of mosquito by an immuno-proteomic approach was assessed. The second step was to design peptide sequences, from one selected mosquito salivary protein using a bioinformatic approach, taking into consideration i) their potential antigenic properties and ii) the absence of cross-reactivity with other arthropods/organisms. For malaria, the specific IgG Ab levels were then evaluated in African children in different context of malaria. From five peptides, only one peptide (gSG6-P1) presented all criteria to be an optimal candidate biomarker for evaluating human exposure to An. gambiae and An. funestus bites and interestingly for evaluating the efficacy of vector control. This new "salivary" biomarker of *Anopheles* exposure could be used as a geographic indicator for mapping the risk of malaria transmission and especially in low *Anopheles* density conditions, where entomological methods are limited in sensitivity (dry season, altitude or urban malaria). It also represents a direct criterion of efficacy in the evaluation of anti-vector strategies.

1466

MALARIA IN SCHOOL CHILDREN UNDER A NEW POLICY OF UNIVERSAL COVERAGE OF NETS: RECENT DATA FROM MALI AND SENEGAL

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Malaria control has traditionally focused on pregnant women and children under five years, in whom the risk of malaria-related mortality is greatest. Yet studies have shown that older school-age children can also benefit from malaria control, with potential gains for both health and education. Insecticide-treated nets are a cornerstone of malaria prevention efforts, but in many countries net usage is lowest in school-age children compared with younger children and adults. Universal coverage of nets will help address this gap, and is increasingly being adopted into policy by national control programmes in malaria-endemic countries. Senegal and Mali recently introduced universal coverage of nets, with national roll-out of community-wide distributions of long-lasting insecticidal nets (LLINs) starting in 2010 and 2011 respectively. The coverage of LLINs amongst schoolchildren in these two countries was examined through school surveys 6-12 months after the net distributions, and the prevalence of malaria parasitaemia and anaemia measured at the end of the transmission season. Data was collected in 38 primary schools (1900 children) in Sikasso, Mali and 6 primary schools (865 children) in Kedougou, Senegal. Our data provide evidence that the new strategy was successful in achieving coverage in this previously neglected age group: reported and observed use of nets was high in both countries, with over 80% of schoolchildren (age 7-14 years) using nets. Yet paradoxically, levels of malaria infection remained high. Overall 83% of primary schoolchildren in Mali (range: 46-98%), and 54% of schoolchildren in Senegal (range: 20-81%) had asymptomatic parasitaemia in December 2011. Factors which may account for this apparent paradox will be discussed, and data presented on patterns of net use by child and household characteristics, including time of going to bed and discontinued use of nets in later months. We shall also present findings from an alternative malaria control strategy in schools, intermittent parasite clearance, which is currently being trialed in these two sites.

1467

THE EFFECT OF LIMITED RESIDUAL LIFE OF INSECTICIDE AND OUTDOOR BITING ON MALARIA INFECTION IN CHILDREN ON BIOKO ISLAND, EQUATORIAL GUINEA: AN EXAMINATION OF TWO KEY ASSUMPTIONS OF INDOOR RESIDUAL SPRAYING

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Malaria is endemic on Bioko Island, Equatorial Guinea, with year round transmission. In 2004 an intensive malaria control strategy primarily based on Indoor Residual Spraying (IRS) was launched. The limited residual life of IRS poses particular challenges in a setting with year round transmission such as Bioko. Recent reports of outdoor biting by An. gambiae are a further cause for concern. In this study the effect of the short residual life of bendiocarb insecticide and of children spending time outdoors at night on malaria infection prevalence was examined. Data from the 2011 annual malaria indicator survey and from standard WHO cone bioassays were used to examine the relationship between time since IRS, mosquito mortality and prevalence of infection in children. Children spending time outside at night and the association of this behavior with malaria infection were also examined. Prevalence of malaria infection in 2 to 14 year-olds was 18.4%, 21.0% and 28.1% in communities with median time since IRS of three, four and five months respectively. After adjusting for confounders, each extra month since IRS corresponded to an odds ratio (OR) of 1.44 (95% CI 1.15 - 1.81) for infection prevalence in 2 to 14 year-olds. Mosquito mortality was 100%, 96%, 81% and 78%, at month two, three, four and five respectively after spraying. Only 4.1% of children spent time outside the night before the survey between the hours of 10pm and 6am and were not at a higher risk of infection (OR 0.87, 95% CI 0.50 - 1.54). Sleeping under a mosquito net provided additive protection (OR 0.68, 95% CI 0.54 - 0.86). The results demonstrate the epidemiological impact of reduced mosquito mortality with time since IRS. The study underscores that in settings of year round transmission there is a compelling need for longer lasting IRS insecticides, but that in the interim high coverage of long lasting insecticidal nets (LLINs) may ameliorate the protective effect conferred by current shorter lasting IRS insecticides.

1468

EARLY MORNING BITING BY *ANOPHELES* VECTORS: A POTENTIAL RISK PERIOD FOR MALARIA INFECTION IN AN AREA WITH HIGH AND SUSTAINED USE OF INSECTICIDE TREATED BED NETS IN WESTERN KENYA

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As malaria vector control activities using insecticide-based interventions such as indoor residual spraying and insecticide treated nets (ITNs) expand, the question emerges as to how these activities shape the distribution, population and behavior of the vectors; as these processes will influence the effectiveness of each intervention. Outdoor and early evening biting have been suggested as possible vector behavior changes resulting from indoor vector control interventions. Therefore, we conducted a study during the peak malaria season in an area of high ITN ownership in western Kenya to quantify the biting behavior of the local malaria vectors, and related results to the behavior of people in the community. A total of 150 adult men were recruited as human landing catchers who collected and stored mosquitoes hourly at indoor and outdoor fixed positions, from 5 PM to 7 AM, for 4 nights per week, in a total of 75 villages for a period of 6 weeks. The main malaria vectors were *Anopheles* arabiensis (n=153, sporozoite rate SR=0.04); *A. gambiae* s.s (n=241, SR=0.12) and A. funestus (n=1169; SR=0.09). More than a third of bites by each of the main vectors occurred outdoors. However, by 9 PM, 88% of the human population was indoors and were presumably not at risk for malaria infection by outdoor biting mosquitoes. Indoors, the peak biting for all three species occurred after midnight, and biting continued to 7 AM. Net use was high with 77% of the population reporting the use of a net the previous night. By 11 PM, 96% of the population reported going to bed and those who reported using a net were likely at a low risk of mosquito bites and malaria infection. In the morning hours, about 52% of the population was awake before 6 AM, a time when vector mosquitoes, particularly An. funestus were still active, suggesting a window of risk for malaria infection. The temporal distribution of risk of infectious bites among the population and implications for vector control will be discussed.

1469

CLUSTER-RANDOMIZED TRIAL OF TEXT MESSAGE REMINDERS TO RETAIL STAFF OF APPROPRIATE PRACTICES FOR DISPENSING ARTEMETHER-LUMEFANTRINE IN DRUG SHOPS IN TANZANIA: EFFECT ON DISPENSER KNOWLEDGE AND PATIENT ADHERENCE

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Patient adherence, the extent to which patients promptly and correctly take the full course of a drug, is a key component in ensuring drug effectiveness. As artemisinin-based combination therapies (ACTs) for malaria become more widely available in the private sector, there are concerns that patient adherence might be low due to insufficient or incorrect advice provided by dispensers with limited training. In this cluster-randomized trial in drug shops in southern Tanzania, we assess the effect of text message reminders to retail staff on advice to provide when dispensing artemether-lumefantrine (AL) on dispenser knowledge and patient adherence. Of 72 randomly selected drug shops in Mtwara region, 36 were randomized for dispensers to receive text message reminders once per day five days per week beginning a month prior to the study. No intervention was delivered in the control arm. Patients desiring to purchase ACT at study drug stores were eligible to receive AL from a mixed supply of regular blister packs and identical-looking blister packs containing devices to record the date and time each blister was opened to remove pills. From each arm, 468 patients receiving study AL were followed up at home a minimum of 75 hours after drug purchase; consenting patients or their caregivers were administered a detailed questionnaire about when and how each dose of AL was taken. Patients were asked to present their blister packs for a pill count and extraction of timestamp data. Following patient data collection, dispensers were interviewed regarding their knowledge of AL dispensing practices, and mobile phone usage and receipt of malaria-related messages. Using data from questionnaires, pill-counts, and timestamps, we will report the effects of the intervention on dispensers' knowledge, the proportion of patients completing all doses within 75 hours of purchase and those adhering to the correct timing of each dose, and the advice patients received from the dispenser. These data will be useful for designing strategies to enhance the effectiveness of ACTs in the private sector.

1470

ANALYSIS OF FACILITY-LEVEL STOCKOUTS OF ACTS IN ZAMBIA: THE IMPACT OF INVENTORY MANAGEMENT

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Despite remarkable and successful recent improvements efforts by the government and its partners, the current public distribution system of essential medical drugs in Zambia still results in low availability to patients relative to private sector standards. Many possible causes have been cited, including procurement financing and processes, supply capacity, communication and road infrastructure, distribution resources and planning methods, personnel staffing and training, coordination among stakeholders. A field experiment in Zambia's public distribution system conducted from Q3 2009 to Q2 2010 involved a high adherence to recommended inventory control policies and offers an opportunity to isolate their impact. To do so we collected daily clinic storeroom stock levels of Arthemeter-Lumefantrin (AL) antimalarial products in up to 90 facilities through photography and manual transcription, then used that data to estimate demand patterns and service levels. Delivery lead-times and estimates of monthly facility accessibility were obtained through survey of health workers. Monthly national warehouse stock levels were extracted from a software database. A simulation model was constructed to reproduce and interpret observations of stock-out patterns. We found that up to 30% of surveyed facilities stocked out of all AL products at certain times of the year despite ample inventory being available at the national warehouse. The simulation model closely reproduced these results and linked them to the use of average past monthly issues and failure to capture lead-time variability in current inventory control policies. These results suggest that inventory control policies widely recommended and used for distributing medicines in Sub-Saharan Africa directly account for some of the stockouts observed in situations involving demand seasonality and/or clinic access interruptions. They also suggest specific improvement opportunities for pharmaceutical inventory control systems that include digital transmission of inventory transactions through mobile wireless devices, standard forecasting algorithms and mathematical optimization.

1471

A REVIEW OF THE CAUSES OF ACT STOCK-OUTS IN BURUNDI

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Prompt treatment of malaria cases with an effective antimalarial is a key global strategy for malaria control. Despite global efforts to scale up the use of artemisinin-based combination therapies (ACTs), coverage across Africa remains poor, with public sector health facilities frequently plagued by stock-outs. The causes of stock-outs vary, but often reflect poor planning and weak supply chain management systems. Data from public health facilities in 6 African countries illustrated that in some cases up to 90% of health facilities lacked the full range of weight-specific packs of the recommended ACT treatments in stock. Stock-outs often last several weeks, leaving malaria patients dangerously vulnerable. We analyzed the root causes of these stock-outs in Burundi using both record reviews and in-depth interviews with providers at the national, district, and facility levels between June and December 2011. Results indicated that districts required five signatures with their monthly requisition for ACTs, which led to delays and a resignation to use other treatment options or split blister packs of other age groups, which skewed consumption data. The districts did not receive sufficient stock to cover their health facilities, and little provision was made for safety stock or emergencies, resulting in partially filled orders. As soon as districts receive monthly orders, they repeat the process without allowing time to monitor stock sent to the facilities. The time between preparing an order and distribution to the facilities is long, and emergency procurements are frequent and expensive. The formulas used to forecast needs at the multiple levels are inconsistent; in addition, data are sometimes "created" in reports to place an order and to meet performance targets. Staff also appeared to be complacent regarding the effect of stock-outs on patient outcomes. While interventions to avert some challenges are being implemented, more efforts are needed to ensure uninterrupted availability of ACTs and to promote the importance of these efforts at all levels.

1472

QUALITY OF UNCOMPLICATED MALARIA CASE MANAGEMENT IN MALAWI-FINDINGS FROM A NATIONAL HEALTH FACILITY SURVEY

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Quality malaria case management is dependent on patients being appropriately assessed, diagnosed, and treated with artemesinin-based combination therapy (ACT) for uncomplicated malaria. We conducted a nationally representative cross-sectional health facility survey in Malawi to examine malaria case management quality and assess factors related to correct treatment. We sampled 107 public health centers and hospitals in all 29 districts of Malawi in April-May 2011, during peak malaria transmission. In all, 2,019 patients seeking curative care at outpatient departments were interviewed after their consultation, and blood smears were taken. Malaria was defined as fever or history of fever and malaria parasitemia on exit interview blood smear. Logistic regression was used to examine factors associated with correct treatment, defined as ACT prescription for patients with malaria. Thirty-four percent of all patients presenting to facilities in Malawi had malaria, including 46% of children <5 years and 27% of patients ≥5 years ($p<0.001$). Among patients with malaria, 67% received correct treatment; the most common reason for incorrect treatment was missed diagnosis (27%). Clinicians did not assess fever/history of fever in 27% of all patients. Only 21% of patients were tested for malaria using microscopy, and rapid diagnostic tests were not yet available. Overtreatment was common with 31% of patients without malaria prescribed an ACT. Patient-level factors, including high temperature (adjusted odds ratio (aOR) = 3.3; 95% confidence interval (CI) 3.3-5.5), spontaneous complaint of fever (aOR = 4.0; 95% CI 3.3-7.2), and complaint of cough (aOR = 0.3; 95% CI 0.2-0.5) were significantly associated with correct treatment. Health worker- or facility-level factors were not. Malawi has a high burden of uncomplicated malaria, but both failure to deliver correct treatment and overtreatment are common. Improved assessment of fever and increased parasitological confirmation of malaria diagnosis are critical to improve malaria case management.

1473

ADVERSE DRUG EVENTS RESULTING FROM USE OF DRUGS WITH SULFONAMIDE AND ARTEMISININ-BASED ANTIMALARIALS: FINDINGS ON INCIDENCE AND HOUSEHOLD COSTS FROM THREE DISTRICTS WITH ROUTINE DEMOGRAPHIC SURVEILLANCE SYSTEMS IN RURAL TANZANIA

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Antimalarial regimens including sulfonamide and artemisinin derivatives have been deployed in many parts of the world in an effort to halt the acceleration of antimalarial drug resistance problem. Access to these drugs has faced multiple obstacles including availability, acceptability, and adherence. Meanwhile, weak public health infrastructures and drug regulatory authorities prevalent in most malaria endemic countries, particularly in sub-Saharan Africa, are partly responsible for poor post-marketing surveillance and have enhanced the proliferation of fake antimalarials. We used active and passive surveillance to identify and document antimalarial-associated adverse drug reactions (ADR) in three rural districts of Tanzania with high malaria transmission. Clinicians were trained to identify, categorize and report ADR cases linked to sulfadoxine/pyrimethamine (SP) and artemisinin (AS) use. Additional questions relating to demographics, care-seeking and treatment costs were asked. A total of 95 suspected ADR cases were identified. 79 were traced and successfully classified. 67 (85%) of the 79 cases were related to use of SP and/or AS antimalarial drugs. 51% of the 67 cases were classified as 'probable' and 49% were classified as 'possible' ADR events. Annual ADR incidence per 100,000 was calculated at 5.6 for AS/SP and 25.0 for SP monotherapy. Treatment costs per episode ranged from a median of US $2.00 for those making a single visit to US $21.13 for patients with 4 visits to healthcare providers. Drug costs constituted 43% of the treatment costs. Faith-based and NGO facilities were the most expensive source of care. 85% of the patients used out-of-pocket funds to pay their bills. 21% of the patients had to sell assets or borrow from relatives to settle their bills. Costs of treatment of ADR episodes were substantially catastrophic.

1474

PROVIDER AND COMMUNITY RESPONSES TO THE NEW MALARIA TREATMENT REGIME IN SOLOMON ISLANDS

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Improvements in availability and accessibility of artemisinin-based combination therapy (ACT) for malaria treatment and the emergence of multi-drug-resistant parasites have prompted many countries to adopt ACT as the first-line drug. In 2009, Solomon Islands (SI) likewise implemented new national treatment guidelines for malaria. The ACT, artemether-lumefantrine is now the primary pharmacotherapy in SI for *Plasmodium falciparum* malaria, *Plasmodium vivax* malaria and mixed infections. Targeted treatment is also recommended in the new treatment regime through maintenance of quality microscopy services and the introduction of Rapid Diagnostic Tests (RDTs). Ascertaining the factors that influence community and provider acceptance of and adherence to the new treatment regime will be vital to improving the effectiveness of this intervention and reducing the risk of development of drug resistance. To understand community and prescriber perceptions and acceptability of the new diagnostic and treatment regime, 12 focus group discussions and 12 key informant interviews were carried out in rural and urban villages of Malaita Province, Solomon Islands, four months subsequent to roll out of these interventions. Lack of access to microscopy or distrust in the accuracy of diagnostic tools were reported by some participants as reasons for the ongoing practice of presumptive treatment of malaria. Lack of confidence in RDT accuracy negatively impacted its acceptability. Artemether-lumefantrine had good acceptability among most participants; however, some rural participants questioned its effectiveness due to lack of side effects and the larger quantity of tablets required to be taken. Storing of left over medication for subsequent fever episodes was reported as common. To address these issues, further training and supportive supervision of healthcare workers will be essential, as will the engagement of influential community members in health promotion activities to improve acceptability of RDTs and adherence to the new treatment regime. Exploring the extent of these issues beyond the study population must be a priority for malaria programme managers. Practices such as presumptive treatment and the taking of sub-curative doses are of considerable concern for both the health of individuals and the increased risk it poses to the development of parasite resistance to this important first-line treatment against malaria.

1475

N-ACETYL TRANSFERASE GENE TYPE 2: PREDOMINANCE OF SLOW ACETYLATORS AND EFFECT ON RESPONSE TO ARTESUNATE AMODIAQUINE

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Inter individual differences in the metabolism of the antimalarials could be due to polymorphism of NAT2 gene. We determined the genotypic frequencies of single nucleotide polymorphism (SNP) of NAT2 gene and it's implication in antimalarial treatment during a vitamin A and zinc supplementation intervention in children less than 5 years in Bangolan, Cameroon. A total of 100 children aged 6 to 24 months were recruited into the study after obtaining informed consent from parents or guardians. Participants were randomized to receive vitamin A +placebo or vitamin A+Zinc supplements. All participants received artesunate-amodiaquine(ASAQ) -toddler 50/135mg at baseline to clear any parasites, vitamin A administered and followed up for 30days. This was followed by daily administration of Zinc or placebo and follow up for 6 months for incidence of clinical malaria and other diseases. Blood was spotted on filter paper for DNA extraction by chelex method. RFLP-PCR was performed with restriction enzymes KpnI, TaqI, and BamHI for detection of NAT2*5, NAT2*6, NAT2*7 SNPs respectively. Allelic frequencies and phenotypes were compared between participants with or without adverse reactions . A total of 55% of the participants had slow acetylator, 30% intermediate acetylator, 11% rapid acetylator and 4% an unknown genotype. NAT2 genotypes observed to be associated with susceptibility to develop anorexia were NAT2*5/5 (OR=13,000) and NAT2*4/6 (OR=6,538). Those likely to develop fever were NAT2*4/7 (OR=5,082), NAT2*5/6 (OR=2,389), NAT2*6/7 (OR=1,481) and NAT2*5/7 (OR=1,156). Those likely to develop fever of unknown etiology were NAT2*6/6 (OR=23,467), NAT2*4/5 (OR=2,933), NAT2*5/5 (OR=2,048) and NAT2*4/6 (OR=1,026). Those likely to develop skin rash were NAT2*4/5 (OR=2,857), NAT2*5/7 (OR=2,483), NAT2*6/7 (OR=1,385), and NAT2*4/7 (OR=1,357). Those likely to develop cough, catarrh (common cold) and fever were NAT2*4/6 (OR=2,255), NAT2*6/6 (OR=1,895), NAT2*4/5 (OR=1,850), NAT2*5/5 (OR=1,200) and NAT2*5/5 (OR=1,016). The slow acetylator genotype NAT2 gene was the most predominant in the study population. Both slow and intermediate acetylators were more likely to the develop adverse reactions to ASAQ, vitamin A and Zinc supplements.

1476

EXPLORING HOW LARGE-SCALE IMPLEMENTATION OF MALARIA CONTROL PROGRAMS MEDIATES THE RELATIONSHIP BETWEEN HOUSEHOLD SOCIOECONOMIC STATUS AND VARIOUS CHILDHOOD MALARIA CONTROL INDICATORS: EXPERIENCE FROM THREE PRESIDENT'S MALARIA INITIATIVE COUNTRIES IN SUB-SAHARAN AFRICA

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Following the first Global Malaria Eradication Program in the 1950's malaria was confined almost exclusively in the poorest nations of the world, particularly in sub-Saharan Africa and Southeast Asia. While macroeconomic studies consistently show a strong and stable relationship between malaria and poverty, most microeconomic studies have largely been inconclusive. This study explores how variations in implementation of large scale malaria control programs may explain the reason why microeconomic studies remain inconclusive. It sets out to explain the critical role played by large scale implementation of malaria control strategies such as those financed by Global Fund and the President's Malaria Initiative (PMI) in mediating the relationship between households' socioeconomic status (wealth, education and place of domicile) and key malaria control indicators. The study focuses on malaria parasitemia, bed-net ownership and bed-net use among children aged less than five years as key outcome variables of interest. We analyzed Malaria Indicator Survey data for the first three PMI countries: Angola, Tanzania and Uganda. A multilevel-hierarchical cluster analysis restricted to malaria program implementation regions was used. SES interaction terms with malaria program implementation were found to have significant bearing across the three countries. In Angola, programs were more likely to benefit households headed by individuals or mothers with higher education levels whereas household wealth status was less important. In Tanzania, wealth, education and living in urban or rural settings were all significant determinants of which households benefited more from the programs while in Uganda programs were more likely to benefit poorer households. Following these findings, policy relevant conclusions are drawn to help design more pro-poor malaria control policies in light of the renaissance of malaria eradiation policy debates.

1477

PREVENTION OF NEONATAL HYPOTHERMIA IN SOUTHERN PROVINCE, ZAMBIA

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Newborn hypothermia is associated with increased neonatal mortality. Zambian guidelines recommend facility-based delivery by skilled birth attendants and immediate postpartum skin-to-skin care to provide thermoprotection of the neonate. This study assessed institutional capacity to prevent neonatal hypothermia in Zambia. We conducted comprehensive health center (HC) surveys in Southern Province, Zambia, and pregnant women were recruited at the same HCs during routine antenatal care to participate in a neonatal study (ZamCAT). Enrollees were interviewed 4 days post-delivery about the delivery and immediate postpartum care. Of the 90 primary HCs surveyed, only 8.8% had a neonatal warmer and 6.7% had heat control for the delivery room. When HC directors were asked about delivery practices, 36.7% said the newborn was placed the mother's abdomen after delivery, 46.7% put the baby next to the mom and 15.6% placed the baby in a cot. Nearly all HCs (94.4%) reported drying and wrapping the baby in a new cloth, and, in the last month, 92.2% recommended skin-to-skin contact to new mothers. Among 9,816 deliveries [63% at a facility, 36% at home], the baby was placed on mother's skin after delivery 49.9% of the time; this was significantly higher in facility compared to home deliveries ($p<0.001$). Women delivered by a nurse/midwife or trained TBA were more likely to have the baby put on the mother's skin afterwards compared to those delivered by family members, self or untrained TBAs (61.8% vs. 21.5%, $p<0.001$). In 98% of deliveries, the baby was wrapped in a dry cloth; this did not differ by delivery location. Southern Province health centers are not well equipped to prevent neonatal hypothermia although evaluation of actual practices suggests that efforts are made to warm the newborn and recommend skin-to-skin care. These practices are less common in home deliveries, thus increasing risk of hypothermia in newborns delivered by unskilled birth attendants.

1478

USING CURRENT AND EXTENDED ROUTINE PREVENTION VISITS TO HEALTH FACILITIES ACHIEVE HIGHER COVERAGE WITH CHILD-SURVIVAL INTERVENTIONS IN SUB-SAHARAN AFRICA

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Interventions to improve child survival in sub-Saharan Africa are frequently administered during mass campaigns (MCs), particularly for interventions targeting children 1-4 years old. Integrated delivery of interventions in MCs, including Vitamin A supplementation, insecticide-treated bednets, supplemental immunizations, and deworming, has been promoted to decrease program costs and to expand coverage. However, integrated preventive services have long been provided during routine preventive visits (RPVs) to health facilities by pregnant women and mothers and their children during the child's first year of life. While the interventions offered during RPVs are currently limited, they could be expanded and offered as well at new RPVs scheduled up to the age of five. To assess RPVs as a platform for expanded service delivery and compare them to MCs, we analyzed data from Demographic and Health Surveys in 12 sub-Saharan African countries in which mothers were asked about receipt of services for themselves or their children in one or more MCs (median number of MCs: 4.5; range: 2-11). RPV coverage demonstrating access (the percentage of mothers seen at least once for an RPV) was high in all countries (range: 80.6 [Nigeria]-99.9% [Swaziland]; median: 96.7), typically exceeding the percentage of eligible 1-4 year-old children receiving an intervention in at least one MC (range: 36.3 [Sierra Leone]-89.5% [Eritrea], median difference: 28.1 percentage points). The median number of RPVs among mothers of 1-4 year-old children ranged from 4.5 in Niger, to 12.9 in Swaziland. The percentage of children aged 1-4 years missed by all MCs but whose mothers and made at least one RPV ranged from 62.7% in Nigeria to 99.5% in Sao Tome & Principe. The median number of RPVs among these children ranged from 2.3 in Niger to 11.0 in Ghana. Among 1-4 year-olds whose mother made no RPV, the percentage receiving at least one MC intervention for which they were eligible was lower (range: 0.0 [Sao Tome & Principe]-35.5% [Niger]; median 4.8%). Current and extended RPVs may reach children missed by MCs, and are potentially an effective alternative to MCs for delivering some child-survival interventions, particularly to 1-4 year-old children.

1479

IMPACT OF A BRIEF IN-HOME NEONATAL HEALTH PROMOTION ON SELF-REPORTED BIRTH AND NEONATAL CARE PRACTICES AMONG PRIMIPAROUS WOMEN IN THE THIRD TRIMESTER IN RURAL BANGLADESH

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Over 75% of neonatal deaths occur in the first week of life. Community-based promotion programs that have promoted safe birthing and early infant care practices have decreased neonatal mortality; but they included prolonged staff training and antenatal visits as early as 12-16 weeks gestation. Although desirable, reaching women early in pregnancy with highly trained staff is difficult and expensive, potentially limiting the scale of these programs. We sought to describe the behavioral impact of safe birthing and neonatal care messages delivered to primiparous women in the third trimester as part of a randomized controlled trial of handwashing promotion in rural Bangladesh. We promoted delivery at a medical facility, use of a clean delivery kit for a home delivery, recognition of maternal and neonatal danger signs, and essential neonatal care to all participants and their families. Field workers received four days of training and delivered the messages during one home visit between 33 and 35 weeks gestation and two visits within one week after birth. We compared self-reported changes in knowledge and beliefs of these practices before and after the intervention. Of 250 women in the study, 212 completed interviews before and after the intervention. Prior to the intervention, 57% of the women had ≥1 prenatal visit to a health care provider, which increased to 94% after the intervention ($p<0.01$). Only 2% planned to deliver at a medical facility but 41% reported delivering at a medical facility ($p<0.01$). Before the intervention, 32% of women reported a foreign substance (such as oil or dung) should be placed on the umbilical cord after cutting, and 72% agreed a baby should be bathed immediately after birth. However, only 6% reported placing anything on the cord after it was cut ($p<0.01$) and 2% of neonates were reportedly bathed <5 hours after birth ($p<0.01$). This program requiring minimal training of field staff resulted in reports of improved birth and neonatal care practices compared to reported prior beliefs. Neonatal care and birthing practices can be improved, even when women are identified late in pregnancy. A brief training of community health workers may be feasible and effective for reducing risky health behaviors in the antenatal and neonatal period, and may be scalable.

1480

THE ASSOCIATION BETWEEN COGNITION AND ACADEMIC ACHIEVEMENT IN UGANDA CHILDREN SURVIVING MALARIA WITH NEUROLOGICAL INVOLVEMENT

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An understanding of the contribution of different cognitive abilities to academic achievement in children surviving cerebral injury can guide the choice of interventions to improve cognitive and academic outcomes. This study's objective was to identify which cognitive abilities are associated with academic achievement in children after an episode of malaria with neurological involvement (MNI). 62 children with a history of MNI were assessed for cognitive ability (working memory, reasoning, learning, visual spatial skills, attention) and academic achievement (arithmetic, spelling, reading) three months after recovery from the illness. Linear regressions were run for each academic score with the five cognitive outcomes entered as predictors. Adjusters entered in the analysis were age, sex, education level, nutritional status and quality of the home environment. Exploratory factor analysis (EFA) and structural equation models (SEM) were used to determine the nature of the association between cognition and academic achievement. In regression of a single academic score on all five cognitive outcomes and adjusters, only Working Memory was associated with Reading (coefficient estimate=0.36, 95% confidence interval=0.10 to 0.63, $p<0.01$) and Spelling (0.46, 0.13 to 0.78, $p<0.01$), Visual Spatial Skill was associated with Arithmetic (0.15, 0.03 to 0.26, $p<0.05$), and Learning was associated with Reading (0.06, 0.00 to 0.11, $p<0.05$). A single latent cognitive factor was identified using EFA. The SEM demonstrated a strong association between this latent cognitive ability and each academic achievement measure ($P < 0.0001$). No additional association between the academic scores and the individual cognitive measures was found beyond the latent cognitive ability. Academic achievement is best predicted by a latent variable, cognitive ability, which captures most of the variation in the individual cognitive ability measures. EFA and SEM can help to define how cognitive testing outcomes relate to academic achievement in children with disease-associated brain injury.

1481

BELIEFS AND CULTURAL PRACTICES TOWARDS MEASLES AND MEASLES VACCINATION PROGRAMS IN A MULTI-ETHNIC URBAN NEIGHBORHOOD IN KENYA: A QUALITATIVE STUDY

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A recent measles outbreak in Kenya began in late 2010 and by April 2011 had spread across the country, with the highest number of cases reported in Eastleigh, Nairobi, a community with a high proportion of refugees and migrants from neighbouring countries. To better understand cultural perspectives and community awareness of measles, and assess response to immunization activities, we conducted a series of focus group discussions (FGDs) in Eastleigh. Six FGDs were held (during April 23-29, 2011) before a supplementary immunization activity (SIA) for children <5 years and another 6 FGDs were held (during May 18-20, 2011) after the SIA. Between 6 and 10 individuals matched for primary language and gender participated in every session. Sessions were facilitated by persons with similar primary language and gender using facilitation guides with similar questions for all the groups. The sessions were recorded, transcribed and translated into English. Qualitative data were analyzed using NVivo 2.0. A total of 103 individuals (mean age, 30.5 years) representing three language groups (Oromo, Somali and Swahili) participated in the 12 discussions. Participants in all groups were able to identify measles and associated it with poverty, poor sanitation and dirty environment. The Oromo and Somali speakers mentioned home remedies as first-line therapy. Cost, long queues, distance to immunization sites, perceived discrimination by non-nationals, lack of understanding of health messages due to language barriers, belief that injections could cause death or exposure to disease, and belief that vaccinated children were not protected were some of the barriers to vaccination mentioned. Somali and Oromo participants recommended providing information through trusted community leaders and community health workers who speak their primary languages. Failure to provide linguistically and culturally appropriate health education materials may negatively impact disease prevention efforts in this setting with ethnically diverse populations.

1482

PRACTICES OF ANTIBIOTIC USE IN CHILDREN LESS THAN FIVE OF MEDICAL PERSONNEL IN PRIMARY CARE CENTERS IN PERI-URBAN AREAS OF LIMA, PERU

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The increase of antibiotic resistant pathogens acquired in the community is a growing problem worldwide which requires prompt intervention. The overuse and misuse of antibiotics in children is a practice rooted in developing countries, and it has been assumed that the use of antibiotics without prescription is one of the main causes of this misuse. However, previous studies showed that physicians had prescribed more than 80% of the antibiotics used and have the main responsibility in the overuse of antibiotics. The objective of the study was to describe the practices of antibiotics use in children under 5 years by the medical staff of primary health care. A structured questionnaire was applied in 218 general practitioners of primary care facilities of three districts of peri-urban Lima. It consisted of 6 typical clinical cases that may occur in children less than 5 years. 75.6% of the doctors affirmed that of the total of patients attended, more than 25% were children under the age of 5 years. Only 3.2% doctors had received training in pediatric care. When asked if necessary the use of antibiotics in the case of common cold, 15.6 would used an antibiotic, mainly amoxicillin (76.5%).78.9% of the physicians would use antibiotic in dysentery, mainly furazolidone (39.9%) and TMP-SMX (43.9) . 84.2% of the doctors would recommend an antibiotic for pharingitis and would use amoxicillin (54.3%) and amoxicillin -clavulanic acid (22.3%). 33.2% of the doctors responded that an antibiotic was needed for watery diarrhea treatment, they mainly used furazolidone(42.3%) and TMP-SMX (40.8%). 73.3% would recommend an antibiotic for bronchospasm. 28.3% would use amoxicillin -clavulanic acid and 28.9% amoxicillin. 98.1% would recommend an antibiotic in the case of pneumonia, mainly amoxicillin -clavulanic acid (30.7%) and cephalosporins (26.7%). Approximately half of patients treated in the study primary care establishments are children under five. However the doctors didn't receive training in pediatric care. An overuse of prescribed antibiotics in children less than 5 years was observed, especially in diagnoses as watery diarrhea, pharyngitis and bronchospasm. Misuse of antibiotics that are not considered first line of action on the pathogens or to which the pathogens are highly resistant show that training of medical personnel should be improved in order to reduce unnecessary antibiotic use.

1483

RECONSTRUCTING THE POPULATION HISTORY OF *WUCHERERIA BANCROFTI* IN A POST-MDA REGION

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Wuchereria bancrofti (Wb) is the primary causative agent of lymphatic filariasis (LF). Our studies of LF in Papua New Guinea have shown that it is possible to reduce the prevalence of Wb in human and mosquitoes through mass drug administration (MDA; diethylcarbamazine with/without ivermectin). While MDAs through 1998 significantly reduced prevalence of Wb infection, interruption has allowed parasite populations to recovering to pre-MDA levels. We collected genetic data with the objectives to i) reconstruct Wb population dynamics post-MDA, ii) document contemporary levels of genetic diversity, and iii) differentiate mechanisms of population connectivity. We sequenced Wb infections from 17 patients across 8 villages encompassing both high and moderate annual transmission potentials (ATP).We confirmed the presence of a genetic bottleneck consistent with past MDA treatment with a successive period of exponential growth following treatment interruption. We characterized 175 unique maternal haplotypes currently segregating in the Wb population, with one common haplotype present in 75% of infections. Finally we describe the spread of haplotypes between villages corresponding to the period of population growth following interruption of MDA. We conclude that while the MDA was successful in reducing the Wb genetic diversity, it was not prolonged enough to eliminate all genetic diversity. Interruption of treatment allowed the parasite population to recover and consequently disperse across the landscape via host and vector migration. We hypothesize that through the combined use of long-lasting insecticide treated bed nets (LLINs) in conjunction with MDAs we can eliminate all but the most common haplotypes as well as prevent migration of drug resistant strains both among patients and among villages. Through examining genetic diversity, we have been able to make insights into the demographic history of the parasite population and estimate the most effective strategies to reduce genetic diversity.

1484

COMPARATIVE PROTEOMICS OF *WOLBACHIA* STRAINS BETWEEN INSECTS AND NEMATODES

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The symbiont *Wolbachia* is of intense interest for tropical medicine, both as a drug target in filarial nematodes and as an inhibitor of pathogen transmission in insect vectors. Research on *Wolbachia* has been accelerated by genome sequencing from the major taxonomic "supergroups", including "A" (strain *w*Mel from *Drosophila melanogaster*, 1.3 Mb), "C" (*w*Oo from *Onchocerca ochengi*, 1.0 Mb) and "D" (*w*Bm from *Brugia malayi*, 1.1 Mb). However, proteomic analysis of *Wolbachia* remains scant, despite its potential to illuminate the apparent divide between "parasitic" (group A) and "mutualistic" strains (C and D). Here, we present absolute abundance data for ~30% of the *w*Mel proteome, compared with semi-quantitative estimates for *w*Bm and *w*Oo. Strikingly, the chaperonin GroEL represents 20% of *w*Mel protein, and also dominates in *w*Bm and *w*Oo, alongside six other conserved proteins [*Wolbachia* surface protein, elongation factor (EF)-Tu, co-chaperonin GroES, chaperone DnaK, peptidoglycan-associated lipoprotein, and a porin]. Despite the larger genome of *w*Mel, only two proteins absent from *w*Bm and *w*Oo (a hypothetical protein and a NAD-dependent epimerase) are highly expressed in *w*Mel; although EF-G, heat-shock protein 90 and ribosomal protein L7/L12 are quantitatively elevated. Surprisingly, the profiles of proteins involved in the stress response, nucleotide salvage, transcription and DNA binding are more similar between *w*Bm and *w*Mel than *w*Bm and *w*Oo. The abundance of many proteins in *w*Bm and *w*Oo is not concordant, with increased representation of Zn peptidases, Lon protease and an ankyrin protein in *w*Bm; in contrast with ClpB protease, the copper chaperone SCO1, and oxoglutarate dehydrogenase in *w*Oo. However, shared overrepresentation of two proteins (ATP synthase and HtrA protease) may constitute a "mutualistic signature". Thus, proteome evolution in *Wolbachia* is shaped by compensatory mechanisms to maintain protein metabolism during genome reduction. However, that hypothesis that ATP is a key metabolite provisioned by the mutualistic strains is also supported.

1485

WOLBACHIA-LIKE TRANSCRIPTS AND PROTEINS IN THE WOLBACHIA-FREE FILARIAL PARASITE *ONCHOCERCA FLEXUOSA*

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Most filarial parasites that infect humans require *Wolbachia* endobacteria for normal development and reproduction. It would be interesting to know how *Wolbachia*-free filarial worms function without an endosymbiont. We have previously reported that two *Wolbachia*-free filarial species contain *Wolbachia*-like sequences in their nuclear genomes and that some of these sequences are expressed at the RNA level in a tissue- and stage-specific manner. In the present study, we sequenced the transcriptome of adult *Onchocerca flexuosa* in order to further explore the phenomenon of horizontal gene transfer between *Wolbachia* and presently *Wolbachia*-free filarial species. We estimate that 40% of all *O. flexuosa* protein-coding genes are represented in our dataset, and we were able to detect regions with homology to 97 different *Wolbachia*-like genes. The transcriptome data facilitated a follow-up proteomic analysis in which 1,800 *O. flexuosa* proteins were identified, including two candidate *Wolbachia*-like proteins. Peptide antibodies raised against the two mass-spectroscopy identified and other computationally predicted *Wolbachia*-like proteins were used to further confirm their expression. Immunohistochemistry studies indicated that these proteins were present in many body regions in adult worms. However, *in situ* hybridization studies showed that the *Wolbachia*-like transcripts are expressed in the lateral chords, the tissues where *Wolbachia* are concentrated in species that harbor the *Wolbachia* endosymbiont. Future studies will attempt to demonstrate the functional significance of remnant *Wolbachia* genes and proteins in *Wolbachia*-negative filarial worms.

1486

PROTEOMIC ANALYSIS OF EXCRETORY-SECRETORY PRODUCTS OF THE FILARIAL NEMATODE *LITOMOSOIDES SIGMODONTIS*

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The excretory-secretory (ES) products of a parasitic worm represent the 'frontline' in its interaction with the host. These products are known to have immunomodulatory roles in parasite invasion and long-term persistence of infection. The filarial nematode *Litomosoides sigmodontis* is a tractable experimental model for filariasis, as it can produce transmissible offspring in BALB/c mice. Proteomic analysis of adult female *L. sigmodontis* ES products was performed using shotgun LC-MS/MS, identifying several hundred proteins against a draft *L. sigmodontis* genome assembly. The protein abundance profile of the ES differed greatly to that of the somatic protein extract. The predominant ES protein families were protease inhibitors, proteases, lipid-binding proteins and antioxidants. The cysteine protease inhibitor Ls-cystatin, a key vaccine candidate, was the most abundant species present in the ES. Members of the transthyretin-like protein family were also well represented, consistent with earlier studies on the ES of *Ostertagia ostertagi* and *Brugia malayi*. Several previously characterised filarial antigens, including FAR1, leucyl aminopeptidase and RAL2 were also highly enriched in the ES material. In addition, a novel protein product highly expressed in the ES exhibited homology to an apolipophorin from *Ascaris suum* (a lipid-binding protein). However, only three proteins from the *Wolbachia* endosymbiont of *L. sigmodontis* were detected and at low abundance. These initial proteomic data from the adult females will be compared to the ES protein profiles of the adult male, microfilaria and L3 life stages of *L. sigmodontis* to obtain a comprehensive representation of the quantitative changes in the secretome during filarial development.

1487

IDENTIFICATION OF GENES CONTAINING ECDYSONE RESPONSE ELEMENTS IN THE GENOME OF *BRUGIA MALAYI*

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Recent studies have demonstrated that filarial parasites contain a functional homologue of the insect ecdysone receptor (EcR). As a first step in deciphering the physiological role that ecdysteroids play in filarial parasites, adult female parasites cultured in the presence and absence of 20-OH ecdysone were metabolically labeled. Gel electrophoretic analysis of proteins extracted from the cultured parasites revealed changes in the level of expression of several proteins, indicating that adult female parasites contained an ecdysone-responsive gene network. A bioinformatic analysis was then conducted to identify putative ecdysone response elements (EcREs) in the *B. malayi* genome. A total of 18 genes were identified that contained putative EcREs located in the 4 kbp upstream from the start of their open reading frames. The most common functional classifications of the encoded proteins were factors involved in transcription and metabolism. These genes revealed a number of different developmental patterns of transcription. The promoter of one EcRE-containing gene was cloned into an luciferase reporter vector and transfected into *B. malayi* embryos. Reporter gene expression from embryos transfected with this construct was up-regulated by 20-OH ecdysone, a response which was dependent upon the putative EcRE. These results demonstrate the presence of endogenous functional EcREs in the *B. malayi* genome and provide insights into the role that ecdysteroids may play in the developmental processes of *B. malayi*.

1488

STRUCTURAL ELUCIDATION OF *WUCHERERIA BANCROFTI* GLUTATHIONE-S-TRANSFERASE BY X-RAY CRYSTALLOGRAPHY TO EVALUATE ITS ROLE AS A THERAPEUTIC TARGET FOR HUMAN LYMPHATIC FILARIASIS

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Human lymphatic filariasis is an incapacitating vector borne disease and is the world's second leading cause of long-term disability. To worsen the condition there are no vaccines yet and vector control programs have limitations of insect resistance. The current drugs have limited ability in removing adult worms and do not remedy chronic morbidity and are suitable only for preliminary control measures. Further their broad use would increase the likelihood of accelerated drug resistance. With this distressing scenario there is a growing demand to identify new molecular targets for lymphatic filariasis towards development of drugs and prophylactics. The current study involved in structurally characterizing filarial glutathione-S-transferase (*Wb*-GST) as a drug target for lymphatic filariasis. Accordingly, the recombinant *Wb*-GST was expressed, purified and co-crystallised along with its native substrate glutathione. The structure was solved at a resolution of 2.3Å by X-ray crystallography. The structure resembles ϖ-class GSTs. The superimposed structures of *Wb*-GST and *Hu*-GST (human host) monomers showed an r.m.s. deviation of 1.2Å for all Cα atoms. The G-site residues were highly conserved (differed by 8%), whereas the H-site residues revealed a significant difference (62%) between *Wb*-GST and *Hu*-GST. The H-site of *Wb*-GST showed greater accessibility for electrophilic substrates compared to *Hu*-GST. The electron

density map of *Wb*-GST showed that the catalytic residue Tyr^7 swings off and works as a proton shuttle for catalytic stabilization. The *Wb*-GST structure also revealed the presence of non-catalytic ligand binding sites (ligandin function) in the intersubunit cleft, which can serve as a binding site for hydrophobic ligands. These crucial insights from structural data could be exploited for developing parasite-specific inhibitors.

1489

A VALIDATION STUDY FOR A MULTIPLEX QPCR ASSAY FOR THE DETECTION OF *WUCHERERIA BANCROFTI* AND *BRUGIA MALAYI*

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Responsible for causing infection in more than 120 million individuals, *Wuchereria bancrofti* and *Brugia malayi* are the primary causative agents of lymphatic filariasis. As a number of Southeast Asian and South Pacific countries exist that are co-endemic for both parasite species, diagnostic tools capable of simultaneous detection of both filarial nematodes are an attractive option for infection monitoring and surveillance efforts. We previously described the development of a multiplex qPCR assay for the detection of both filarids within a single pool containing DNA extracted from larval worms of both species. However, the usefulness of this assay as a time and money-saving tool is dependent upon the assay's ability to accurately and repeatedly detect parasite DNA extracted from human bloodspots and from mosquito vectors. Here we describe a validation study using both vector mosquito DNA extracts and human bloodspot DNA extracts. This study demonstrates the sensitivity of this multiplex qPCR assay at the 1 pg level, which is as sensitive as the established singleplex assays for the detection of *W. bancrofti* and *B. malayi*.

1490

HISTAMINE RELEASE DURING *LITOMOSIDES SIGMONDONTIS* INFECTION ENHANCES ADULT WORM BURDEN

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Numerous studies have demonstrated that helminth antigens induce release of histamine from basophils and mast cells of infected hosts. To date, however, the role histamine plays in the immune response against helminths has not been well characterized. In this study, we evaluated the role of histamine in mice infected with ***Litomosoides sigmondontis,*** a tissue-invasive filarial infection of rodents that lives for months in immunocompetent Balb/c mice. Extended time-course studies revealed that histamine in plasma peaked at 8 weeks of infection whereas expression of histidine decarboxylase mRNA in circulating blood cells increased throughout the course of infection. Mice vaccinated with irradiated L3 larvae demonstrated substantial increases in circulating histamine levels 30 minutes after challenge infection, but administration of HR1 and HR2 receptor blockers did not attenuate the protective efficacy of vaccination. Interestingly, short time course measurements demonstrated that primary infection of unvaccinated mice with L3s also causes histamine release into the bloodstream 30 minutes following infection indicating a non-specific mechanism of histamine release. To evaluate the role histamine may play during infection, mice were chronically administered HR1, HR2, and a combination of HR1 and HR2 blockers in their drinking water and assessed for adult worm survival after inoculation with 40 L3 larvae. Surprisingly, at 8 weeks post-infection all groups of mice treated with antihistamine antagonists had significantly reduced numbers of adult worms compared to untreated controls. Taken together, these data indicate that histamine, rather than being involved in vaccine-mediated protection, may be induced by filarial parasites for their growth and/or survival ***in vivo.***

1491

EOSINOPHILS AND STAT6 REGULATE *TRICHINELLA SPIRALIS* MUSCLE INFECTION BY CONTROLLING PARASITE GROWTH

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The parasitic nematode ***Trichinella spiralis*** establishes chronic infection in skeletal muscle. The muscle phase of infection is characterized by tissue and blood eosinophilia. Using two models of eosinophil-ablated mice, we have previously shown that larval growth and survival are significantly compromised in the absence of eosinophils and that this correlates with reduced Th2 immunity. We show here that reduced Th2 cell accumulation at infection sites is caused by impaired Th2 cell production in draining lymph nodes. Defective Th2 cell accumulation did not correlate with the expression pattern of chemokines that direct the migration/activation of T cells, nor the ability of T cells to enter antigen-bearing tissue. Moreover, studies using STAT6-/- and IL-13-/- mice revealed that the IL-4/STAT6 axis regulated parasite growth. Impaired parasite growth in eosinophil-deficient mice correlated with increased expression of genes associated with nutrient deprivation (AMPK and INSR), but neither muscle nor larval glycogen content were affected. Our results support a pivotal immunoregulatory role for eosinophils in acquired immunity and nutrient acquisition during nematode infection.

1492

EVIDENCE FOR THE SEQUESTRATION OF DEVELOPING *PLASMODIUM FALCIPARUM* GAMETOCYTES IN THE BONE MARROW

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A hallmark of ***Plasmodium falciparum*** infection is the sequestration of asexual stages in deep tissue, which has been linked to cerebral malaria and other disease outcomes. Like late asexual stages, immature sexual stages are visibly absent from the bloodstream and are hypothesized to sequester. However, unlike asexual stages, their localization and mechanism for sequestration is largely unknown. In the current study, we systematically quantified gametocyte sequestration in autopsy cases from an ongoing study of fatal pediatric malaria in Blantyre, Malawi. An organ survey using immunohistochemistry (IHC) on tissue sections from nine body sites (brain, lung, heart, intestine, liver, kidney, subcutaneous fat, spleen, and bone marrow) suggested enrichment of gametocytes in the bone marrow. Quantitative real time RT-PCR supported this finding and revealed transcriptional signatures specific to young gametocytes, confirming that we are in fact observing gametocytes during development. Following up on these significant findings, we performed electron microscopy and observed the presence of knobless parasites in the bone marrow. We are currently performing detailed IHC studies on bone marrow samples using additional tissue markers, and complementary ***in vitro*** experiments to test alternative models of gametocyte development in the human bone marrow. The identification and characterization of a genuine bone marrow cycle of ***P. falciparum*** gametocytes is of

great relevance, in particular considering the fieldβs renewed focus on understanding the dynamics of malaria transmission and the development of new strategies to interrupt it.

1493

A NOVEL *PLASMODIUM FALCIPARUM* SR PROTEIN IS AN ALTERNATIVE SPLICING FACTOR THAT IS REQUIRED FOR PARASITE PROLIFERATION IN HUMAN ERYTHROCYTES

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The malaria parasites have a complex life cycle, during which it undergoes significant biological changes to adapt to different hosts and changing environments. ***Plasmodium falciparum***, the deadliest form of human malaria, has adapted to its complex life cycle with relatively small number of genes. Alternative splicing (AS) is an important post-transcriptional mechanisms that enables eukaryotic organisms to expand their protein repertoire out of relatively small number of genes. SR proteins are major regulators of splicing in higher eukaryotes. Nevertheless, the splicing as well as the AS machinery in ***Plasmodium spp***. are still elusive. We show that PfSR1 is a putative SR protein that can mediate RNA splicing ***in vitro***. In addition, we demonstrate that PfSR1 functions as an alternative splicing factor in a mini-gene system similar to the mammalian SRSF1. Expression of PfSR1-***myc*** in ***P. falciparum*** shows distinct patterns of cellular localization during intra erythrocytic development. Furthermore, we determine that the predicted RS domain of PfSR1 is essential for its localization to the nucleus. Finally, we demonstrate that proper regulation of ***pfsr1*** is required for parasite proliferation in human RBCs, and affect the splicing pattern of endogenous genes.

1494

A CELL INTRINSIC ROLE FOR MUC5AC IN MEDIATING THE TH2-RESPONSE TO HELMINTHES AND ALLERGIC ASTHMA

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MUC5AC is a secreted mucin known to be upregulated in response to IL-13 as part of the Th2-mediated response that occurs during helminth infection, fibrotic disease and allergic asthma. IL-13 signals through the Type II IL-4 receptor by binding the IL-13Rα1 chain to drive complex formation and phosphorylation of STAT6, thus mediating downstream effects such as upregulation Fizz1, Ym1 and Arg1, as well as increased eosinophilia, tissue remodeling and mucus hypersecretion. The purpose of this study was to investigate the role of Muc5ac in mouse models of helminth infection, fibrosis and allergic asthma using wildtype C57BL/6 (WT) and Muc5ac knockout (KO) mice. The three models used included a model of *Nippostrongylus brasiliensis* infection, a pulmonary granuloma model using *Schistosoma mansoni* eggs, and a model of allergic asthma using house dust mite. In all three models, a significant reduction in airway eosinophilia in conjunction with reduced expression of Fizz1, Ym1 and Arg1 was observed in KO compared to WT mice; however, no differences in the expression or production of IL-4, IL-5 and IL-13 were observed. To determine if the KO mice were capable of responding to IL-13, rIL-13 was delivered i.t. to WT and KO mice. No tissue inflammation, airway eosinophilia or increase in IL-13 regulated genes was observed in the KO mice in response to rIL-13. Alveolar macrophages and lung fibroblasts isolated from naïve WT and KO mice were grown in culture and treated with either IL-4 or IL-13. Isolated cells from KO mice had reduced expression of Fizz1 and Ym1 compared to WT in response to IL-13, however did not have reduced expression levels of these genes in response to IL-4. Phosphorylation of STAT6 in response to IL-13 but not IL-4 was ablated in KO macrophages, and phosphorylation of STAT6 in response to IL-4 was also ablated after pretreatment with a blocking antibody against the Type I IL-4 receptor. These data identify Muc5ac as a novel component of the Type II IL-4 receptor and thus a novel target to disrupt IL-4/IL-13-mediated inflammation.

1495

TLR3-DEPENDENT RECOGNITION OF A PROTOZOAN PARASITE

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Despite striking similarities in invasion, intracellular growth and cellular ultrastructure between ***Toxoplasma gondii*** and its close relative ***Neospora caninum***, these two protozoan parasite species exhibit different host ranges and are associated with distinct disease pathogenesis. The molecular mechanisms underlying these differences have not been well characterized. To address this, we utilized comparative genomics of the host response to these parasites in order to identify host pathways induced during ***Neospora***, but not ***Toxoplasma*** infection. Our results revealed that ***Neospora*** is a potent activator of innate immune signaling and canonical antiviral responses, whereas representative members of the three archetypal strains of ***Toxoplasma*** failed to trigger this host response. Recognition of ***Neospora*** by macrophages occurs via ***Tlr3*** and the adapter protein ***Trif***, and is conserved across multiple species and cell types. RNA isolated from ***Neospora***, but not ***Toxoplasma***, is able to induce potent antiviral responses when targeted to the host endosomal system. Surprisingly, we found that although live ***Toxoplasma*** failed to trigger type I interferon production, heat-killed parasites were potent activators of this response. Direct competition experiments between ***Toxoplasma*** and ***Neospora*** revealed that ***Toxoplasma*** potently suppresses innate immune signaling to prevent type I interferon production and that this is the dominant phenotype, suggesting that ***Toxoplasma*** acquired and retained a suppressive factor after divergence from ***Neospora***.

1496

TOXOPLASMA SUBVERTS HOST CELL IMMUNE RESPONSE VIA ASSOCIATION WITH HOST MITOCHONDRIA

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As with some bacterial pathogens, the tachyzoite stage of the intracellular parasite ***Toxoplasma gondii*** is often found specifically and extensively associated with host mitochondria at the parasitophorous vacuole membrane (PVM) (Jones and Hirsch, 1972; Sinai et al., 1997). Although previously assumed to be metabolically beneficial for the parasite, the actual consequences of host mitochondrial association (HMA) and the molecules that mediate it have not been determined. We have observed

that HMA is substantially diminished in Type II parasites relative to Types I and III. This has enabled us to use genetic analysis of F1 progeny from a cross between Type II and Type III parasites to map the parasite locus involved. Through a candidate gene approach, we have identified the specific gene involved and dubbed it ***Mitochondrial Association Factor 1 (MAF1)***. Introduction of a Type I allele of ***MAF1*** into Type II parasites is sufficient for conferring a strong HMA phenotype and this is associated with dramatic global changes in the host cell's transcriptional and induced cytokine response to parasitic infection. These results support a growing body of literature that mitochondria are a "hub" of innate immune responses. HMA may represent, therefore, an important adaptation by some strains of ***Toxoplasma*** to subvert host immunity, as well as a new mechanism by which an intracellular pathogen can interact with its host and manipulate this interaction.

1497

CHEW YOUR FOOD: "PARTIAL INGESTION" PLAYS A ROLE IN HUMAN CELL KILLING BY *ENTAMOEBA HISTOLYTICA*

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Entamoeba histolytica is the causative agent of amoebiasis, a diarrheal disease that is a major source of morbidity and mortality in the developing world. Pathogenesis is associated with profound tissue destruction, manifesting as ulceration of the intestinal epithelium or abscesses in extraintestinal sites. The cytotoxic activity of the parasite is central to tissue destruction, but the mechanism by which human cell death is induced is unknown. We sought to elucidate the mechanism by first employing live cell fluorescence video microscopy to observe host-parasite interactions in real time. Surprisingly, we found that within one minute, the amoebae internalize distinct "pieces" of the targeted human cell. These "pieces" contain target cell membrane and cytoplasm and organelles. "Partial ingestion" of the target cell precedes death, as assessed by irreversible calcium elevation and membrane permeabilization. We employed multiple independent strategies to inhibit amoebic phagocytosis in order to determine if ingestion is required for cell killing. By using Amnis Imagestream analysis to simultaneously quantify ingestion and killing, we find that the inhibition of partial ingestion prevents host cell killing. Live two-photon microscopy of amoebae with ***ex vivo*** mouse colon tissue demonstrates that the amoebae also partially ingest enterocytes, suggesting that partial ingestion is relevant to ***in vivo*** tissue destruction. Notably, we have rarely detected complete internalization of the human cells, and we find that once cells have been killed, they are not further ingested by the parasite. Therefore we speculate that complete ingestion of the killed cell may not be the "goal" and that rather partial ingestion represents an unusual, "offensive" mechanism to elicit cell killing. Thus, through these studies we have uncovered surprising host-parasite interactions and are beginning to get a clearer picture of how this parasite effects such devastating tissue destruction.

1498

A MITOCHONDRIAL CATION/PROTON ANTIPORTER IS ESSENTIAL IN BOTH LIFE STAGES OF *TRYPANOSOMA BRUCEI BRUCEI* AND THE AKINETOPLASTIC *TRYPANONSOMA BRUCEI EVANSI*

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The leucine zipper EF-hand-containing transmembrane protein (Letm1) is a ubiquitous mitochondrial (mt) protein that serves as a cation/proton antiporter across the inner membrane. It remains controversial whether the cation in question is K^+ or Ca^{2+}, as there are data supporting both scenarios. Furthermore, Letm1 is believed to anchor mitoribosomes to facilitate translation of mt genes in yeast. RNAi-silencing of Letm1 in PS and BS ***Trypanosoma brucei brucei***, indicate this protein is essential in both life stages. Its ablation results in mitochondrial swelling, consistent with a role in cation efflux from the matrix. Furthermore, mitochondrial translation is indeed compromised in PS Letm1 knockdowns. However, treatment with ionophores that can mediate K^+/H^+ exchange ameliorates these RNAi phenotypes. Letm1 is also essential in ***T.b. evansi***, where translation is non-existent, not only supporting Letm1's role in K^+/H^+ exchange but also indicating that the energy expenditure needed to maintain an active mitochondrion in the BS, which does not produce energy as in the PS, is cellular ion homeostasis.

1499

INTERACTION AND COEVOLUTION BETWEEN POLYMORPHIC IRG PROTEIN FAMILY AND *T.GONDII* VIRULENCE FACTORS

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Immunity-related GTPases (IRGs) are important cell-autonomous resistant factors in mice against ***Toxoplasma gondii*** and ***Chlamydia trachomatis***. We will report on significant polymorphism and copy number variation of IRG genes. In this study, the sequences of IRG genes were assembled from 18 strains of mouse from the Sanger Mouse Genomes Project and NIG. Further IRG genes were amplified and sequenced from genomic DNA samples of wild derived mouse strains and wild mice. The results reveal a gene family with haplotypic polymorphism apparently on the scale of the MHC in mouse populations. The ***IRG*** genes are essential for resistance against ***T. gondii*** and are the targets of virulence factors. Our experiments show an interaction between some IRG members and two polymorphic ***T. gondii*** virulence factors, the secreted kinase ROP18 and the secreted pseudokinase ROP5. Together these proteins inactivate IRG proteins by targeted phosphorylation of the switch I loop of the nucleotide binding domain. Certain ***IRG*** haplotypes may confer differential resistance of wild derived mouse strains against virulent ***T. gondii*** strains.

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