The association between malaria parasitemia, erythrocyte polymorphisms, malnutrition and anaemia in children less than 10 years in Senegal
a case control study

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HOST SELECTION, DEFENSIVE BEHAVIORS AND FEEDING SUCCESS OF CULEX QUINQUEFASCIATUS IN EXPERIMENTAL TRIALS

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Studies describing common blood sources of field collected mosquitoes are inconsistent in their description of the host selection behavior of Culex quinquefasciatus. Host selection is an important determinant of pathogen transmission, and this knowledge gap in mosquito behavior is limiting our understanding of vector-host contacts and the importance of reservoir hosts in West Nile virus (WNV) transmission. We conducted host-choice experiments under semi-natural conditions to quantitatively host feeding preference by Cx. quinquefasciatus mosquitoes when presented with an array of common passerine hosts: Northern Cardinals, American Robins, Blue Jays, Brown Thrashers, and Gray Catbirds. The experimental design consisted of: 1) a 1.5m x 0.75m x 0.75m enclosure inside of which two bird cages were placed, 2) 30 recently emerged female Cx. quinquefasciatus originating from wild eggs, and 3) an infra-red camera recording system. We performed 12 two-bird choice experiments in which we calculated the feeding index for each potential host and tested the null hypothesis of random host selection. We also quantified the number of defensive behaviors exerted by each bird. The blood sources for the 168 mosquitoes that successfully obtained a bloodmeal were assessed by amplifying a fragment of the 16s ribosomal gene using generalist avian primers, sequencing each amplified fragment, and comparing the fragment to reference sequences. Host selection differed significantly from random, exhibiting the following preference structure: American Robins preferred over Blue Jays and over Northern Cardinals, and Northern Cardinals preferred over Brown Thrashers. The most common types of defensive behaviors were those protecting the feet and head, but the number of defensive behaviors did not differ significantly between hosts. Further experiments are needed to determine the role of these defensive behaviors in host selection and feeding success by vectors. Our results indicate a non-random pattern of host selection by vectors that needs to be considered when modeling WNV transmission.

INTERACTIVE TOOLS FOR IDENTIFICATION OF MOSQUITO AND SAND FLY VECTORS OF INFECTIOUS DISEASES

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Computerized interactive tools to identify mosquito and sand fly vectors of infectious human diseases were developed for various regions of the world (see Walter Reed Biosystematics Unit/WRBU website, www.wrbu.org). Using LUCID programs, WRBU identification keys for mosquito and sand fly vectors and their associated groups included morphological diagnostic characters primarily of the head, thorax, abdomen, legs and wings. Automontage images of diagnostic characters of various insect body parts were attached to each key. Genus and species pages for selected vectors and related groups were developed, including brief basic taxonomy, distribution, bionomics, medical importance, selected references, and detailed photos of habitus and other morphological parts. World catalogs of mosquitoes and sand flies, with updated taxonomy and hierarchical classification were linked to each key. In addition, comprehensive lists of known and potential vectors, and their associated taxonomic information, were included in the WRBU website. New LUCID identification keys were recently developed, namely: African Anopheles adult and larval keys (include 140+ species and groups for adult key, 120+ for larval key); South American Culicine mosquitoes (include vector adult and larval keys of Aedes, Culex, Coquillettidua, Haemagogus, Mansonia, Psorophora, Trichprosphon); South American Phlebotomine Sand flies (include male and female keys of genera, subgenera, and vector species of Dampomyia, Evandromyia, Helocrotryia, Lutzomyia, Nyssonomyia, Pintomyia, Psathyromyia, Psychodopygus, Sciopemyia, Trichophoromyia, Verrucarum Group). Diagnostic characters, updated taxonomy and related information of new vector identification tools are noted and discussed.

SRPN2 DEPLETION REDUCES MOSQUITO FITNESS AND BITING FREQUENCY

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The mosquito’s immune system is at the vector-pathogen interface and largely determines susceptibility. One consequence of its manipulation can be the reduction in vectorial capacity. Therefore, the mosquito immune system provides potential targets for novel intervention strategies aimed to reduce vector-borne disease burden. Melanization is a powerful immune response in arthropods that leads to encapsulation and killing of invading pathogens. This process renders some mosquito species partially or completely resistant to infection with pathogens of global public health significance. One of its rate-limiting steps of melanization is the activation of prophenoloxidase (PPO), which is controlled by an extracellular proteinase cascade and serpin inhibitors. The molecular composition of this system is largely unknown in mosquitoes with the exception of Anopheles gambiae SRPN2 and CLIPB9, which constitute the first known regulatory unit that controls melanization. If uncontrolled, e.g. by the depletion of the inhibitor SRPN2, melanization can kill adult females late in life, and thus potentially reduce the vectorial capacity of An. gambiae . This feature makes PPO activation, which is a rate-limiting step in melanin production, a potential target for novel malaria control strategies. Using life table analyses, we determined the consequences of SRPN2 depletion by RNAi on several demographic growth parameters under standard laboratory settings. Net reproductive rate (Ro) was decreased by 29%, while mean generation time was unaffected. As a consequence, doubling time (Td) was moderately increased by 9%. The negative effect on net reproductive rate is largely attributable to a significant decrease in bloodfeeding propensity. Bloodfeeding propensity and survival were disproportionally reduced in older mosquitoes after the first two gonotrophic cycles. As a consequence, the number of potentially infectious bites is at least reduced by 83%. Taken together, these data suggest that SRPN2 constitutes a viable target for novel malaria intervention strategies.

SPATIAL DISTRIBUTION, SEASONALITY AND BEHAVIOR OF NOVEL MALARIA VECTORS IN THE WESTERN KENYAN HIGHLANDS

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Results from a light trap study carried out in 2010, presented previously, revealed the presence of previously unidentified mosquito species carrying Plasmodium falciparum sporozoites in Kisii district in the western Kenyan highlands, an area prone to epidemics of malaria. The majority of these specimens could not be definitively identified to the species level using the commonly used morphological keys, and sequencing revealed that there were no matching published sequences available at ribosomal ITS2 and
mitochondrial CO1 loci. To further morphologically describe these species, study their behavior and investigate their temporal and spatial distribution, mosquitoes collected during both longitudinal and transect studies were identified morphologically and sequenced at rDNA ITS2 and mtCO1 loci. To study spatial and temporal distribution of any novel vectors, monthly pyrethrum spray catches were made to sample indoor resting mosquitoes in 10 villages across Kisii Central, Kisii South and Rachuonyo South districts over an 18-month period between 2009 and 2011. To determine host-seeking activity and peak biting times, mosquitoes were collected from light traps set up indoors and outdoors over a 12-month period from June 2011. To fully describe larval and adult stages, a wide range of larval breeding sites were sampled across Rachuonyo South in November 2011 and 400 Anopheles larvae were reared to obtain adults with associated larval and pupal exuviae. For specimens found to have novel unpublished sequences, taxonomic studies were conducted to establish the identity of the species. Results on the presence of unusual species, their associated behaviors, spatial distributions and seasonality are presented.

INVESTIGATING THE ROLES OF ANOPHELES GAMBIAE G PROTEIN-COUPLED RECEPTORS IN GUSTATION
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Vector-targeted control strategies remain our most effective tools for reduction of malaria transmission and incidence. However, the threat and continuing increase in insecticide-resistance motivate discovery of novel insecticides. G protein-coupled receptors (GPCRs) are well known as one of the most “druggable” targets in many organisms. Numerous GPCRs mediate developmental, sensory or other physiological pathways that can greatly impact vectorial capacities of Anopheles gambiae and other malaria vectors. Gustatory GPCRs are central to the ability of insects to identify foods, including sugars, and detect noxious compounds in the environment. We are investigating the abilities of An. gambiae to detect various sugars and noxious compounds when given a choice between sugar meals, or between a sugar meal and a sugar/compound meal. Dye-labeling of meals enables colorimetric detection of intake. By using RNA interference to independently knockdown individual gustatory receptors, we will identify specific GPCRs required for sugar and noxious compound gustation. We have begun by analyzing sugar preferences among glucose, sucrose, fructose, galactose and mannose. Among these sugars, preliminary results imply mosquitoes prefer glucose and sucrose. Initial data suggests that mosquitoes exhibit strong aversion to berberine, a canonical noxious compound used in insect gustatory preference assays. We will report further progress in the analysis of sugar preference and noxious compound sensing in An. gambiae, and initial RNA interference results characterizing requirements for different GPCRs in sugar and noxious compound gustation. The overall goal of this project is to understand the functional roles of the mosquito gustatory GPCRs and exploit this system to enhance development of sugar-meal based, vector-targeted interventions that will decrease vectorial capacity of An. gambiae and other malaria vectors.

SCHISTOSOMIASIS AND SOIL TRANSMITTED HELMINTHIASIS IN KAÉDI, SOUTHERN MAURITANIA
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We report findings of a cross-sectional parasitological study on schistosomiasis and soil transmitted helminthiasis among school aged children since the establishment of irrigation schemes in Kaédi, located at the confluence of the Gorgol and Senegal Rivers in Southern Mauritania. Stool and urine samples were obtained from 246 children between the ages of 5 to 15 years recruited from randomly selected households followed by administration of a parental questionnaire. Urine samples were analysed the same day using the centrifugation method, while stool samples were collected early the next morning and analyzed by the Kato-Katz technique. We found a low prevalence of Schistosoma haematobium (3.7 %) with a mean infection intensity of 38 eggs per 10 ml of urine, while no cases of S. mansoni were detected in all participants. Only one participant was infected with a soil transmitted helminth (A. lumbricoides). Working in the rice paddies during the annual flood recession (Oualo farming) was the strongest predictor of infection intensity among participants when we fitted a zero-inflated negative binomial (ZINB) model for egg counts with potential confounding factors controlled for in multivariate analyses.Prevalence of S. haematobium seems to have decreased compared to last estimates before the establishment of the irrigation schemes (14 %). These findings highlight a need for an integrated surveillance system aimed at transmission control targeting various aspects of the transmission cycle S. haematobium.

OUTBREAK CLUSTERS OF FASCIOLA HEPATICA IN ARGENTINA
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Fasciola hepatica is a trematode responsible for the human disease fascioliasis. It differs from other human liver flukes in that it is present worldwide. Fascioliasis is transmitted through the ingestion of the metacercaria stage parasite found in infected vegetables such as watercress. Although it generally presents as chronic indolent individual infections, outbreaks of acute fascioliasis can occur in endemic areas related to ingestion of a contaminated food item. Despite the high prevalence of F. hepatica in many areas, its importance has been largely neglected. We investigated clusters of F. hepatica which occurred in four family groups in rural Argentina. A total of 34 confirmed cases of acute fasciolasis from 4 different families (15, 6, 8, and 5 affected members) were investigated. Three of the four families were residents of endemic areas, while a fourth family vacationed in an endemic area. All members from the 4 families reported ingestion of watercress. The most common clinical symptoms were right upper quadrant pain and fever, present in all subjects. All cases had elevated liver transaminase tests as well as absolute eosinophilia. Fasciola ova were detected in stool samples in all 34 subjects. Serology was performed in 16 subjects and was positive in 13 (81%). Abdominal ultrasound (performed in 22 subjects) showed diffuse hepatomegaly in 19 cases (86%). Twenty patients were treated with intravenous emetin and 14 patients with triclabendazole. Of 29 subjects who presented for post-treatment stool ova and parasite examination at 60-days, 26 (90%) became negative, with the remaining 3 patients becoming negative by 90-days post-treatment. In the triclabendazole group, 2 subjects had relapse with one subject needing repeat treatment and another subject needing two additional treatments. In the emetin group one patient needed repeat treatment, however three individuals developed hypotension as a treatment complication requiring hospitalization. F. hepatica remains an under-recognized infection. In fact, most subjects reported here did not seek medical care since they had only mild clinical symptoms and were only detected through the outbreak investigation. There is a need for increased education and awareness of fascioliasis in patients living and traveling to endemic areas.
DEVELOPMENT OF A RECOMBINANT PROTEIN VACCINE AGAINST SCHISTOSOMA MANSONI INFECTION USING CATHEPSIN B AND PEROXIREDOXIN 1 ANTIGEN

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Schistosomiasis is a fresh-water-borne parasitic disease caused by trematode worms of the genus Schistosoma. Due to its morbidity and mortality, Schistosomiasis is the most important helminth infection. The pathology of the disease is due to egg deposition, by the female worm, which will trigger an immune reaction and consequently cause progressive damage to the organs. The lack of therapeutic drugs and preventative measures, as well as the high disease burden caused by the infection are justifications for developing a vaccine against schistosomiasis. The development of a recombinant protein vaccine against this parasitic disease has the potential to contribute a long-lasting decrease in disease spectrum and transmission. Furthermore, it would relieve some of the concern surrounding the potential emerging resistance to praziquantel; the drug which is solely being used to treat the infection. Our group has chosen to focus on the S. mansoni antigens Cathepsin B and Peroxiredoxin 1 (Px1) as vaccine candidates. It is hypothesized that immunization with either recombinant Cathepsin B or recombinant Px1 in the presence of an adjuvant can elicit protective immunity against Schistosoma infection. The objective of this research project is to develop a safe recombinant protein vaccine against schistosomiasis that will stimulate an optimal immune response which will prevent pathology. Upon cloning, expressing, and purifying the proteins of interest, mice were initially immunized with recombinant Cathepsin B in the presence of either synthetic oligodeoxynucleotides (ODN) containing unmethylated CpG dinucleotides or Montanide ISA 720 VG. The mice received two booster injections following the first immunization. The vaccine formulations were not toxic, and all of the mice survived until the end of the study. The vaccine elicited a pronounced production of S. mansoni Cathepsin B specific antibodies whereas no antigen-specific antibodies were found in the control animals. Splenocytes proliferated in response to Cathepsin B and produced elevated levels of Th1, Th17, and inflammatory cytokines. These results highlight the potential of S. mansoni Cathepsin B as a promising vaccine candidate for schistosomiasis. The investigation concerning Px1 is ongoing.

PROJET-CREVETTE: AN INTERNATIONAL COLLABORATION TO REDUCE PARASITIC DISEASE, RESTORE THE ENVIRONMENT AND IMPROVE LIVELIHOODS IN WEST AFRICA THROUGH AQUACULTURE

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“Projet-Crevette” is a collaborative research project to investigate novel ways to combat the spread of schistosomiasis in rural Africa. In 1986, the Diama Dam was built on the Senegal River in West Africa in order to stabilize river flow, reduce drought conditions, and support a growing agriculture industry. Within 5 years after dam construction, schistosomiasis spread rapidly, leading to an epidemic that has persisted until today, with > 90% prevalence among some rural villages along the river. River prawns, which at one time were voracious predators of snails, have recently been decimated in the Senegal River due to habitat loss above the Diama Dam. Thus, Projet-Crevette aims to develop an aquaculture program to supplement prawn reproduction and re-introduce native river prawns to the Senegal River basin as predators of snails that carry schistosome parasites. Projet-Crevette has monitored the distribution and abundance of prawns, snails, and schistosome parasites at 11 sites throughout the Senegal River Basin over the course of one full year during 2011. This baseline data will pave the way for development of an innovative parasite-control strategy that promises to simultaneously combat disease transmission, restore the environment, and improve livelihoods by restoration of an artisanal prawn fishery.

IN VITRO, HUMAN EOSINOPHILS DOWN MODULATE PERIPHERAL BLOOD MONONUCLEAR CELLS RESPONSES TO SCHISTOSOMA MANSONI ADULT WORM ANTIGENS

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Eosinophils have been regarded as terminally differentiated non-replicating effector cells observed in a number of health disorders including parasitic infections and allergic diseases where they play a beneficial role in the host defence against helminth infections or cause a harmful inflammatory response respectively. In schistosomiasis eosinophils have been associated with direct or indirect killing of schistosomula. However, there is growing evidence that eosinophils can play an additional immunoregulatory role in both adaptive and innate immunity to parasitic infections. Here we report results of a study, using samples from Schistosoma mansoni infected individuals, in which we investigated the effects of co-culturing human eosinophils with peripheral blood mononuclear cells (PBMC)
on in vitro cytokine production in response to S. mansoni adult worm antigen. PBMCs obtained from 26 S. mansoni infected adults were examined for cytokine responses to S. mansoni adult worm antigen (SWA) when stimulated alone or when enriched with autologous eosinophils. Production of IL-4, IL-5 and IL-13 was lower (p = 0.017, 0.018 and < 0.001 respectively) in PBMC+eosinophil cultures than in PBMC-only cultures stimulated with SWA. IL-13, IL-10, IFN and TNF were released in eosinophil-only cultures but none of these cytokines produced by the eosinophils showed a significant association with the observed eosinophil-induced drop in cytokine responses of PBMCs. This preliminary study shows that eosinophils can exert a down-modulatory effect on schistosome specific responses. The mechanism of this immune-modulation remains to be elucidated.

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**Schistosoma Haematobium Recombinant Proteins as a Vaccine Candidate for Human Schistosomiasis**

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In Sub Saharan Africa human schistosomiasis is largely caused by *Schistosoma mansoni* and *S. haematobium*. The current strategy for controlling morbidity of the disease is through mass drug administration using praziquantel, the drug of choice. This dependency of using only one drug can possibly induce praziquantel resistance of the parasite and could render this method of intervention ineffective. This has therefore necessitated the urgency for the development of a vaccine to combat the disease. With the focus of most schistosomiasis vaccine developments on *S. mansoni* parasite, it could be challenging for identified putative vaccine candidates to elicit the required immunological responses in *S. haematobium*-endemic communities in African populations, where the disease is caused by either *S. haematobium* or in regions of co endemicity. If this condition arises, it could lead to the detriment of full potential of *S. mansoni* vaccine candidates. It will therefore be complementary for vaccine design efforts to strive into proteomic and immunology of *S. haematobium* counterpart as well. Here we discovered 17 orthologs hits (5 Tetraspanin proteins, 5 CD59-like proteins, 2 MEG-8 proteins, 2 Saponin proteins, 1 FOG precursor, 1 Stomatin-related protein) from *S. mansoni* sequences. This bio-informatic lead analysis was performed using tBLASTn search of Wellcome Sanger S. haematobium ESTs libraries against 52 *S. mansoni* vaccine candidates (with e-value 3.90e–30–3.00e–110 and identity value 56%–93%). These 17 orthologs were characterised by non-allergen, had no human orthologs and was expressed during vertebrate infections (definitive host). If such orthologs are identified from *H. mansoni*, it will not only mark a crucial milestone in terms of vaccine development for disease but will drastically facilitate the reduction of schistosomiasis in Africa.
Markov Model profiles. We were able to identify around 60 HMEs with some variation within the three Schistosoma species. From the identified enzymes, 24 were tested individually as therapeutic targets using RNA interference in cultured larval stages (schistosomula) to invalidate each corresponding gene. Although, gene knockdown of up to 90% could be achieved, no phenotype could be observed after 7 days of dsRNA exposure. Loss of motility could be observed as a phenotype for two HDOMs after 30 days of dsRNA exposure. In addition, in order to assess the role of genes in the presence of the host environment under immunological pressure, knockdown parasites for four HMEs (HDAC8, KDM1/KDM2 and PRMT3) were tested in vivo. A significant reduction of worm burden (50%) could be observed in mice infected with knockdown parasites for HDAC8 when compared to unspecific control. Finally, egg count was significantly reduced in mice livers for all tested HMEs. In conclusion, our work improved the functional annotation of over 20% of S. mansoni HAT and HDAC proteins. Parasites with reduced levels of HDAC8, KDM1/KDM2 and PRMT3, seem to diminish the oviposition and ability to survive (for HDAC8) in the host milieu, indicating that these enzymes could be good target candidates for drug development.

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REDOX BIOLOGY AND DRUG DEVELOPMENT FOR SCHISTOSOMIASIS

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Schistosomiasis remains an important neglected disease with 200 million infected individuals. Individual treatment and large-scale control campaigns rely primarily on the use of praziquantel, the only available drug for schistosomiasis treatment. There is concern that praziquantel resistance will evolve and, in the absence of alternative therapies, control measures will be imperiled. Enzymes in the redox pathways of schistosomes have been found to be suitable targets for schistosomiasis drug development and schistosome antioxidant enzymes have been shown to be essential and druggable proteins. Of particular interest is thioredoxin glutathione reductase (TGR), which plays a central role as a multifunctional protein entirely providing the activity of several distinct enzymes present in the human redox network. Therefore, TGR is a redox bottleneck in schistosomes. Oxadiazole-2-oxides have been identified as TGR inhibitors, acting through both nitric oxide production resulting from TGR activity and TGR inhibition. We will present results defining the role of nitric oxide in the action of oxadiazole-2-oxides and other nitrosating agents both in the local context of TGR S-nitrosylation and global context of other schistosome proteins susceptible to modification by nitric oxide. In addition, a rescreen of the NIH Chemical Genomics Center compound library has identified many new classes of small-molecule TGR inhibitors. Mechanisms of action, activities against ex vivo parasites, and structure-activity relationships of these compounds will be discussed.

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SIGNIFICANCE OF THE SO-CALLED “APO-AMEBOCYTE PRODUCING ORGAN” IN BIOMPHALARIA GLABRATA

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Internal defense against microorganisms are performed in mollusks by a single cell type: the hemocyte or amebocyte. Their place of origin in Biomphalaria glabrata has nowadays become a matter of controversy. Initially, the hypothesis maintained by several authors was that the amebocytes had a multicentric origin. However, more recently it has been postulated that B. glabrata amebocytes are instead formed within a central special organ. The initial argument for the Amebocyte Producing Organ (APO) being considered as the locale of origin for hemocyte production in B. glabrata was the finding of hyperplasia and mitoses in its cells during the course of Schistosoma mansoni infection. The present investigation was concerned with a morphological analysis, with histological, immuno-histochemical, morphometrical, and ultra-structural findings, from the so-called B. glabrata APO. Its structure was identified as a collection of epithelial basophilic cells, disposed on one-cell-thick layer or in small round collections, covering a small area of the pericardial surface in the reno-pericardial region. Sometime it vaguely resembled the epithelial component of the vertebrate juxta-glomerular apparatus of the kidney. During our studies, mitoses were only occasionally found, either in normal or infected mollusks. Also our quantitative studies failed to demonstrate the presence of APO cellular hyperplasia, either in normal or schistosome-infected B. glabrata.
ASSESSING THE HEALTH IMPACT OF IMPROVED RURAL SANITATION: DESIGNING AND CARRYING OUT A CLUSTER-RANDOMIZED, CONTROLLED TRIAL IN ORISSA, INDIA

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Despite progress on other MDG targets, sanitation coverage continues to fall behind with 2.6 billion people still lacking access to even basic sanitation. More than one billion people still practice open defecation, including an estimated 636 million in India alone. One possible reason for the slow progress in sanitation is the lack of clear, compelling evidence about the effectiveness and cost-effectiveness of sanitation. To date, there is no randomized controlled trial of sanitation interventions to prevent diarrhoea diseases. We describe the design and execution of a large-scale study that seeks to help close the evidence gap on rural sanitation in low-income settings. Using a cluster-randomized trial design, the study aims to assess the effectiveness of a project by Water Aid India to promote the construction and use of individual household latrines in accordance with the Indian Total Sanitation Campaign. The study population consists of 100 villages (about 12,000 people) in a costal district of Orissa, India. The main objective of the trial is to assess whether improved sanitation reduces diarrhoeal and helminth infection among young children. The presentation will focus on five additional aspects that we believe necessary in designing evaluations of sanitation interventions: (a) comprehensive process evaluation, carefully documenting the manner in which the intervention is actually implemented rather than intended or reported by the program implementers; (b) documenting uptake, the actual use of the intervention by the target population, since there is widespread evidence that latrine use is sub-optimal in India; (c) assessing whether the intervention has actually reduced exposure, a condition to securing health outcomes; (d) spatial analysis and spill-over effects from sanitation interventions, and (d) longer-term assessments due to (i) the longer time required to implement the intervention, (ii) the potential persistence of excreta-related pathogens in the environment even after the widespread uptake of an effective sanitation intervention, (iii) the need to investigate longer-term changes in uptake, and (iv) the need to follow whether safe and effective pit-emptying is underway.

STUDIES ON THE PRESENCE OF CRYPTOSPORIDIUM SP AROUND WATER TREATMENT PLANTS THAT SUPPLY WATER TO GREATER ACCRA REGION OF GHANA

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Cryptosporidium sp a Protozoan parasite. These parasites of the Apicomplexan family were found in association with diarrhea in calves and are water-borne. The organism is second only to rotavirus as a causative agent of diarrhea in newborn calves and infants. As such, it is a potentially serious contaminant in water where cattle graze. In order to estimate the human health risk in cattle rearing areas around water treatment plants, we measured the prevalence of Cryptosporidium oocysts in the fecal matter from four cattle ranges upstream in Joma near the Densu Dam at Weija in the Ga South Municipality and Kpong in the Lower Manya District all of Southern Ghana. The Modified Ziehl-Neelsen staining technique (MZN) for Cryptosporidium oocysts was used. Of the 320 fecal samples for each species screened, 63 (19.7%) were positive for Cryptosporidium. Prevalence was higher in calves younger than three months of age, as compared to weaned calves and adults. Oocysts were detected in both diarrheic and non-diarrheic samples, with a significantly higher prevalence (p< 0.05) of oocysts shedding in diarrheic samples.

LACK OF EDUCATION OF HOUSEWIVES CONCERNING THE TRANSMISSION RISKS FACTORS OF TYPHOID FEVER IN THE DR CONGO

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DRC is still characterized by a critical socioeconomic situation impacting negatively the quality of health services offered to the population, creating a permanent problem of hygiene and prevention of diseases. Every year the Country faces various epidemic outbreaks of avoidable diseases such as typhoid fever (TF). In addition, more than 50% of Congolese women are illiterate, whilst it is established that the mother’s education level is the most determining factor for the family’s health and nutritional status. A proper washing of the hands especially before preparation of food, before the meal, after the toilet, access to healthy source of water; safe elimination of human excreta, are the key factors to help reduce the frequency of diseases with oro-faecal transmission. To assess this assertion, we conducted a Community based survey from June 1st, to August 31st, 2008 to assess the knowledge and practices relative to the prevention of TF transmission by questioning 500 domestic women of the City of Bunia in the northeast of the DRC. The study used a randomization method for the selection of the Housewives by Quarter of the City of Bunia (40% subset of the Quarter population) Of the 500 women interviewed: 288 (56.7%) were 20-24 years old; 325 (65%) unemposed, 261 (52.2%) have not attended school or only primary school; only 198 (39%) have some sufficient knowledge of the transmission of TF; 267 (53.40%) have little or no knowledge of the good qualities of drinking water; 265 (53.00%) are unaware of the rules of food hygiene; only 137 (27.00%) practice correctly the washing of the hands; all households use unsafe sources of drinking water and 380 (78.8%) among them do not treat the drinking water (boil or treat with Chloramines); 202 (42.60%) of women use non hygienic latrines and 85 (17%) of households do not have latrines at all. The promotion of hygiene and specifically education of housewives remain fundamental in the improvement of national educational strategies in the DRC. This finding might be relevant to all Stakeholders involved in the fight against TF.

AN UNUSUAL PARTNERSHIP TO ENSURE SAFE DRINKING WATER TO THE RURAL POPULATION IN INDIA

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Water treatment at the point-of-use (POU) can reduce diarrhea caused by waterborne pathogens by 30 to 50% (WHO 2007). The goal of the project was to demonstrate a comprehensive strategy aimed at increasing use of POU water treatment methods among poor rural and urban populations and thereby reduce childhood diarrhoea in the state of UP in India. The at-scale goal was to achieve 30% rural and 40% urban use of an effective POU method. By January of 2009, the partnership between POU manufacturers and NGO partners was formalized through MOUs. The project reached 674,064 households residing in 1120 urban slum areas and 1350 rural villages in UP. A quantitative study of 1400 households at baseline, showed only 2.5% of households (4.1% urban and 1.1% rural) reported ever using a POU method promoted by the project (boiling, disinfection products, or filtration). In contrast, the outcome evaluation found very high rates of POU use in both the intervention and comparison areas, with 96.8% of intervention households reporting they had ever used a recommended POU method, along with 71.0% of households in the comparison areas. The biggest difference between intervention and comparison districts was in the use of chlorine liquid for
disinfection (56.9 % versus 0.3 %). No difference was found in the use of water filters (about 7 % in both areas). Among urban households, 50 % reported current use of chlorine tablets, vs. 3 % of rural households. Conversely, 60 % of rural households reported current use of liquid chlorine, vs. 11 % of urban households. This clear preference for different products cannot be explained by any difference in intervention approach, and bears further investigation. An engaged commercial sector was able to reach a substantial new market by partnering with NGOs and micro-finance institutions. NGOs can be trained to become effective product demonstrators and micro-distributors. The long-term viability of NGO POU product distribution should be monitored. Commercial partners are now expanding the model in other states in India.

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DO THE CHILDREN GETTING WHAT DO THEY NEED TO WASH HANDS IN SCHOOL? EXPERIENCE FROM BANGLADESH

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Schools are common sites for the spread of gastro-intestinal and respiratory diseases. There are a variety of hygiene interventions linked to hand washing, respiratory hygiene, sanitation and water quality, which have shown some success in preventing and /or reducing these diseases. However, little research has been done on the feasibility and effectiveness of these interventions. The objective of this study was to understand the current practice regarding hand washing and facilities needed to wash hands from primary school children in a low income country like Bangladesh. The study used data from in-depth interviews and observations conducted with purposively selected school children in Bangladesh. The interviews were conducted with a topic guide line developed based on existing literature and in consultation with study investigators. Transcripts were processed using a thematic analysis approach. Major findings indicated that increasing hand washing in low resource setting is a complex process, it included that after giving knowledge, knowledge increased but lack of hand washing facilities in school premises influence the practice of hand washing in school. A greater number of informants stated that availability of resources like soap and water supply is important to keep the practice of hand washing in school. Most of the schools do not have fund and capacity to supply soap and water. Children are motivated to wash hands due to school hygiene program but cannot practice their knowledge as schools are not able to supply those facilities. School Authority suggested for better communication with government before implementing intervention so that government can help to generate fund to continue the program.

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PRELIMINARY ASSESSMENT OF THE POTENTIAL EFFECTIVENESS OF WATER FILTERS TO REDUCE DIARRHEAL DISEASE BURDEN IN CHILDREN YOUNGER THAN FIVE YEARS OLD IN A PACIFIC ISLAND NATION

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A novel household water treatment device was proposed for use in the Kiribati, a small, remote pacific island nation. In collaboration with the Kiribati Ministry of Health and the WHO (South Pacific) we conducted a field study to understand the extent to which water-borne diarrheal disease is an important health issue, and whether a domestic water filter used in this setting is likely to reduce the incidence of diarrhea. As far as we are aware, this is the first investigation of its kind in any pacific island nation. In this field study of 97 randomly selected households of 802 individuals we found that 7% of participants, and 25% of children under 5 years old had experienced diarrhea in the past month and 7% of children under 5 had experienced diarrhea in the past week. Participants reported high levels of open defecation (59% children) combined with low knowledge of the danger of children’s feces and low levels of handwashing, especially after defecation and the handling of children’s feces. It is highly likely that contamination from hands and flies goes on to contaminate food and individuals directly leading to high levels of ‘water-washed’ (rather than water-borne) endemic diarrhea. Most individuals we interviewed (86%) reported that their household normally boils their water for drinking. Water samples were highly contaminated, and there was not a statistically significant difference in fecal coliforms between source water and drinking water. In households that boiled their drinking water, it was less contaminated than the source water in only half the samples, suggesting that significant recontamination occurs following boiling, this would likely happen following filtration. Almost all (91% of households) store drinking water and only 24% use safe storage containers, while the remainder access drinking water by dipping dirty cups, vessels, and hands into the water container. Information on behaviour and water quality indicates that the transmission of endemic diarrhoea is likely to be through many pathways other than drinking water, and even treated water is highly susceptible to recontamination. Filtration as a form of household water treatment is likely to have limited effect in this setting.

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DEVELOPING A SOCIAL ECOLOGICAL MODEL FOR VIBRIO CHOLERAE TRANSMISSION DYNAMICS IN HAITI: IMPLICATIONS FOR CONTROL STRATEGIES AND PUBLIC POLICY INTERVENTIONS

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An evaluation of Catholic Relief Service’s (CRS) post-earthquake cholera education programming in Haiti was conducted in June 2011 to evaluate the efficacy of their social marketing efforts for cholera prevention. A Knowledge, Attitude, and Practice (KAP) survey implemented throughout Haiti provided cholera incidence data as well as social and behavioral data that indicate sources of disease transmission and water contamination. Evaluation results indicate that there remain gaps in practices such as hand washing and open defecation. These results highlight the importance of monitoring of incidence data and surveillance in countries where poor infrastructure and a lack of proper sanitation facilities necessitate changes in routine behavior to prevent outbreak. Monitoring and prevention, coupled with mathematical compartmental models of transmission dynamics, would enable prediction of future cholera outbreak risk per commune and also would enable the Haitian Ministry of Health, HSPR, and the population to take preventative measures well in advance. Thus, a novel SIR-type social ecological model for Vibrio cholerae transmission dynamics in resource-poor settings has developed, incorporating behavioral data from the KAP evaluation. Furthermore, the behavioral and ecological factors that have been integrated into the base model ensure greater predictive ability at the commune level when the model is back-fitted to prior incidence data. Such models hold the key to affecting control strategies and public policy interventions in ways that ensure a given population is prepared for a potential outbreak when conditions are ideal.
CHOLERA OUTBREAKS IN URBAN BANGLADESH IN 2011

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In February 2011, an outbreak of severe diarrhea was reported at a tertiary medical college hospital campus in Bogra District in northwest Bangladesh. In April 2011, a similar outbreak was reported at 3 urban communities in the northeastern district of Kishorganj. We investigated these outbreaks to determine the etiology and pathways of transmission. We visited the tertiary hospital in Bogra and the secondary hospital serving the affected communities in Kishorganj. We listed the admitted cases of severe diarrhea (passage of ≥ 3 loose stools per day) from the affected areas. We interviewed the admitted cases, physically examined them and collected rectal swabs in bacterial transport media to test for enteric pathogens including Vibrio cholerae. We visited the affected communities to explore the water supply and sanitation. We collected water samples from selected cases’ household taps, tube wells and central pumping stations to test for microbes including Vibrio. We identified 21 cases from Bogra and 84 cases from Kishorganj. The median age was 23 years in Bogra and 21 years in Kishorganj. There were no reported deaths. We isolated Vibrio in 29% (5/17) of the rectal swab samples from Bogra and in 40% (8/20) of the rectal swab samples from Kishorganj. We found Vibrio in 1 out of 8 tap water samples from Bogra and both the tap water samples from Kishorganj. We did not find Vibrio in the water samples from central pumps or tube wells. Ground water extracted from deep tube wells was supplied intermittently through interconnected pipes without treatment in both outbreak areas. We found visible leakages in pipelines in Bogra. Though we found no visible leakages, but pipes passed through open sewers in Kishorganj. The rapid onset of severe watery diarrhea in adults and isolation of cholera organisms from their rectal swabs confirmed that the outbreaks were caused by Vibrio cholerae. The detection of Vibrio in the tap water samples but not from central pumps or tube wells, suggested water contamination in the pipelines. Safe water provision is difficult in municipalities where water supply is intermittent, and where pipes commonly leak; and requires actions outside of the health sector. Collaborative research exploring effectiveness of water purification strategies, including chlorination in areas with intermittent purification, may identify appropriate approaches for ensuring safe water until improvement of the water and sanitary infrastructure.

ACCEPTABILITY, FEASIBILITY AND SUSTAINABILITY OF DUAL PIT LATRINES FOR RURAL HOUSEHOLDS IN BANGLADESH

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In Bangladesh, single pit pour-flush latrines that separate human feces away from a child’s environment are commonly installed. But latrine water seals are frequently broken by owners to reduce the volume of water used, thus extending the time for pits to fill. They also break the pit liner and allow latrine contents to overflow. Up to 70% of rural households have visible feces on or around latrines. Latrines with two pits, one for current use and a second for subsequent use when the first is filled and composting, may reduce fecal exposure. We investigated an on-going dual pit latrine construction project to identify the acceptability, feasibility, sustainability, perceived benefits and barriers among impoverished rural households. From October 2010 to April 2011 we enrolled households in a communities where BRAC, a non-governmental organization, implemented a project in 2007 providing dual pit latrines for households that met the Government of Bangladesh definition of ‘hardcore poor’ who shared the cost of transportation and labor. We conducted group discussions (5), interviewed household members (15) and observed their latrine use at four key stages: immediately after installation, upon switching to the second pit when the first was full, during decomposition of the first pit contents, and during emptying and disposing of the first pit contents. None of the households reported latrine overflow or breaking the pit liner or water seals and we observed no visible feces on or around latrines. Participants perceived the main benefit of using the dual pit latrine was when the first pit became full; households immediately started using the back-up pit without having to empty the fresh feces. After approximately one year, households could empty the decomposed pit contents themselves. This saved money and the decomposed excreta of the first pit could be used as manure. Disgust when switching pits was described as a barrier, but did not discourage switching. 8 households successfully completed pit switching at least once. Subsidized dual pit latrines were acceptable to impoverished households in rural Bangladesh and provided safe and effective separation of feces from the environment. The dual pit latrine should be evaluated among other groups on a larger scale.

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emphasized that the encouragement provided by the CHPs motivated them to use NaDCC tablets in spite of their initial reaction to stored treated water, their heavy workload, and the reluctance of males to drink treated water. Despite initial concerns with smell, taste and temperature, the majority of study participants continuously treated their water for 13 months and at least one month after active promotion ended. Combining access to effective, easy-to-use water treatment technology with trained, confident and knowledgeable local community health promoters can help improve the uptake of POU water treatment.

THE NEED FOR POINT OF USE WATER TREATMENTS IN AREAS OF PERI-URBAN POVERTY: CASE STUDY OUTSIDE IQUITOS, PERU

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This study aimed to determine the water collection and home treatment methods in Belen, Iquitos, Peru to elucidate the cause of the high incidence of gastrointestinal diseases in this neighborhood. The results of this study emphasize the importance of point of use water treatment in the home. Belen is a sector of urban poverty on the outskirts of Iquitos, capital of the Loreto region of Peru, where people suffer from gastrointestinal diseases at higher rates than the rest of the city. While many previous studies have highlighted the prevalence of several specific pathogens in this neighborhood, there is little information regarding water-collection methods and treatment in this region. In July 2011, 50 households located in Belen were surveyed using stratified random sampling. Surveys were administered to the head of household in Spanish. In each house, a water sample was collected from the primary drinking water source in a sterile cup with an airtight screw cap then transported to the Universidad Nacional de Amazonica Peruana microbiology lab for fecal coliform (FC) testing. The American Public Health Association guidelines were used for FC testing. The overall rate of contamination was 11.1%. Most of the water samples (92.5%) that were negative for FC were untreated in the home, suggesting that treatment methods used by the local water provider are sufficient at the point of treatment. The positive FC sample results most likely represent contamination during the time of storage or use in the home. No sample that had been treated at home had a positive FC test. Therefore, it will be critical to emphasize to residents of Belen the importance of home water treatment before consuming water. It is possible that water is contaminated during storage, so residents should be urged to keep their water storage containers disinfected as well. Iquitos boasts a water plant with treated water, but the amount of chlorine may not be adequate to cover contamination en route or in the home. Although a region may have access to treated water, residents should continue to practice point of use treatment to ensure the safety of their drinking water. Point of use contamination could be a substantial source for fecal contamination and therefore point of use treatment should be encouraged in the homes of communities of peri-urban poverty similar to Belen.

MEASURING CONTAMINATION OF CHILDREN’S TOYS TO EVALUATE HOUSEHOLD SANITATION IMPROVEMENTS IN RURAL BANGLADESH

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The impact of modest improvements in sanitation facilities and practices on community health are unknown. As one step to better understand the potential contribution of such modest improvements, we evaluated whether different levels of sanitation are associated with environmental contamination, as indicated by fecal contamination of children’s toys, in rural Bangladesh. We assigned 100 households to the “clean” category if they had an improved latrine and no visible human feces in the living or adjacent space, or to the “less clean” category if they had an unimproved latrine and visible human feces in living or adjacent space. We distributed two non-porous toy balls to each household, washed each toy in 200 ml of Ringer’s solution 3-4 days later, and repeated the process with two new toys. We enumerated fecal coliforms and fecal streptococci in the wash fluid from each toy following standard procedures. Toys from 39 clean households had lower average fecal coliform contamination than toys from 61 less clean households (mean of log10 transformed values 2.4 versus 3.2, p= 0.03). Fecal streptococci contamination was not significantly different between clean and less clean households (mean of log10 transformed values 4.7 versus 4.8, p = 0.37). There was substantial variability in fecal coliform contamination of two toys in the household at the same time (Coefficient of Variation (CV)=36.5), and toys in the household at two different times (CV=37.6). In rural Bangladesh improved sanitation structures and practices were associated with less environmental contamination. Whether this level of difference in environmental contamination improves child health merits further study. The level of variation of this measure was typical for measures of environmental contamination, such as measures of water quality. Sentinel toy contamination may be a useful objective measure to assess the ability of sanitation interventions to reduce fecal contamination.

ETHNOGRAPHIC AND DIARRHEA PREVALENCE RESULTING FROM COMMUNITY BASED WATER TREATMENT SYSTEMS: A COMPARISON BETWEEN FINDING IN UGANDA AND HONDURAS

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Using a combination of ethnographic methods, healthcare facility chart reviews, and individual waterborne parasite tests, this paper presents the results of a three year investigation comparing the health impacts of providing water treatment systems for communities in Uganda versus Honduras. The Honduras project provided treated water and flush toilets for an approximated 340,000 people. Improvements in health were documented in Honduras by ethnographic findings, parasite surveys, and medical chart reviews, and were confirmed by local public health officials. In Uganda, no such impact was documented despite provision of access to treated water meeting US, EPA standards and the universal knowledge of waterborne illnesses and their causes within the six communities studied. Ethnographic data and subsequent KAP survey data confirmed accurate local understandings of water and health issues as well as significant gaps in the water safety behavior. A total of 19,420 patient interactions were searched for possible waterborne illnesses within both test and control communities and showed no significant differences in rates of diarrhea and/or dysentery. Random selection of subjects for parasite surveys by
rapid stool tests also showed no significant differences between test and control communities. Likely causes of these findings will be discussed including the probability that exposure to contaminated water in Lake Victoria, animal feces, and open-air food sources contribute to ongoing disease loads within the test communities. Methods developed and implemented for this study represent a significant advance over commonly used survey techniques.

### CLINICAL TOLERABILITY OF ARTESUNATE-AMODIAQUINE VS. COMPARATOR TREATMENTS FOR UNCOMPLICATED FALCIPARUM MALARIA IN SUB-SAHARAN AFRICA, AN INDIVIDUAL PATIENT DATA ANALYSIS

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The wide-spread use of artesunate-amodiaquine (ASAQ) for treating uncomplicated malaria makes it important to gather and analyse information on its tolerability. An individual-patient tolerability analysis was conducted using data from eight randomized controlled clinical trials conducted at 17 sites in nine sub-Saharan countries comparing ASAQ to other antimalarial treatments. All patients who received at least one dose of the study drug were included in the analysis. Differences in adverse event (AE) and treatment emergent adverse event (TEAE - AE which were absent pre-treatment or worsened with treatment) were analysed by Day 28. A total of 6,179 patients were enrolled (74% < 5 years of age), of whom 50% (n=3,113) received ASAQ, 20% (n=1,217) another ACT, and 30% (n=1,849) a non-ACT (combination or single-agent) treatment. Overall, 8,542 AEs and 3,943 TEAEs were recorded. The proportion of patients experiencing at least one gastro-intestinal AE on ASAQ was 43% (higher than with artemether-lumefantrine and dihydroartemisinin-piperaquine at two sites only), and was 23% for any other AEs (not different from other treatments). Specifically, the risk of another AE on ASAQ was 43% (higher than with artemether-lumefantrine and dihydroartemisinin-piperaquine) which was absent pre-treatment or worsened with treatment. AEs were significantly related to the pre-treatment prevalence of the symptom (p=0.001, Fischer test); AEs overestimated TEAEs by a factor ranging from none to 5-fold. The overall incidence of serious AEs (SAEs) with ASAQ was nine per thousand (29/3,113) and a mortality of one per thousand (three deaths, none drug-related) and similar to other treatments. ASAQ was comparatively well-tolerated. Safety information is important, and must be collected and analysed in a standardised way.

### TOWARDS RATIONAL USE OF ANTIBIOTICS FOR SUSPECTED SECONDARY INFECTIONS IN BURULI ULCER PATIENTS

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The emerging neglected disease Buruli ulcer is treated with streptomycin and rifampicin and surgery if necessary. Frequently other antibiotics are used during treatment. Information on prescribing behavior of antibiotics for suspected secondary infections and for prophylactic use was collected together with cultures from ulcers. Of 185 patients that started treatment for Buruli ulcer in different centers in Ghana and Bénin 51 were admitted. Forty of these 51 admitted patients (78%) received at least one course of antibiotics other than streptomycin and rifampicin during their admission. The median number (IQR) of antibiotic courses for admitted patients was 2 (1, 5). Only twelve patients received antibiotics for a suspected secondary infection, all other courses were prescribed for use as prophylaxis during 10 days on average after excision, debridement or skin grafting. Antibiotic regimens varied enormously per indication. Cultures from superficial swabs showed the expected bacteria from a chronic wound, but 13 of the 34 (38%) S. aureus showed to be MRSA. A guide for rational antibiotic treatment for suspected secondary infections or prophylaxis is needed. Adherence to the proposed guideline may reduce and tailor on antibiotic use other than streptomycin and rifampicin in Buruli ulcer patients. It may save costs, reduce toxicity and limit development of further antimicrobial resistance. This topic should be included in general protocols on the management of Buruli ulcer.

### THE ASSOCIATION BETWEEN MALARIA PARASITEMIA, ERYTHROCYTE POLYMORPHISMS, MALNUTRITION AND ANAEMIA IN CHILDREN LESS THAN 10 YEARS IN SENEGAL: A CASE CONTROL STUDY

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Malaria and anaemia (Haemoglobin <11 g/dl) remain frequent in sub-Saharan Africa. The etiology of anaemia is known to be multi-factorial, most studies in malaria endemic areas, have been confined to analysis of possible associations between anaemia and individual factors such as malaria. A case control study involving children aged from 1 to 10 years was conducted to assess some assumed contributors to anaemia in the area of Bonconto Health post in Senegal. Study participants were randomly selected from a list of children who participated in a survey in December 2010. Children aged from 1 to 10 years with haemoglobin level below 11 g/dl represented cases (anaemic children). Control participants were eligible if of same age and their haemoglobin

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level was >= 11 g/dl. For each participant, a physical examination was done and anthropometric data collected prior to a biological assessment which included: malaria parasitemia infection, intestinal worm carriage, G6PD deficiency, sickle cell disorders, and alpha-talassemia. Three hundred and fifty two children < 10 years of age were enrolled (176 case and 176 controls). In a logistic regression analysis, anaemia was significantly associated with malaria parasitaemia (OR=5.23, 95%CI [1.1-28.48]), sickle cell disorders (OR=2.89, 95%CI [1.32-6.34]), alpha-thalassaemia (OR=1.82, 95%CI [1.2-3.3]), stunting (OR=3.37, 95%CI [1.93-5.88]), age ranged from 2 to 4 years (OR=0.13, 95%CI [0.05-0.31]) and age > 5 years (OR=0.03, 95%CI [0.01-0.08]). No association was found between G6PD deficiency, intestinal worm carriage and children’s gender. Malaria parasitaemia, stunting and haemoglobin genetic disorders represented the major causes of anaemia among study participants. Anaemia control in this area could be achieved by developing integrated interventions targeting both malaria and malnutrition.

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**ASSESSMENT OF THE ULTRASOUND EXAMINATION AS AN EPIDEMIOLOGICAL TOOL FOR THE SECONDARY AND TERTIARY PREVENTION IN A MALIAN RURAL AREA**

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Rural populations have less access to preventive health care and routine medical tests than residents of large cities. Ultrasonography is a non-invasive method that can aid in the diagnosis of a variety of conditions that require medical intervention. To assess the utility of ultrasound as a diagnostic screening test in a rural setting, five ultrasound examination visits were held in the 11 villages of Sabougou health area in Kolokani district (population 10,999 inhabitants in 2010). The village chief provided the examination site in 10 of the study villages and the local health clinic (CSCOM) was used in Sabougou. The motorcycle of the CSCOM was used, as well as a power generator and a portable ultrasound machine equipped with two probes of different frequencies. All volunteers (n=782) underwent a brief medical examination and ultrasound examination of the abdomen and heart performed by two physicians, including a well-trained ultrasonographer. In addition, women older than 15 years of age (n=416) underwent uterine ultrasound, male subjects of all ages (n=272) underwent scrotal ultrasound and all individuals older than 15 years of age (n=588) underwent thyroid ultrasound. Of the 782 subjects examined, 194 (25%) were less than 15 years old, 64 (8%) were pregnant women and 53 (7%) were > 65 years old. The overall prevalence of cardiac valvular calcification was 5% (39/782), and 0.64% (5/782) subjects had evidence of ventricular dilatation. Among the 272 men examined, 22 cases (8.09%) of subclinical hydrocele, 11 cases (4.04%) of hydrocele, 5 cases of testicular cysts (1.84%), 3 cases of prostatic adenoma (1.1%) and 1 case of prostatic cancer were identified. Two of the 510 women (0.39%) examined had uterine fibromas and one case of uterine malignancy was detected. Among the 64 pregnant women, one case of fetal demise (1.6%) and one case of extra-uterine pregnancy (1.6%) were detected. One case of multiple abnormalities of the thyroid, heart and tests was also observed. A total of 117 and 28 subjects were referred for further management to the Sabougou community health center and the Kolokani district reference center, respectively. Given these results, ultrasound examination in remote rural areas is a practical and non-invasive method for the identification of individuals requiring referral for medical care in rural Mali and its use should be considered at a regional and national scale.

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**EVALUATION OF NEW TECHNOLOGY MULTIPLEX NUCLEIC ACID TESTS FOR EMERGING AND TROPICAL BLOODBORNE PATHOGENS**

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Testing of bloodborne pathogens has reduced the risk of transfusion-transmitted infections significantly and use of molecular diagnostic tools has further improved the accuracy of diagnosis. However, with increasing numbers of emerging pathogens that can impact blood safety and the potential for multiple infections in a tropical setting, it is becoming burdensome to conduct separate tests for each agent. Devices that allow simultaneous testing for multiple pathogens (multiplex testing) can potentially streamline blood donation and diagnostic testing. We evaluated two devices, the OpenArray® by Life Technologies Corp. (Carlsbad, CA), and the Resequencing Pathogen Microarray (RPM) by TessArae, LLC (Potomac Falls, VA), for their potential ability to enable multiplex testing in whole blood and plasma samples. The OpenArray® system can perform approximately 3,000 individual real-time polymerase chain reaction tests simultaneously on a microscope slide-size wafer. The RPM utilizes an Affymetrix® GeneChip® base and a particular arrangement of oligonucleotides. Hybridization to these oligonucleotides leads to sequence identification. We assembled and tested a blood pathogen OpenArray® by primer and probe sets for viruses (HIV-1, HCV, HBV, WNV), parasites (Trypanosoma cruzi, Leishmania, Plasmodium), and Gram negative bacteria. Simultaneous detection of these 4 viruses in plasma specimens and 5 bacterial or parasite species in whole blood specimens was achieved at limits of detection equivalent to individual assays. An RPM was designed and tested with tiles for 22 viruses, 53 prokaryotes and 25 eukaryotes. We correctly identified nucleic acid from 10 pathogens simultaneously. These two multiplex detection devices are highly specific for known bloodborne pathogens. Future testing will reveal whether the sensitivity of these platforms is adequate and whether they are feasible for use in clinical diagnostic and blood donation settings.

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**CENTRE-BASED CLINICAL MANAGEMENT OF CYSTIC ECHINOCOCOSIS**

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Cystic echinococcosis (CE) is one of the world’s most neglected diseases. The lesions, predominantly in the liver and lungs, develop clinically silently over long periods of time until complications suddenly precipitate. The challenge for health care services, in particular in low-resource settings, is twofold, early detection and treatment of cases and very demanding management of complications of late stage disease. In high-income countries mostly migrants from CE-endemic areas are affected. In this setting CE is not only neglected but also rare and health services are, as a rule, not experienced to diagnose, stage and manage this disease appropriately. A centre-based approach of CE is presented with our interdisciplinary clinical CE unit at Heidelberg University Hospital as an example. Infectious disease / tropical medicine physicians, radiologists, abdominal and thoracic surgeons, gastroenterologists and parasitologists work very closely together to stage patients (ultrasound-based cyst classification) and to tailor currently available mostly expert-opinion based treatment options (medical treatment with albendazole, percutaneous cyst-sterilization techniques, surgery and ‘watch and wait’) to the needs of the individual patient. This approach can serve as a model for the clinical management of many other NTDs / NIDs in highly mobile global populations.

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and one suburban village in northeastern Thailand and southern Laos, entomological, bacteriological, and socioeconomic data from one rural contaminated DW, and mosquito production. In 2011 we collected by identifying relationships between household water management, to assess health risks associated with household water storage practices diseases if water is fecally contaminated and the storage containers. This relationship and the role of domestic water water contamination and mosquito production in household water storage containers. This relationship and the role of domestic water management practices as risk factors for both dengue and diarrheal disease will be discussed.

Central nervous system (CNS) infections are significant causes of mortality and mobility in low-middle income countries. To improve clinical diagnosis, management and public health intervention, it is essential to clarify the comprehensive picture of CNS infections. However most published studies focused limited pathogens. The objective of this study is to describe the present picture of whole CNS infections in the Philippines. We conducted a hospital-based retrospective descriptive study in San Lazaro Hospital (SLH), the national referral hospital for infectious and tropical diseases in the Philippines. We collected demographic and clinical information of all patients who were admitted with any suspected CNS infection from 1st January 2008 to 30th September 2011. It included all patients who were diagnosed CNS infections as initial and/or final diagnosis and all hospitalized patients who required a lumbar puncture (LP) examination, except for patients in HIV ward. A total of 1,264 patients were analyzed, 937 of them showed CNS infections as final diagnosis. There were more males (62%) and nearly half of the cases (43%) was under 12 years old. Tuberculous meningitis (TBM) and Rabies were the most common CNS infections with 312/2750(12%) cases of TBM and 217/195(10%) of rabies. This was followed by other bacterial meningitis 169(15%) and viral encephalitis 135(12%). Case fatality rate (CFR) for rabies was 100%; likewise, the CFR for non-rabies CNS infection was also high at 238/703(33.9%). 187(16%) of the patients who were initially diagnosed as CNS infections were confirmed not CNS infection in the final diagnosis. Febrile convulsions and seizures were the most common non-CNS infections. 131(11%) of the patients who were initially not diagnosed as CNS infections were later diagnosed as CNS infection: typhoid fever was the most common misdiagnosis upon admission. LP was performed in 277(22%) cases but its performance was often substantially delayed since many of the patients were critically ill upon admission and none of CSF was positive for bacterial culture. There is ample room for improvement of clinical diagnosis and management of CNS infections.

Household drinking water (DW) storage can be a determinant for both diarrheal and dengue fever are major global health problems. Household drinking water management practices as risk factors for both dengue and diarrheal disease will be discussed. In Thailand almost 10% of the Aedes positive containers were used for drinking, whereas in Laos as many as 25% were used for drinking. Of the Aedes infested DW containers 26% in Thailand and 51% in Laos were also contaminated with Escherichia coli. The results suggest an intricate relationship between water contamination and mosquito production in household water storage containers. This relationship and the role of domestic water management practices as risk factors for both dengue and diarrheal disease will be discussed.

Dengue and diarrheal disease risk factors in rural and suburban villages in Thailand and Laos

DENGUE AND DIARRHEAL DISEASE RISK FACTORS IN RURAL AND SUBURBAN VILLAGES IN THAILAND AND LAOS

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Diarrheal diseases and dengue fever are major global health problems. Household drinking water (DW) storage can be a determinant for both diseases if water is fecally contaminated and the storage containers provide breeding sites for dengue mosquitoes. The aim of this project is to assess health risks associated with household water storage practices by identifying relationships between household water management, contaminated DW, and mosquito production. In 2011 we collected entomological, bacteriological, and socioeconomic data from one rural and one suburban village in northeastern Thailand and southern Laos, respectively. In rural Thailand, almost 100% of the study population use rainwater as DW. In rural Laos 83% use unprotected wells in the dry season and 92% use rainwater in the rainy season. In the suburban

Settings DW sources are rainwater and bottled water. There was an average of 2.5 DW containers per household. Only 6% of households in rural Thailand and 43% in rural Laos treat their DW. These figures were higher for the suburban areas (Thailand: 65%; Laos: 84%). Water holding containers were found in >93% of the households, of which ~19% were positive for Aedes aegypti immatures. The most productive containers were cement tanks in both countries, representing 15-17% of all encountered pupae. The Breteau index (BI) was higher in Thailand than in Laos (140 vs 845, p<0.01). In Thailand the BI was higher in the rural area than in the suburban area (147 vs 134, p<0.01), whereas in Laos the opposite was observed (112 vs 56; p<0.01). In Thailand almost 10% of the Aedes positive containers were used for drinking, whereas in Laos as many as 25% were used for drinking. Of the Aedes infested DW containers 26% in Thailand and 51% in Laos were also contaminated with Escherichia coli. The results suggest an intricate relationship between water contamination and mosquito production in household water storage containers. This relationship and the role of domestic water management practices as risk factors for both dengue and diarrheal disease will be discussed.

SAFETY AND EFFICACY OF ARTEMETHER-LUMEFANTRINE AGAINST UNCOMPPLICATED PLASMODIUM FALCIPARUM MALARIA DURING PREGNANCY: A SYSTEMATIC REVIEW

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Plasmodium falciparum malaria during pregnancy, linked to increased morbidity and mortality, must be reduced by preventive measures and effective case management. Although, the World Health Organization (WHO) recommends artemisinin-based combination therapy (ACT) to treat uncomplicated P. falciparum malaria during the second and third trimesters of pregnancy, and quinine plus clindamycin during the first trimester, the national policies of many African countries currently recommend quinine throughout pregnancy. Our objective is to analyze available data on the safety and efficacy of artemether-lumefantrine (AL) in pregnancy. English-language search identified 16 publications from 1989 to October 2011 with reports of artemether or AL exposure in pregnancy, including randomized clinical trials, observational studies, and systematic reviews. There were 1,103 reports of AL use in pregnant women: 890 second/third trimester exposures; 212 first trimester exposures; and 1 case where the trimester of exposure was not reported. In the second and third trimesters, AL was not associated with increased adverse pregnancy outcomes compared with quinine or sulphadoxine-pyrimethamine, showed improved tolerability relative to quinine, and its efficacy was non-inferior to quinine. Few reports suggest that the pharmacokinetics of anti-malarial drugs may change in pregnancy, however, the majority of studies reported high cure rates and adequate tolerability. Additional data are required to assess the potential to use AL in the first trimester. These findings reinforce the WHO recommendation to treat uncomplicated P. falciparum malaria with quinine plus clindamycin in early pregnancy and ACT in later pregnancy.
MICROBIAL ETIOLOGY OF TRAVELERS’ DIARRHEA: EXPERIENCE OF A TRAVEL CLINIC IN TOKYO

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Travelers’ diarrhea (TD) is the most common illness in international travelers visiting developing regions of the world. Published studies provide relatively limited data on the microbial etiology of TD from South and Southeast Asia, which is popular destinations for tourism and business from Japan, compared with that from Africa and Latin America. Travelers who visited at the Travel Clinic of the National Center for Global Health and Medicine, Tokyo, with acute diarrhea (<14 day) that started during or shortly after a stay abroad during December 2009 and March 2012 were eligible for this study. After the participants provided informed consent, clinical data and stool samples were collected. The stool samples were screened by PCR for conventional diarrheagenic bacterial pathogens and cultured by standard methods. Commercially available antigen detection kits for Giardia, Cryptosporidium, rotavirus, norovirus, and adenovirus were also used. A total of 121 cases were analyzed. The major destinations included Southeast Asia (44%), South Asia (30%), and Africa (13%). Diarrheagenic pathogens were detected in 66% of the TD cases. In 23% of them, multiple pathogens were detected in the stool samples. Enterotoxigenic Esherichia coli was the most common pathogen in all the destinations (36%). Enteraggregative E. coli was the second most common pathogen overall (12%) and more frequently detected in the cases who had returned from Southeast Asia. Campylobacter, Shigella, and rotavirus followed in this order. Rotavirus was more frequently detected in the cases who had returned from South Asia (P <0.05). Ciprofloxacin resistance in diarrheagenic E. coli was rare in all the destinations, but broad-spectrum β-lactam resistance was found in the strains from South Asia. Further investigation focusing on antimicrobial resistance of pathogens of TD is needed.

DISTRIBUTION OF RUBELLA INFECTIONS IN RWANDA SINCE 2003

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Rubella virus is the causative agent of the disease known more popularly as German measles and is predominantly a childhood disease, endemic throughout the world. Natural infections of Rubella occur only in humans and are generally mild but complications, most commonly polyarthralgia in adult women, do exist. RV infection of women during the first trimester of pregnancy can induce a spectrum of congenital defects in the newborn, known as congenital rubella syndrome (CRS). Since 2003, the National Reference Laboratory of Rwanda has been involved in the surveillance of Rubella infection throughout the country. Cumulative data show that of the 1,778 samples suspected of Rubella, 362 were positively identified by ELISA (21.5%). In Rwanda, geographical data indicates that the Rubella is equally distributed in all provinces of Rwanda with small pockets of infections in Kigali city and Ruhango district, close to the border of Burundi. According to sex and age, infections occur equally in males and females but the majority of infections were in patients older than 5 years old (69.9%).
DECREASING OCCURRENCE OF TROPIC NEUROINFECTIONS: CEREBRAL MALARIA, MENINGOCOCCAL MENINGITIS AND SLEEPING SICKNESS IN SOUTH SUDANESE RURAL HOSPITALS

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With advantages of AIDS treatment, 20 years ago some of tropical neuroinfections (e.g. cryptococcal meningitis, tuberculosis meningitis, CNS toxoplasmosis or CMV encephalitis) showed increasing incidence. However, with the era of HAART they again become to be rare. Also severe malaria, manifesting as cerebral malaria with hypoglycemia acidosis and severe anemia, is less frequent after the introduction of intermittent preventive treatment (IPT) and artesimisin in early treatment. In this study, two rural hospitals - Mapuordit in Yirol country and Gordim in Aweil country (both with 5-20 beds) - were compared. Totally, 18,027 patients were treated in Mapuordit and 9,358 in Gordim. Most patients (90%) were treated on outpatients` basis, the rest as inpatients. Both hospitals offered two doctors with surgical and medical qualification and mobile team from Italy is coming for elective surgeons two times a year. Occurrence of severe malaria in Mapuordit (close to Nile River) cases was more common in Gordim. Trends in malaria occurrence correlated with dry and rainy season. Diarrheal diseases showed slight increase in 2011. Pneumonia represented about 20-25% of all respiratory tract infections. Skin and soft tissue infections (STI) were the most common infection after RTI, diarrheal diseases, malaria and sexually transmitted diseases with urinary tract infections showed stable trend and not significant difference between Mapuordit (40-50 a month in average) and Gordim (33-40 per month). However, trends in malaria correlated with rainy season, with maximum of 329 and 367 cases in August and 30 - 60 new cases per month. However, trends in malaria correlated with rainy season, with maximum of 329 and 367 cases in August and September, respectively. Fortunately, complicated malaria (cerebral malaria, renal failure) were extremely rare, probably because of early treatment with artesunate or artesunate/fanfimfantrin. Second commonest diseases were respiratory tract infections (RTI) with mostly stable occurrence of 260 - 293 cases per month, followed by diarrhea and sexually transmitted diseases (STD). Only 1 case of tetanus occurred during 2010 - 2011. There was observed only 1 case of cerebral malaria, 8 cases of meningococcal meningitis and 1 case of sleeping sickness in period of 2010 - 2011, which is very low in comparison with 32 cases of cerebral malaria, 119 cases of meningococcal meningitis and 9 cases of sleeping sickness in period of 2005 - 2006. We can conclude that in Gordim there was no significant difference in tropical ID incidence before and after South Sudan has been completely opened for foreign travellers. Also, occurrence of tropical neuroinfections, such as sleeping sickness, cerebral malaria and meningococcal meningitis, was sporadic.

HEALING OF CUTANEOUS LARVA MIGRANS AFTER A SINGLE DOSE OF IVERMECTIN IS ACCOMPANIED BY CHANGES IN CYTOKINE PATTERNS IN PERIPHERAL BLOOD

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Cutaneous larva migrans (CLM) is a neglected tropical skin disease caused by the migration of animal hookworm larvae in the epidermis. The disease is common in resource-poor communities in developing countries. Patients with CLM were identified through active case finding in two resource-poor communities in Manaus, Brazil. Patients were diagnosed clinically, and severity of the disease was assessed using a semi-quantitative severity score. Clinical pathology was assessed and hematological and immunological investigations were performed before, and two and four weeks after treatment with ivermectin (200µg/kg). Leucocytes and eosinophils were counted and total serum IgE was determined. The concentration of IL-4, IL-5, IL-10, IFN-γ, TNF-α and TGF-β was determined in serum using commercially available ELISA kits. 92 patients were included in the study: 69.6% were male and 30.4% were female. Median age was 9.5 years (IQR 5-44). At baseline, 93.4% of all patients complained about severe pruritus and 73.6% about insomnia. The median severity score was 4 points (IQR 3-6). 87.8% of the patients had eosinophilia. Patients with CLM had significant higher concentrations of IgE, eosinophils, IL-4, IL-5 and IL-10 in serum than age- and sex- matched controls living in the same community. Four weeks after treatment, clinical pathology and eosinophilia decreased significantly. While the serum concentration of IL-4, IL-5 and IL-10 decreased, the concentration of IFN-γ increased significantly. It is concluded that in an impoverished community CLM is associated with considerable morbidity. After treatment with ivermectin, clinical pathology, eosinophilia and cytokine patterns normalize rapidly.

SPECTRUM OF TROPICAL NEUROINFECTIONS AND OTHER INFECTIOUS DISEASES IN OUR LADY OF FATIMA HOSPITAL, GORDIM, SOUTH SUDAN, BEFORE AND AFTER DECLARATION OF INDEPENDENCE

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For 10 years, the area of South Sudan was under control of Sudan’s People Liberation Army (SPLA) and medical services were served by Médicins Sans Fronières (MSF) and other humanitarian organizations after the declaration of independence in May 2011, when country was completely opened for foreign travel. Aim of this study is to compare the spectrum of tropical infectious diseases (ID) before and after independence declaration (2010 vs. 2011) in rural hospital in Gordion, South Sudan. In 2010, together 5097 outpatients were compared to 3612 outpatients treated in 2011. Diagnostic of malaria, geohelmintus and tuberculosis (TB) has been performed by microscopy, as rapid diagnostic tests (RDT) were not available until spring 2012. HIV was not observed in none of outpatient department (OPD) patients. Trends in TB were stable with 30 - 60 new cases per month. However, trends in malaria correlated with rainy season, with maximum of 329 and 367 cases in August and September, respectively. Fortunately, complicated malaria (cerebral malaria, renal failure) were extremely rare, probably because of early treatment with artesunate or artesunate/fanfimfantrin. Second commonest diseases were respiratory tract infections (RTI) with mostly stable occurrence of 260 - 293 cases per month, followed by diarrhea and sexually transmitted diseases (STD). Only 1 case of tetanus occurred during 2010 - 2011. There was observed only 1 case of cerebral malaria, 8 cases of meningococcal meningitis and 1 case of sleeping sickness in period of 2010 - 2011, which is very low in comparison with 32 cases of cerebral malaria, 119 cases of meningococcal meningitis and 9 cases of sleeping sickness in period of 2005 - 2006. We can conclude that in Gordion there was no significant difference in tropical ID incidence before and after South Sudan has been completely opened for foreign travellers. Also, occurrence of tropical neuroinfections, such as sleeping sickness, cerebral malaria and meningococcal meningitis, was sporadic.
EPIDEMIOLOGY OF FEBRILE ILLNESSES AMONG INFANTS: A CASE CONTROL STUDY IN KINTAMPO NORTH AND SOUTH DISTRICTS

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Information on the burden and risk factors of febrile illnesses in infancy is scarce. Young infants are relatively protected against infant illnesses during the first six months of life due to the presence of maternal antibodies and foetal haemoglobin, and have received relatively little attention with respect to research and treatment guidelines. To add to the limited data available, this study therefore sought to determine the predisposing factors to febrile illnesses among infants (0-11months). A case control study was conducted in Kintampo North Municipality and South District. We randomly selected 230 cases and 454 controls from infants with and without infant febrile illnesses and were participants of an ongoing study. Standard questionnaires were administered by blinded interviewers to randomly selected cases and controls. Variables compared in both groups included birth weight, breastfeeding practices, immunization status, household background characteristics and socio-economic status of mothers. Data collected was entered on Microsoft Access and analysed using STATA Version 11. Results of the study showed that malaria was the most prevalent febrile illness. Analysis showed that 70% of febrile cases were above 6 months of age, whilst 91.85% were exclusively breastfed. A significant difference was shown between cases and controls in terms of age and breastfeeding status. Apparent similarity was shown between cases and controls with respect to birth weight, household background characteristics, immunization status, ITN use and socio-economic levels of mothers. The study showed that infants above 6 months and those not exclusively breastfed are more likely to develop febrile illnesses. Information on the epidemiology of febrile illnesses among infants will be essential for designing and interpreting results of clinical trials of drugs, vaccines and other interventions for this vulnerable group.

PROVIDING HIV EDUCATION TO HEALTH CARE PROVIDERS IN COCHABAMBA, BOLIVIA IN EXCHANGE FOR AMERICAN STUDENT ROTATION AT THE MAIN LOCAL PUBLIC HOSPITAL

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American physicians in training seldom experience the florid variety of infectious diseases found in developing countries and tropical areas. The management of patients is also different in limited resource settings. The number of reported cases of HIV in Bolivia is growing exponentially, but the number of HIV providers is not. Hospital Clinico Viedma is a public hospital in the heart of South America that serves the most underprivileged population in the city. It also is a referral center for the surrounding tropical areas. In February, 2012, the Division of Infectious Diseases at the University of Massachusetts coordinated an elective for UMass Medical Students in Cochabamba Bolivia. The elective consisted of 3 medical students, 4 faculty members and a Bolivian Infectious Diseases fellow who served as the course coordinator/director. A major goal of the interchange between the two institutions is to provide basic HIV education for Bolivian health care practitioners in the Cochabamba area in Spanish, the main native language. We accomplished this by teaching an intensive course, involving 10 hours of didactics and 5 hours of case presentation. This course was judged to be outstanding by the participants, although they felt that the course should be expanded to teach the care of pediatric patients with HIV. This year we are expanding our course to pediatrics and live state of the art recording. A secondary benefit of expanding our HIV course will be that UMass faculty members will be on site to precept UMass students on elective in Cochabamba in the diagnosis and management of infectious illnesses not commonly seen in the US setting. This DVD will then be available for distribution to additional health care providers in Bolivia and other Spanish speaking countries.

THE HEALTH AND DEMOGRAPHIC SURVEILLANCE SYSTEM IN RURAL WESTERN KENYA: RELEVANCE TO PUBLIC HEALTH AND RESEARCH ENDEAVORS

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The Health Demographic Surveillance System (HDSS) set up in Kisumu West district, rural western Kenya, is designed to track on a bi-annually basis, evolving health status, demographics and health threats within the catchment area. The program has GPS-located every dwelling unit that exists within the catchment area and has conducted baseline population and housing survey that is monitored through regular surveys. The primary goal for the program is to provide an exhaustive health and demographic data set throughout the catchment area that would be of great interest to potential research collaborators and the local ministry of Health. This paper examines the contributions made by the program towards advancement of public health and research agenda in the catchment area. The Kisumu West HDSS has provided the first steps in developing the linkage of extensive demographic and health data that is tracked over time to patient health care records, beginning with PEFFAR subjects and later expanding to all patients in the study area. The linkage will greatly aid in subject/patient tracking and linking of disease and patient to specific locations with a view of designing targeted interventions. During a recent polio outbreak in western Kenya, the Kisumu West HDSS provided information towards the successful implementation of the immunization campaign. The information included baseline population figures for the target population and village maps to aide movement of the MOH staff in the field. The KWHDSS provides an ideal research platform for clinical and epidemiological studies. Specific examples of how the KWHDSS supports these studies-including the Phase III Malaria vaccine trial will be provided to showcase its relevance to research endeavors. In conclusion, the KWHDSS continues to provide a central analytical framework for work on clinical trials, disease surveillance and public health intervention in the Kisumu West District. The longitudinal nature of the KWHDSS allows better matching of volunteers for clinical trials such as those involving post-marketing surveillance and studies assessing the impact of other health care interventions.

BEYOND SIMPLE PREVALENCE: ENHANCED DISCRIMINATION OF INFECTIOUS DISEASE-RELATED DATA PATTERNS BASED ON THREE-DIMENSIONAL ANALYSIS

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One important challenge in infectious disease research is to reduce the rate of information loss and/or errors associated with data analysis, including those generated by prevalence-based analyses. To develop and evaluate an alternative approach that addresses these issues, leukocyte
data collected from humans, birds, and bovines affected by different pathogens were assessed with two approaches: (i) non-structured indicators, such as the neutrophil percent, which were determined with bi-dimensional plots and considered the overall (population) disease prevalence, and (ii) structured indicators (indices designed to generate a single line of observations), which were explored with three-dimensional (3D) plots and considered subset-specific prevalence. These approaches revealed that population-based prevalence analyses did not distinguish the leukocyte profiles of disease-negative (D-) and disease-positive (D+) subsets. In contrast, structured indicators assessed with 3D plots revealed patterns which, when used to partition the data, enhanced discrimination of infection: (1) non-overlapping D- and D+ subsets were generated, (2) observations suspected to be false were detected, and (3) in humans infected with malaria, four disease classes were distinguished. Results presented here demonstrate that patterns previously unrecognized in D+ and D- individuals can be identified with structured, 3D analysis, leading to more informative, subset-specific prevalence estimates.

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METRICS OF SUCCESS FOR SOCIAL DETERMINANTS OF HEALTH AND TROPICAL DISEASES

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Numerous measurement problems emerge when considering neglected tropical diseases (NTDs) on their own, and especially in the social determinants of health (SDH) framework. Burden of NTDs is miscalculated because of underestimation of mortality, long-term sequelae, effects on fertility and on pregnancy, cross-generational effects, and synergies of multiple morbidities. In addition, burden-of-disease methodology specifically abstracts from socio-economic context. Disability-Adjusted Life Years (DALYs) attempt to aggregate the effects of every disease on mortality and morbidity, based on prevalence and specific effects of each disease. DALYs were derived so that a life has the same value, and a disease has the same burden, regardless of place of residence, occupation, or income. The SDH framework embodies a different principle of fairness that requires society to prioritize problems of poor and marginalized people. In the SDH approach, it is necessary to allocate investment disproportionately to diseases of poverty and also to the structural determinants that promote poor health in poor populations. Invisibility of socially excluded populations and their health concerns is another methodological challenge. National and subnational averages can show important progress in achieving disease-reduction goals, while obscuring the persistence of NTDs and the concentration of multiple NTDs in family and community clusters. Global campaigns, including the Millennium Development Goals, use state targets as national and global averages. Reliance on them as sole indicators of progress in disease reduction reinforces invisibility of persistent clusters afflicted with multiple morbidities of diseases of poverty, even while national statistics improve. ‘Elimination as a public health problem’ is a term that definitionally could be at odds with the spirit and practice of reducing health inequities. Continued existence of even low levels of impoverishing and often stigmatizing diseases is evidence of persistent inequities.

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RECLAIMING THE ‘BETTER HEALTH FOR ALL’ MANDATE: A CASE FOR INTEGRATING GLOBAL HIV PROGRAMMING WITH COMPREHENSIVE PRIMARY HEALTH CARE SYSTEMS IN SUB-SAHARAN AFRICA

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Twelve years into the twenty-first century, our world is still grappling with an HIV/AIDS epidemic that has placed great strain on global human and material resources and compounded human suffering; especially in sub-Saharan Africa. While the discovery and improved access to antiretroviral therapy and allied treatment has significantly reduced mortality and morbidity from this disease, it is still positioned to continue to garner prime attention in health discourse and in the allocation of global resources. However, competing health demands in developing countries such as the rising threat of non-communicable diseases and often-neglected communicable diseases amidst a slowly recovering global financial economy are timely prompts for a careful reconsideration of the prevailing approach to HIV funding and programming which has so far privileged this disease to the detriment of overall health. At this critical juncture, a re-evaluation is invaluable if we are to make the smart health investment decisions that would protect better health in the future. I argue that a departure from the current vertical nature of HIV programming is needed to curb its detraction from the development of effective health systems that are sensitive to the totality of local health realities and needs in sub-Saharan Africa. Using support from relevant literature, I trace the evolution of this vertical handling of HIV, its origins in the selective health care model that eclipsed the Alma Ata affirmation and its deleterious effects on health systems; present policy options and recommendations in making the case for an integration of HIV programming with comprehensive primary health care and discuss some of the few available cases that have pursued integration in various forms. Despite the litany of practical difficulties that may dissuaded a global adaptation of such integration, this step is vital if developing countries are to achieve sustainable, efficient and locally owned comprehensive health systems capable of safeguarding better overall health.

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COMBINING HIV/AIDS AND MALARIA INDICATOR SURVEYS IN TANZANIA TO LEVERAGE EXPERTISE AND MAXIMIZE EFFICIENCY IN LARGE HOUSEHOLD SURVEYS

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Development assistance to Tanzania for HIV/AIDS and malaria ($2.3 billion in 2005-10) has permitted intensive scale-up of multiple interventions. Separate, labor-intensive, nationally-representative household surveys are a cornerstone of monitoring and evaluation (M&E) for National AIDS and Malaria Control Programs. In 2007/8 and 2011/12, the National Bureau of Statistics (NBS) and Zanzibar’s Office of Chief Government Statistician (OCGS) succeeded in meeting the needs of multiple stakeholders by creating a combined Tanzania HIV/AIDS and Malaria Indicator Survey (THMIS). The THMIS used a two-stage probability sample design implemented by NBS/OCGS, overseen by the Tanzania Commission for AIDS, with technical guidance from Mainland and Zanzibar malaria and AIDS control programs, and MEASURE DHS. Demographic data and HIV/AIDS and malaria knowledge and attitudes, risk behaviors, and intervention use were collected from adults aged 15-49 years. Dried blood spots for HIV testing of adults and malaria rapid tests, thick blood films, and hemoglobin measurement for children were prepared from
fingerstick capillary blood specimens. In December 2011, data collection for the 2011/12 THMIS was initiated in over 9,700 households, with approximately 10,800 women and 8,000 men expected to be interviewed and tested for HIV and 7,500 children tested for malaria and anaemia by April 2012. The 2007/8 HIV and malaria prevalence (5.7% and 17.7%, respectively) will be compared to 2011/12 estimates. The THMIS required four months to complete data collection compared to three to four months per each stand-alone survey. In-country costs for 2011/12 THMIS ($2.1 million vs. $900,000 for 2003/4 AIDS survey) were shared by two U.S. Government initiatives (74%), Government of Tanzania, and others. Careful coordination and planning by multiple stakeholders from HIV/AIDS and malaria control produced a single, mutually appealing, nationally representative household survey. This efficiency helps conserve resources needed to document progress toward Millennium Development Goals.

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EVALUATIONS OF HEALTH RESEARCH CAPACITY DEVELOPMENT: A REVIEW OF THE EVIDENCE

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Local research and innovation capacity is essential to improving health outcomes prompting significant investment in strengthening health research capacity (HRCS) in low and middle income countries LMICs. Although funding agencies need to show value for money and implementers want to demonstrate HRCS impact, empirical evaluation evidence on HRCS seems scarce. We conducted a scoping review of published evaluations of HRCS to learn lessons about how to assess its effectiveness and impact especially in the longer term. We searched electronic bibliographic databases, reference lists of relevant articles, reports of funding agencies, and websites, and consulted ‘experts’ to identify relevant publications using search terms covering training, mentorship, collaborations, partnerships and networks. We assessed the quality of these evaluations using an instrument developed for reviews of community interventions, and synthesized information about the types and design of the evaluation, and the measurement tools and indicators that were used. We identified 593 publications from health, education and management literature that focused on evaluating the development of health research capacity. 31 were primary studies; only 4 (0.7%) were from LMICs; Ghana (2), Vietnam and Pakistan and the quality of these four studies was variable. None used a comparator group; two were retrospective and two used validated tools. All four studies specified objectives and outcome measures, and stressed the importance of engaging senior managers in developing research capacity. Most provided descriptive analyses including both qualitative and quantitative results. HRCS literature is dominated by recounting of programs and experiences with little published evaluation. A much more substantial evidence base on HRCS interventions reported in peer-reviewed publications is needed before we can develop robust evaluations of impact and value for money of investments in HRCS.

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STRENGTHENING RESEARCH CAPACITY WITHIN A GHANAIAN TEACHING HOSPITAL: TEN YEAR PROSPECTIVE STUDY

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A strong research culture within teaching hospitals, supported by robust research infrastructure and the ability to demand and utilise research, is essential to promote evidence-based practice and improve health outcomes. For 10 years senior managers and academics at Komfo Anoyke Teaching Hospital (KATH), Ghana have been strengthening the hospital’s research systems and creating a critical mass of research expertise among mid-career health professionals (eg. doctors, nurses, managers, ancillary cadres). The research capacity strengthening programme was designed prospectively using a rigorous implementation research approach for designing and monitoring complex interventions. We adapted a published framework for institutional change and used this to design and monitor the programme in collaboration with key stakeholders. The framework enabled us to use mixed methods flexibly and systematically to plan, regularly review and adapt the programme, to identify and prioritise gaps in KATH’s research systems and infrastructure, and to derive and use indicators to monitor progress in closing the gaps. One component of the programme was an innovative 1 year, part-time Professional Diploma course (UK award) which taught ~20 students/year to undertake a research project important to their department. Our published course evaluation demonstrated graduates were competent and confident to design and conduct research. Through the programme KATH now has a dedicated research support office and administrators, a biostatistics unit and better successes with exams, grants and publications. By 2007 the Diploma course was managed and taught entirely by Ghanaian faculty (KNUST) and it is sustainable through locally generated funds. KATH/university faculty have extended the course to other sites in Ghana and Zimbabwe (2009-12). There has been some impact on clinical practice (eg. birth injury prevention programme; reduced needlestick injuries). Next priorities are to strengthen systems for utilisation of research results and to consolidate departmental research incentives.

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STRENGTHENING PATIENT-CENTERED COMMUNICATION THROUGH WORKSHOPS AND SELF-REFLECTION: A CLUSTER RANDOMIZED TRIAL AT PUBLIC HEALTH CENTERS IN UGANDA

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The quality of health care at African health facilities is often reported to be poor, linked to low patient attendance and poor health outcomes. In western settings, patient centred approaches, focused on communication between health workers and care seekers, have been advocated to improve quality of services. Such approaches have received little attention in Africa. Rigorous evaluations are required to inform best practice for improving quality of care in resource limited settings. A cluster randomised trial of the PRIME intervention to enhance quality of services is underway at health facilities in rural Uganda. One component of the intervention is a Patient Centred Services package, intended to improve communication with health care seekers, increase attendance at health facilities and improve overall population health indicators. This paper presents the first step in this hypothesised mechanism of change: the
impact of the intervention on health worker communication. We assessed communication between health workers and care seekers at baseline and immediately after the implementation of the intervention in 20 health facilities randomly assigned to intervention or standard care. A total of 26 health care workers and 213 health care seekers participated. Consultations were recorded and rated using the Measurement of Patient Centred Communication method and care seekers were interviewed on exit to provide their assessment of the quality of communication. Patient-centred communication was rated 10% higher (p<0.008) by care seekers consulting with health workers who had recently participated in the PRIME intervention compared with those in the standard care arm. A per protocol analysis suggests this increase may be plausibly attributed to the Patient Centred Services component of the intervention. Improvements to quality of care in resource limited settings may be achieved by approaches that reorient services towards patients.

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INScale Cluster Randomized Trial Evaluating the Effect of Innovative Motivation and Supervision Approaches on Community Health Worker Performance and Retention in Uganda and Mozambique: Intervention Design

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If properly trained, equipped and utilized, community health workers (CHWs) delivering integrated community case management (ICCM) for children with diarrhea, pneumonia and malaria can potentially reduce deaths from these infections by 60%. To achieve this outcome it is essential to maintain CHW performance and retention. The iNScale project aims to increase sustainable coverage of ICCM in Uganda and Mozambique by designing and evaluating innovations for increased CHW supervision and motivation. A combination of participatory research methods were used to identify program gaps, best practices and potential interventions. Quantitative baseline surveys with household members, CHWs and health facility staff were conducted to establish key outcomes and to inform the randomization process. Following extensive formative research and national stakeholder consultations, two interventions were developed in Uganda and one in Mozambique. In Uganda approximately 3500 CHWs in 39 clusters were randomized into a mobile health (mHealth) arm, a community engagement arm, and a control arm. In Mozambique 300 CHWs in 12 clusters will be randomized into a mHealth arm and a control arm. The mHealth interventions in Uganda and Mozambique encompass three main activities: 1) closed user groups to enable free two-way communication between CHWs and their supervisors; 2) weekly ICCM data submission using phones with automated motivational feedback, SMS to supervisors flagging problems for target supervision, and summary ICCM statistics made accessible online to district stakeholders; and 3) monthly motivational and constructive SMS to CHWs. The community engagement arm in Uganda will establish health clubs which seek to improve child health and identify health challenges through a community led model with the CHW as its focal point, potentially resulting in 1) improved status and standing of CHWs as key health assets; 2) increased demand for CHW services, and 3) communication to CHWs and other village members that CHW work is important, of value and appreciated. In both countries process evaluation will be conducted and endline surveys will establish impact after 12 months. Main outcomes will be the proportion of sick children appropriately treated, CHW performance and motivation, and cost effectiveness of interventions.

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Evaluation of Low-Cost Open-Source mHealth Tools to Support a Longitudinal Pediatric Dengue and Influenza Cohort Study in Nicaragua: Improving Quantity, Quality, Traceability and Timeliness of Data Collection and Management

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Dengue and influenza are major problems worldwide. The Pediatric Dengue Cohort Study began in 2004 as a collaboration between the University of California, Berkeley, the NGO Sustainable Sciences Institute, and the Ministry of Health in Managua, Nicaragua, to study the natural history and transmission of dengue in children in a community setting in a developing country. In 2007, the Nicaraguan Influenza Cohort Study was added to study the burden and seasonality of pediatric influenza. These studies provide critical epidemiology and transmission data to support detection and prevention approaches around the world, and clinical data and biological samples are used to study viral and immunological determinants of protection and pathogenesis and for development of novel diagnostic assays and algorithms. Currently, ~3700 children aged 0-14 receive medical care through the studies, and data from all clinical visits are systematically recorded. Participants with suspected dengue, influenza or undifferentiated fever are tested by serological, virological, and molecular biological assays, and yearly blood samples are analyzed to detect inactive infections. To facilitate the logistics and operations, a set of information technologies have been implemented by the study team since 2004. These eHealth tools - electronic medical records, patient and sample tracking systems using barcode and fingerprint IDs, and field logistics support tools for household visits - help to maintain quality control and facilitate compliance with established Good Clinical Practice (GCP) and Good Laboratory Practice (GLP) standards. In the 2012 annual blood collection conducted in the study Health Center and through household visits, a mixed methods approach was used to assess the impact of a new mobile data collection and management technology package using low-cost Android tablets and cell phones with the free open-source software ODk Collect and OpenMRS. Specific advantages in data entry/processing time, accuracy and accessibility, user experience, and cost savings were observed compared to paper and PDA-based tools. Results were shared with the Ministry of Health, along with lessons learned about implementation, for potential scale-up for routine data collection needs in the national public health system.

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Prevalence of Purchase of Antibiotics Without Prescription in Children Under Five in Private Pharmacies Close to Primary Care Centers in Peri-Urban Areas of Lima, Peru

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The overuse of antibiotics is associated with the increase of resistant pathogens. In developing countries, antibiotics are commonly purchased
at private pharmacies, which are important suppliers of health tips and low-cost medicines. The objective of this study was to determine the prevalence of purchase of antibiotics without a prescription for use in children under 5 years in private pharmacies of peri-urban areas of Lima, Peru. A survey was applied in adults who bought an antibiotic for a child up to five years in a private pharmacy close to a health center in a peri-urban area of Lima, Peru. 287 of the surveyed bought an antibiotic. The prevalence of purchase without prescription was 13.2%. From these 1.7% were due to self-medication and 11.5% were due to indication of the pharmacist. The diseases that were most often associated with the use of antibiotics were 45.8 common cold (45.8%) and acute diarrhea (18.5%) and bronchospasm (18.5%). Diseases that were mostly associated with use of antibiotics without prescription was common cold (50%) and watery diarrhea (28.9%). An overuse of antibiotics in children less than 5 years exists in this setting, especially in diagnoses as watery diarrhea, common cold and bronchospasm, mainly due to medical prescription. Self-medication was found in a very low percentage as well as pharmacy personnel recommendation. Training of medical personnel should be prioritized and legislative measures in relation to the purchase of prescription antibiotics should also be strengthened.

BUILDING CAPACITY FOR CONDUCTING CLINICAL TRIALS IN VIETNAM

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Vietnam has seen the emergence of new diseases such as SARS and highly pathogenic avian influenza. Other infectious diseases are endemic. Many parties, including the Vietnamese Ministry of Health (MOH) have a strong interest to conduct clinical trials in Vietnam. The MOH is faced with an urgent challenge to develop a system of oversight that follows the Principles of Good Clinical Practice (GCP), which protects the rights and well-being of human subjects. The MOH also recognizes a need to develop the capacity of local research institutions and their personnel to conduct quality research. With the cooperation of FHI 360 and other partners, MOH has made several interventions to improve capacity to conduct clinical trials in Vietnam. As part of the Southeast Asia Influenza Clinical Research Network (SEA ICRN), a new role titled Clinical Trial Support Specialist was developed within FHI 360. Local health professionals were trained in clinical trial regulatory, ethics and operations processes and then provided formal as well as side by side training to study staff of the local hospitals. Existing health system structure and operation were challenges. In a separate but complimentary effort, MOH partnered with FHI 360 to host a series of workshops which included government regulators, and other stakeholders. Open discussions among the groups revealed and prioritized gaps in ethics knowledge, systems, and infrastructure, from which an MOH strategy to build capacity developed. In 2008, MOH issued a GCP document for Vietnam and a regulation to define and operate an Independent Ethics Committee (IEC). The MOH committed to developing an independent ethics system consistent with international standards. Further workshops hosted by the MOH built capacity within the ministry for ethics review. In conclusion, the oversight of clinical trials in Vietnam and the related capacity of Vietnamese institutions have shown significant improvement since 2005. This has been enabled through a coordinated and strategic approach by the MOH and included partnerships with several international institutions. For next steps in the emerging model the MOH will need continuing partnerships that provide technical assistance, monitoring, and support in order to continue this growth.

REDUCING HEALTH AND HEALTH SERVICE DISPARITIES IN AN ETHNICALLY DIVERSE, HIGH MIGRATION AREA ON THE THAI-MYANMAR BORDER

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Trans-border migration is increasing rapidly worldwide and already involves almost one-quarter billion people. Ethnic diversity among migrants and between migrants and national majority populations into which they move, plus legal eligibility for residence and access to services pose numerous problems for providing health services and control of transmissible diseases. Transborder migration to Thailand, mostly from Myanmar, now accounts for at least 3 million people from many different ethno-linguistic backgrounds. Transborder migration is expected to increase markedly in 2015 following the opening of borders between ASEAN countries. Migrants to Thailand have significantly higher prevalence of malaria, TB and probably HIV than non-migrant residents. PHTP’s Access to Care Project to date has surveyed 998 women and men from Chinese, Hmong and Lahu minorities and from the ethnic Northern Thai majority. Survey data show statistically highly significant differences between different ethnic groups, between minorities and the ethnic majority in socioeconomic characteristics (e.g., income, household possession, education, Thai language ability, health insurance), and between migrants and non-migrant members of the same ethnic group living in the same communities, with respect to: health information, (e.g., knowledge of HIV transmission, prevention, diagnosis and treatment); use of health services (antenatal care, HIV counseling and testing); and reported constraints to use of health services (e.g., service delays, transportation, direct and indirect costs, lack of knowledge of health and health services, language). Analysis of hospital records allow analysis of differences in delays and interruptions in services and severity of illness associated with ethnicity, location and migration status. Effects of interventions (e.g., to date, health education) tailored by results from surveys (e.g., ethnicity, education, Thai language ability, knowledge of health and health services, migration status) are evaluated by before and after assessment.

KNOWLEDGE, ATTITUDE AND PRACTICES OF HEALTH CARE WORKERS TOWARDS MALARIA CASE MANAGEMENT IN CHANGING MALARIA TRANSMISSION IN NAMIBIA

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Malaria cases in the last 7 years are on the decline in Namibia due to interventions in malaria control implemented by the Ministry of health and Social Services (MoHSS). There is a shift from control of malaria to its elimination; hence it is necessary for interventions to reflect this shift. Early and proper malaria diagnosis and case management are of paramount importance in reducing the parasite reservoir for elimination of the disease. The knowledge and perceptions of Health Care Workers (HCWs) regarding the prevalence of malaria, its diagnosis and treatment were investigated to provide a basis for aligning the training of health care workers to the objective of elimination of malaria. Three malaria endemic regions of Namibia namely Omusati, Caprivi and Kavango representing different malaria risk strata as well as cultural differences and practices within the country were selected. Six Focus Group Discussions (FGDs) and 7 Key informants Interviews (KI) were conducted. FGDs consisting of 6-10 participants were conducted using semi-structured questions to collect data. Three FGDs were conducted in Caprivi region (Katima Mulilo), 2 in Kavango region (Andara) and 1 in Omusati (Onesi). Each group was primarily composed of registered and enrolled nurses from rural clinics, health centres and regional hospitals. Staff members who were managers
were excluded from the FGDs but were still included in the study and interviewed as key informants. There was a general perception that less malaria cases were presented with 90% of Health Care workers having knowledge of the four species of Plasmodium although 80% of had not participated in a formal, organized malaria case management training session. Only 60% adhered to negative RDT results regardless of persistent symptoms of malaria. There is a need for initial and continuous training of HCWs on malaria diagnosis using RDTs, differential diagnosis and unambiguous case management guidelines to increase their confidence in handling negative results and adherence to RDT results.

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ANALYSIS OF THE AGGREGATE AND DISTRIBUTIONAL WELFARE EFFECTS FROM VACCINE DIFFERENTIAL PRICING, POOLED PROCUREMENT AND POOL MEMBERSHIP
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A well designed vaccine pricing architecture can ensure more equitable vaccine prices and result in greater access to new vaccines globally. Pooled procurement is a mechanism that is commonly used to achieve lower vaccine (and medicine) prices for lower income countries and consequently allow country programs to immunize more people with a given budget. Under pooled procurement, several countries bargain collectively as one unit to achieve one (supposedly) lower price. Currently, there is very little (if any) analytical or empirical research to guide policy around the optimal buy-side market structure for vaccines. Existing vaccine procurement pools are organized in several different ways, each with different implications in terms lowering prices for countries in the pool, and for ensuring more equitable vaccine access globally. Some procurement pools, such as PAHO and GCC, are organized regionally (geographical proximity-based), with high income heterogeneity among the countries in the pool. Other pools such as UNICEF are organized by country income level (income-proximity based), but have to rely on a third party i.e. a UN or multilateral agency led procurement structure. Using game theoretical models this research attempts to answer the following questions: (1) What form of buy-side market structure (single purchasing pool vs. multiple purchasing pools; pools organized by income vs. pools organized by geo-spatial proximity; differential pricing within pools vs. single price within pools) maximizes overall social welfare and vaccine availability? (2) For each type of purchasing structure what is the distribution of welfare benefits across countries in different income groups?; and (3) What opportunities exist for improvement in the current organization of global vaccine pricing and procurement that will increase total social welfare, create more equitable distribution of welfare/benefits between the manufacturer(s), low income countries and middle income countries?

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A PROTOCOL TO OPTIMISE MICROSATELITE DNA AMPLIFICATION OF TRYPANOSOMA BRUCELLI GAMIENSI FROM BODY FLUIDS
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Microsatellite genotyping of Trypanosoma brucei gambiense, the causative agent of human African trypanosomiasis or sleeping sickness, and population genetics tools, are useful for inferring population parameters such as population size and dispersal. Amplifying parasite DNA directly from body fluids (i.e. blood, lymph or cerebrospinal fluid) allows avoiding costly and tedious isolation phases. It is however associated to increased frequencies of amplification failures (allelic dropouts and/or null alleles). In this paper, we present a study focused on improving microsatellite loci amplification of T. brucei gambiense from Guinean sleeping sickness foci. We checked for the real nature of blank and of apparent homozygous genotypes of parasite DNA directly amplified from body fluids. We tested the effect of three different DNA quantities for different microsatellite loci of trypanosomes from different body fluids. Our results show that some initially blanks and homozygous genotypes happen to be actual heterozygous genotypes. In Guinea, lymph from the cervical lymph nodes, known to contain the highest concentrations of parasites, appeared to provide the best amplification results. Simply repeating the PCR may be enough to retrieve the correct genotype, but we also show that increasing initial DNA content provides better results while undertaking first amplification. We finally propose an optimal protocol for amplifying T. brucei DNA directly from body fluids that should be adapted to local characteristics and/or constraints.

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ECOLOGICAL NICHES MODELS FOR CUTANEOUS AND VISERAL LEISHMANIASIS IN BRAZIL BASED ON MAXIMUM ENTROPY (MAXENT)
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Leishmaniasis are diseases of great medical, social, and economic importance in endemic areas and are considered serious public health problems due to its clinical impact and epidemiological diversity. They belong to the group of neglected diseases intrinsically associated with poverty as well as health inquiries. The goal of this work was to identify environmental and socioeconomic factors that may be associated with the occurrence of cutaneous (CL) and visceral (VL) leishmaniasis in Brazil from 2005 to 2009, using ecological niche models to predict the risk of disease at the municipality level. A GIS database was constructed using records of CL cases by municipality available in the national notifiable diseases information system (SINAN) database. Records from the Brazilian Institute of Geography and Statistics (IBGE) and the Pan-American Health Organization (PAHO) unsatisfied basic needs data for people (UBNp) and housing (UBNh)) were used as socioeconomic data variables. Environmental data included long-term normal monthly climate from WorldClim and MODIS remote sensing annual composite image data. Probability distribution models for CL and VL based on environmental and socioeconomic features were executed using Maxent and ArcGIS 10. From 2005 to 2009, a total of 96,351 cases of CL and 13,563 cases of VL were registered by SINAN. No cases of either disease were reported in 83% of municipalities; CL was reported in approximately 13% of the municipalities, mainly in the North and Northeast regions, and VL was reported in less than 1% of the municipalities and mostly in the Northeast. Maxent results showed that variables that contributed most to the environmental model for CL were precipitation of September (26.2%) and annual precipitation (17.3%)(AUC 0.80); for VL, precipitation in October (11.6%) and mean temperature of warmest quarter (14.5%) were the most influencing variables (AUC 0.948). The Maxent socioeconomic model was most influenced by the variables UBNp education (39.6%), UNBH plumbing (11.3%) and number of health units (8.8%) for CL (AUC 0.864) and the variables that most contributed in the VL scenario were human development index (25.7%), literacy rate (24%) and sewage services (18.9%) (AUC 0.928). Results suggest Maxent can be used to generate the probability distribution maps based on limited distribution point data and that models then can be used to improve the allocation of resources in control programs.
ASSOCIATION BETWEEN DISSEMINATED LEISHMANIASIS AND POLYMORPHISMS IN LEISHMANIA BRAZILIENSIS STRAINS

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We have previously described a multiclonal population structure among genotypically polymorphic Leishmania braziliensis from an area with high endemicity for American tegumentary leishmaniasis (ATL) in Bahia, Brazil, named Corte de Pedra. Based on RAPD (Randomly amplified polymorphic DNA) profiles, we also found an association between clinical outcome of ATL and parasite genotypes in this region, indicating a role for the intra-species variability among these microorganisms on form of disease. In order to further explore the hypothesis of association between form of ATL and strain of L. braziliensis, we cloned, sequenced and compared homologous RAPD bands previously explored for genotyping the L. braziliensis of Corte de Pedra. With this strategy we found six genomic loci that were polymorphic between representatives of the different clades (i.e. subpopulations) of parasites described in that region. PCR primer sets were designed for the specific targeting of each locus identified. Using these primers each locus was re-amplified, electrophoresed and the band corresponding to the amplicon gel extracted and cloned into pCRII vectors. Then six clones of each locus were sequenced per leishmania isolate. The cloned amplicons permitted identify that the SNPs and indels defining the polymorphisms at each locus segregate within the population of L. braziliensis in Corte de Pedra according to haplotypes. Several SNPs, indels and haplotypes displayed significant associations with disseminated leishmaniasis (DL). In particular, patients infected with L. braziliensis containing certain SNP genotypes and haplotypes found in the locus starting at position 425451 in chromosome 28 presented significantly increased risk ratios for developing DL. Thus this rapidly emerging form of ATL may have its outcome driven in part by the infecting L. braziliensis strain.

ECOLOGY AND TRANSMISSION DYNAMICS OF VISCERAL LEISHMANIASIS IN ETHIOPIA: RESULTS OF A PROSPECTIVE STUDY TO DETERMINE HUMAN INFECTION RATES IN AN ENDEMIC AREA OF NORTH ETHIOPIA

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Globally, visceral leishmaniasis (VL), a systemic protozoan infection caused by Leishmania donovani spp. is estimated to afflict some 500,000 persons annually. In Africa, the worst affected regions are southern Sudan (15,000-20,000 cases/yr) and Ethiopia (4,000-5,000 cases/yr). VL is considered an emerging disease in north Ethiopia where it is associated with seasonal migration of agricultural laborers to endemic areas and HIV/AIDS. A prospective cohort study of VL in North Ethiopia was designed to elucidate the VL infection dynamics in an endemic setting. A cross-sectional survey was conducted around the town of Sheraro during March 2011 involving 4,883 individuals living in 18 villages. Participating households (1,368) were numbered and their coordinates were recorded. Demographic and socio-economic data were collected. Screening for VL by physical and laboratory examination was performed and previously treated VL cases (PTC) were noted. Exposure to Leishmania was assessed by Leishmanin Skin Test (LST). Sera and dried blood spots were tested by Direct Agglutination Test (DAT) and RT-PCR. The LST rate among 4,554 individuals was 10.1% and remained surprisingly low (35%) among 126 PTCs. Serological screening of 4,788 individuals by DAT identified 3.9% positives. Of 4,757 dried-blood samples tested by RT-PCR, 680 samples (14.3%) were found positive for Leishmania k DNA. Of those, 119 (2.5%) harbored over 100 parasites per ml of blood. To validate these findings ITS1/PCR products were sequenced and 90% (19 of 21) were confirmed to be L. donovani. From March 2011 to February 2012, a total of 34 new VL cases (22 males, 12 females) were found amongst the study population. The mean age of these patients was 16.8 (±12.5). Of these 34 cases, 38.2% were DAT positive in March 2011. Similarly, 15.6%, were positive by LST, and 27% were positive by k-DNA RT-PCR. Based on these data, the annual incidence of VL in the study localities is at least 7.0/1000. The study is ongoing, more data will accrue and the results of in-depth analysis will be reported.

THE ECO-EPIEDEMOLOGY OF Trypanosoma cruzi INFECTION IN RURAL COMMUNITIES OF THE HUMID CHACO OF ARGENTINA

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The eco-epidemiology of the domestic transmission of Trypanosoma cruzi in the humid Chaco region has seldom been investigated. We assessed the household distribution of bug, dog and cat infection in two local ethnic groups (Tobas and Creoles) and investigated differences in transmission risks between them, tested the role of domestic dogs and cats as reservoir hosts, and identified transmission risk factors. We conducted a cross-sectional survey of house infestation with Triatoma infestans bugs and T. cruzi infection in bugs, dogs and cats in a well-defined rural area in northeastern Argentina including 323 households.
Bug infection prevalence among 1,869 bugs examined was highest in domiciles (43%) and in storerooms and kitchens (24%), and was marginal elsewhere (<3%). The composite prevalence of infection was similar for 481 dogs (26%) and for 87 cats (29%) that were examined using serology and/or xenodiagnosis. Vector and host infections were highly aggregated at the household level. Using a catalytic irreversible model, the annual force of infection in dogs was three times higher in Tobas than in Creole households, in agreement with other transmission indices. The demography of dogs and cats differed between ethnic groups in several respects. Random-coefficient multiple logistic regression analysis showed that infection in dogs increased significantly with age of the dog, number of infected dogs or cats in a household, and the relative abundance of infected bugs. The fraction of infected bugs increased significantly and in a dose-response fashion with number of infected dogs in a household. Infected cats also increased transmission to bugs when no infected dog was present. Our results reveal the persistence of domestic transmission of T. cruzi in northern Argentina, especially among Tobas; the occurrence of a peridomestic transmission cycle, and the key role of dogs and cats as domestic reservoir hosts, risk factors and control targets in the humid Chaco.

CHAGAS DISEASE: KNOWLEDGE, ATTITUDES, AND PRACTICES AMONG LATIN AMERICAN IMMIGRANTS LIVING IN LOS ANGELES

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This study was undertaken to examine the knowledge, attitudes, and practices associated with Chagas disease (CD) among Latin American immigrants living in Los Angeles. Background: It is estimated that more than 300,000 individuals are infected with CD in the United States (US), though most were infected via vector-borne transmission in Latin America where it is endemic. We assessed the knowledge, attitudes, and practices associated with CD among 2712 individuals in Los Angeles County, ages 18 to 60 years, who had previously resided in Mexico, Central America, or South America for at least 6 months. Sixty-two percent of participants recall seeing triatomine insects in Latin America, and 27% of participants reported being bitten by triatomine insects at least once per year while living in Latin America. Eighty-six percent of participants had never heard of CD. These results were significantly affected by the participant’s country of birth. Of the 13% of participants who had heard of CD, 62% thought CD was a problem in their native country, 55% thought CD was a problem in the US, and 81% thought CD was not serious. Over 90% of participants who had heard of CD would want to be tested and treated for CD. In conclusion, the majority of Latin American immigrants residing in Los Angeles recall seeing the insects that transmit CD in their native country, yet they have never heard of CD. Of the participants who had heard of CD, the majority believe it is a problem in their native country and the US but do not believe it is a serious problem overall. Nevertheless, they would want to be tested and treated for CD.

MODELING THE DISTRIBUTION OF CUTANEOUS LEISHMANIASIS IN BRAZIL BASED ON ENVIRONMENTAL AND SOCIOECONOMIC RISK FACTORS

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Cutaneous leishmaniasis (CL) presents a variety of casual agents, reservoirs and vectors with different transmission patterns. Out of the 12 new world species known to cause disease in humans, seven can be found in Brazil which makes control of this endemic difficult. Thus, the necessity of a new methodology which would consider a better definition of transmission and risk areas. This study aimed to model the distribution of CL at the municipality level in Brazil based on environmental and socioeconomic factors. The GIS database was constructed using records of CL cases available in the national notifiable diseases information system (SINAN from 2006-2009); records from the Brazilian Institute of Geography and Statistics (IBGE) and the Pan-American Health Organization (unsatisfied basic needs for people (UBNp) and housing (UBNh)) were used to compile the socioeconomic data. The environmental database was constructed using long-term normal monthly climate data from WorldClim and MODIS annual composite data. Distribution models for CL were executed using Maxent and maps of spatial distribution and prediction models were created in ArcGIS 10. A total of 96,351 cases of CL were registered at SINAN (13% of the municipalities). Cases of CL increased as the number of health facilities and UNBp education increased (p<0.0001) but notification of disease decreased as UNBn improved (drinking water; plumbing; sanitation and electricity (p<0.0001)). CL was inversely correlated with Temperature Seasonality (p<0.0001) and directly correlated with annual precipitation (p<0.0001). The environmental variables that most contributed in the Maxent model were precipitation of September (26.2%) and annual precipitation (17.3%) (AUC 0.80). From the socioeconomic data the most influencing variables were gross domestic product per capita (23%) and literacy rate (22%) (AUC 0.795, IBGE model); sanitation (83.9%; AUC 0.76, UBNb model); subsistence (33.7%) and unemployment (26%)(AUC=0.77, UBNb model). A final model was executed combining environmental and socioeconomic data and it was found that the variables contributing in the model were UBNb sanitation (39.6%), UBNb subsistence (11.3%) and annual precipitation (8.8%) (AUC 0.86). Socioeconomic factors may be playing an important role in the occurrence of CL in Brazil and together with environmental features may provide a better understanding of the dynamics of this endemic in Brazil.

CONGENITAL TRANSMISSION OF TRYPANOSOMA CRUZI IN ARGENTINA, HONDURAS, AND MEXICO: AN ONGOING STUDY

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1Tulane University, New Orleans, LA, United States, 2UNICEM, Montevideo, Uruguay, 3Instituto Antonio Vidal, Tegucigalpa, Honduras, 4ECS, Buenos Aires, Argentina, 5Université Libre de Bruxelles (ULB), Brussels, Belgium, 6Universidad Autónoma de Yucatán, Mérida, Mexico, 7National Laboratory, Ministry of Health, Tegucigalpa, Honduras, 8Instituto Nacional de Parasitología “Dr. Mario Fatala Chaben”–ANLIS, Buenos Aires, Argentina, 9National Chagas Program, Ministry of Health, Tegucigalpa, Honduras, 10Trypanosoma cruzi has been divided into different lineages: T. cruzi 1 (TcI) and non-TcI (including lineages II-VII). TcI is predominant in Mexico and Central America, while non-TcI is predominant in most of South America, including Argentina. Little is known about congenital transmission of TcI. The specific aim of this study is to determine the rate of congenital transmission of TcI compared to non-TcI. We are conducting a prospective study to enroll at delivery, 10,000 women in Argentina, 7,500 women in Honduras, and 10,000 women in Mexico. We are measuring transmitted maternal T. cruzi antibodies by performing two rapid tests in cord blood (Stat-Pak, Chembio, Medford, New York, and Trypanosoma Dectec, InBios, Seattle, Washington), and, if at least one of the results is positive, we are identifying infants who are congenitally infected by performing parasitological examinations on cord blood and at 4-8 weeks, and serological follow-up at 10 months. We will also perform standard PCR, real-time quantitative PCR, and T. cruzi genotyping on maternal venous blood and on cord blood, and serological examinations on siblings. Study
enrollment has been staggered and began in Tucuman, Argentina in April 2011, and in Intibucá and Santa Barbara, Honduras in May 2011. Study enrollment began in July 2011 in Merida and Valladolid, Mexico. As of April 2012, recruitment numbers per country are as follows: Argentina - 4,280 births; Honduras - 3,634 births (1,739 in Intibucá and 1,895 in Santa Barbara), and Mexico - 4,002 births (2,161 in Merida and 1,841 in Valladolid). Argentina has reported 80 (1.9%) births of seropositive mothers (with at least one positive serological rapid test result in cord blood), Honduras has reported 144 (4.0%) births of seropositive mothers (96 (5.5%) in Intibucá and 48 (2.5%) in Santa Barbara), and Mexico has reported 28 (0.7%) births of seropositive mothers (21 (1.0%) in Merida and 7 (0.4%) in Valladolid).

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GENETIC DIVERSITY AND POPULATION STRUCTURE OF TRYPANOSOMA BRUCEI IN UGANDA: IMPLICATIONS FOR THE EPIDEMIOLOGY OF SLEEPING SICKNESS AND NAGANA

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Human African Trypanosomiasis has remained a major and long term public health problem in Uganda characterized by recurrent sporadic outbreaks in the traditional endemic areas and continued spread to new unaffected areas. Uganda harbors the two forms of Trypanosoma brucei subspecies; Trypanosoma bbrucei rhodesiense and T. b. gambiae causing two forms of sleeping sickness, the acute and the chronic forms respectively. The third T. brucei subspecies; T. b. brucei is the third leading cause of African Animal Trypanosomiasis or nagana and has a wider geographical distribution. T. b. gambiae remains localized in North West Uganda while T. b. rhodesiense is currently restricted to Central and Eastern regions, although it continues to spread towards the T. b. gambiense foci. All the three forms of parasites are closely related subspecies and remain a major challenge to human health and animal production in Uganda. This is the only country where all three taxa occur. Thus, understanding the population structure of T. brucei in Uganda is critical for disease control. We use a newly developed set of microsatellite loci to investigate two important hypotheses regarding the population processes affecting T. brucei in Uganda: 1) Do temporarily distinct disease foci originate from similar or distinct populations of T. brucei? 2) Does host species influence the genetic population structure of T. brucei? By investigating these hypotheses we aim to inform on evolutionary processes at the population level, which will assist in developing effective control measures and treatment of T. brucei. Results are based on isolate collections from 18 Ugandan sites including 300 Trypanosoma isolates from infected tsetse, vertebrates and humans.

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INFECTIONOUSNESS OF SMALL RODENTS TO THE SANDFLY LUTZOMYIA LONGIPALPS REINFORCES THEIR ROLE AS SOURCES OF INFECTION LEISHMANIA (VIANNIA) BRAZILIENSIS


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There are many records of leishmanial infections detected in wild animals by molecular methods but a major question is: Are they infectious to vectors? The present study was aimed at characterizing the infectiousness of experimental infections of Leishmania (Viannia) braziliensis in the small rodents Necromys lasiurius, Rattus rattus and Lutzomyia squamipes. These animals are incriminated as the major reservoirs hosts of cutaneous leishmaniasis in an endemic area of Pernambuco, northeast Brazil. For these experiments we established colonies of the three rodent species and a colony of Lutzomyia longipalpis in an endemic area of Pernambuco, northeast Brazil. For these experiments we established colonies of the three rodent species and a colony of Lutzomyia longipalpis in an endemic area of Pernambuco, northeast Brazil. For these experiments we established colonies of the three rodent species and a colony of Lutzomyia longipalpis in an endemic area of Pernambuco, northeast Brazil. For these experiments we established colonies of the three rodent species and a colony of Lutzomyia longipalpis in an endemic area of Pernambuco, northeast Brazil. For these experiments we established colonies of the three rodent species and a colony of Lutzomyia longipalpis in an endemic area of Pernambuco, northeast Brazil. For these experiments we established colonies of the three rodent species and a colony of Lutzomyia longipalpis in an endemic area of Pernambuco, northeast Brazil. For these experiments we established colonies of the three rodent species and a colony of Lutzomyia longipalpis in an endemic area of Pernambuco, northeast Brazil. For these experiments we established colonies of the three rodent species and a colony of Lutzomyia longipalpis in an endemic area of Pernambuco, northeast Brazil. For these experiments we established colonies of the three rodent species and a colony of Lutzomyia longipalpis in an endemic area of Pernambuco, northeast Brazil. For these experiments we established colonies of the three rodent species and a colony of Lutzomyia longipalpis in an endemic area of Pernambuco, northeast Brazil. For these experiments we established colonies of the three rodent species and a colony of Lutzomyia longipalpis in an endemic area of Pernambuco, northeast Brazil. For these experiments we established colonies of the three rodent species and a colony of Lutzomyia longipalpis in an endemic area of Pernambuco, northeast Brazil. For these experiments we established colonies of the three rodent species and a colony of Lutzomyia longipalpis in an endemic area of Pernambuco, northeast Brazil. For these experiments we established colonies of the three rodent species and a colony of Lutzomyia longipalpis in an endemic area of Pernambuco, northeast Brazil. For these experiments we established colonies of the three rodent species and a colony of Lutzomyia longipalpis in an endemic area of Pernambuco, northeast Brazil. For these experiments we established colonies of the three rodent species and a colony of Lutzomyia longipalpis in an endemic area of Pernambuco, northeast Brazil. For these experiments we established colonies of the three rodent species and a colony of Lutzomyia longipalpis in an endemic area of Pernambuco, northeast Brazil. For these experiments we established colonies of the three rodent species and a colony of Lutzomyia longipalpis in an endemic area of Pernambuco, northeast Brazil. For these experiments we established colonies of the three rodent species and a colony of Lutzomyia longipalpis in an endemic area of Pernambuco, northeast Brazil. For these experiments we established colonies of the three rodent species and a colony of Lutzomyia longipalpis in an endemic area of Pernambuco, northeast Brazil. For these experiments we established colonies of the three rodent species and a colony of Lutzomyia longipalpis in an endemic area of Pernambuco, northeast Brazil. For these experiments we established colonies of the three rodent species and a colony of Lutzomyia longipalpis in an endemic area of Pernambuco, northeast Brazil. For these experiments we established colonies of the three rodent species and a colony of Lutzomyia longipalpis in an endemic area of Pernambuco, northeast Brazil. For these experiments we established colonies of the three rodent species and a colony of Lutzomyia longipalpis in an endemic area of Pernambuco, northeast Brazil. For these experiments we established colonies of the three rodent species and a colony of Lutzomyia longipalpis in an endemic area of Pernambuco, northeast Brazil. For these experiments we established colonies of the three rodent species and a colony of Lutzomyia longipalpis in an endemic area of Pernambuco, northeast Brazil. For these experiments we established colonies of the three rodent species and a colony of Lutzomyia longipalpis in an endemic area of Pernambuco, northeast Brazil. For these experiments we established colonies of the three rods...
vellerorsus. A total of 15 xenodiagnosis-negative animals were kDNA-PCR-positive, including 4 D. albiventeri opossums, 1 Euphractus sexcinctus and 3 D. novemcinctus armadillos, 5 Thlyamyx pusilla (Chaco fat-tailed opossum), and 2 small rodents (unidentified species). Using SAT-DNA-PCR we confirmed T. cruzi infection in one D. novemcinctus and two T. pusilla positive by kDNA-PCR only. These are the first findings of T. cruzi in T. pusilla and T. tulli in C. vellerorsus and T. mactatus from Argentina. A PCR-based strategy showed that all opossums were infected with DTU TcI and all armadillos with TcIII, implying separate parasite transmission cycles. Wild mammals had no evidence of parasite DTUs infecting local domestic dogs, cats or Triatoma infestans bugs (TcV and TcVI). Sylvic transmission cycles of T. cruzi in the dry and humid Chaco differ in the composition of the main reservoir hosts.

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LEISHMANIA BRAZILIENSIS IS THE ETIOLOGICAL AGENT OF CUTANEOUS LEISHMANIASIS IN LOS MONTES DE MARÍA, COLOMBIA

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Cutaneous leishmaniasis (CL) and visceral leishmaniasis (VL) constitute important public health problems in the Caribbean coastal region of Colombia. These clinical presentations of leishmaniasis are endemic in rural and urban areas of the departments of Sucre and Bolívar, especially in Los Montes de María, an area that constitutes the most important CL macrofocus of the Caribbean coastal region. The objective of the present study was to use sequencing of the subtelomeric region to determine the Leishmania species producing CL among the inhabitants of Montes de María. Thirty-six CL patients from the municipalities of Carmen de Bolívar, Macayepo, Morroa, Sincelejo and Ovejas were analyzed, each receiving a direct parasitological examination before samples were taken for parasite culture in NNN medium to allow molecular identification of the species involved. A sequence from the subtelomeric region of approximately 388 bp was obtained, presenting a 99-100% similarity with sequences of the subtelomeric region of three reference strains of Le. (V.) braziliensis. It was thus determined that is the species responsible for CL in the Montes de María area. Its presence in the area has important implications in selecting the correct medical treatment to be administered.

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HEALTHY LIVING TO CONTROL CHAGAS DISEASE IN ECUADOR

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Chagas disease is caused by the parasite Trypanosoma cruzi and transmitted mainly by the feces of triatomine insects. This disease affects ~10 million people mostly in Latin America. Studies conducted since 2002 by our group have described the biological and epidemiological factors that facilitate transmission of T. cruzi in Southern Ecuador, and have determined that insecticide-based control strategies are effective only in the short term due to frequent bug re-infestation of treated dwellings. To address this issue, we designed in 2010 the Healthy Living Initiative (HLI), a holistic process aimed at facilitating the socioeconomic development of rural communities affected by Chagas disease. The final goal of the HLI is to design, implement and evaluate a sustainable model to eliminate vectorial Chagas disease transmission in Loja province via improvement of the houses and the peridomestic areas. This model is based on community organization and socioeconomic participative development as basic conditions to promote human health. So far it has been possible to facilitate process in five areas: health (community promoters and entomological surveillance network); infrastructure (land entitlement and improvements to local water systems and access road); income generation (ecotourism, artisans’ groups, and local products commercialization); capacity building, and safety/security. Based on these advances, the current phase of the HLI identifies characteristics of a Healthy Housing Model adapted to the cultural and social realities of this area. Positive Deviance methodological framework was used with particular attention to existing knowledge, attitudes, and practices (KAP) of houses that have remained bug free during the last four years. In this process the HLI seeks to unite the efforts of various local, national and international organizations active in Loja by integrating their activities to government plans, as well as facilitating families’ participation through critical analysis of their own reality.
SPATIOTEMPORAL CLUSTERING OF VISCERAL LEISHMANIASIS AND LEISHMANIA DONOVANI INFECTIONS IN BIHAR, INDIA

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In the Indian subcontinent, visceral leishmaniasis (VL), also known as kala-azar, is caused by Leishmania donovani, which is transmitted from man to man by the sand fly Phlebotomus argentipes. VL tends to cluster in certain hamlets in remote rural villages but the spatiotemporal dynamics of the disease and leishmaniasis infection are not fully understood. We analysed the clustering of VL cases and L. donovani infections in a VL endemic area covering over 80,000 people in Muzzafarpur district, Bihar. The people living in the study area are regularly monitored and demographic information is been gathered as part of NIH funded project on VL in India. VL cases occurring from 2007 to 2011 were identified by yearly house to house surveys in the whole study area. Incident L. donovani infections were identified as seroconverters (using DAT and rK39 ELISA) in high transmission areas by means of two serosurveys in 2008 and 2009 (n=1,000 people). Yearly edge-corrected kernel density maps, the K-function and the scan-statistic were used to evaluate the spatiotemporal dynamics of VL and L. donovani infection over the study period. The implications of VL clustering and spatial variation for the VL control program in the Indian subcontinent will be discussed.

EXAMINING LEVEL OF USE OF CHEMOTHERAPY, CHEMOPROPHYLAXIS AND INTERMITTENT-PREVENTIVE TREATMENT OF MALARIA IN PREGNANCY BY PREGNANT WOMEN IN NIGERIA

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The study assessed the nature of health seeking for chemotherapy and chemoprevention for malaria-in-pregnancy (MIP), especially the acceptability and use of intermittent preventive treatment of MIP by pregnant women attending public and private health facilities. The study was undertaken in Enugu, southeast Nigeria. A total of 647 consenting pregnant women (321 in the public hospitals and 326 in the private hospitals) were administered with structured questionnaires. Data was analyzed for the levels of perceptions, acceptability and use of IPTp amongst the pregnant women. Bivariate analysis was used to examine whether the differences in the variables between pregnant women attending public and private facilities were statistically significant. The results showed that the knowledge about MIP was high among the pregnant women. Pregnant women attending private hospitals were less aware of IPTp as a preventive strategy for MIP (p<0.05), but there was no significant difference in the acceptability of IPTp by the pregnant women in public and private facilities (p>0.05). IPTp was consumed more by pregnant women in the private facilities (76.9%) compared to those in the public facilities (27.6%) (p<0.05). Blood tests were used more by consumers in the private facilities (71.3%) compared to those in the public facilities for diagnosis of MIP (50.2%) (p<0.05). It is concluded that health seeking behaviour for MIP by pregnant women attending private facilities was better than for those attending public facilities. Hence, interventions are needed to improve the management of MIP in public facilities, and also enhancing the services of private providers.
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IMPACT OF INTERMITTENT PREVENTIVE TREATMENT IN PREGNANCY WITH SULFADOXINE-PYRIMETHAMINE ON PLACENTAL INFECTION AND INFANT BIRTH OUTCOMES IN MALAWI

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Malaria in pregnancy is associated with severe maternal anemia, placental parasitemia, low birth weight, and increased perinatal mortality, especially among primi- and secundi-gravidae. Sulfadoxine-pyrimethamine (SP) is currently recommended for intermittent preventive treatment in pregnancy (IPTp), despite increasing prevalence of SP resistance that might compromise its effectiveness. HIV-uninfected women with a singleton pregnancy were enrolled at delivery and data on number of SP doses during the pregnancy collected via interview and review of the woman’s antenatal care card. The primary outcome was evidence of past or current placental infection by placental histology. Secondary outcomes included malaria parasitemia at the time of delivery in the cord blood, placenta or maternal peripheral blood, and composite birth outcome (any of small for gestational age (SGA) as assessed by Ballard exam, low birth weight, or preterm). Of 713 women enrolled, 22% received <2 SP doses; 33% were primigravid. About one-third reported sleeping under a bednet the previous night. Receipt of <2 SP doses versus ≥2 doses had no impact on placental infection as measured by placental histology (31.5% vs 31.8%, P=0.94) or blood films (3.8% vs 5.9%, P=0.30) at the time of delivery. Receipt of IPTp-SP was associated with a dose dependent protective effect in primigravid women only on the composite birth outcome due to a reduction in SGA; using 0 doses as the comparison, adjusted prevalence ratio (aPR) =0.69 (95% confidence interval (CI) 0.5–1.01), aPR=0.43 (95% CI 0.3–0.6), and aPR=0.32 (95% CI 0.1–0.9) for 1, 2, and 3 doses, respectively. Receipt of SP was not associated with stillbirths or adverse delivery outcomes. IPTp-SP did not reduce placental infection, but was associated with improved birth outcomes in primigravidae in Malawi, suggesting that IPTp-SP may work primarily by treating infection, rather than prophylaxis. Very few women received 0 doses of SP, so these results may underestimate the true effect of IPTp-SP. IPTp-SP should continue to be provided to pregnant Malawian women, but given the high prevalence of SP resistance in Malawi, alternative antimalarials should be investigated for IPTp.
clients after they had presented their symptoms. The rest did not. The rate of purchase of anti-malarials was not associated with the type of facility. The same proportion of LCSs and pharmacies (i.e. 2.3%) referred the clients to a clinic for diagnostic tests. Management practices of pharmacies and LCSs were encouraging however most of them did not ask about the medication history of the clients. Pharmacy council of Ghana should educate the pharmacies and LCSs on the need to ask their clients about the medication history of their illness before prescribing anti-malarials to them.

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**HOW PATIENTS TAKE MALARIA TREATMENT: A SYSTEMATIC REVIEW OF THE LITERATURE ON ADHERENCE TO ANTIMALARIALS**

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Artemisinin-based combination therapies (ACTs) are the first-line drugs for treatment of malaria throughout sub-Saharan Africa, and are becoming increasingly available in the private sector. However, there are concerns about sub-optimal patient adherence which may have consequences for treatment efficacy and the development of antimalarial drug resistance. In order to identify patterns in how patients use antimalarial drugs and highlight gaps in current knowledge, a systematic literature review was performed. A search was conducted in PubMed using MeSH and free text terms. Of 1242 studies initially identified, 40 met the inclusion criteria of providing quantitative data on patient adherence to antimalarials obtained for treatment. Manual search of reference lists and contacting researchers in the field yielded 11 additional studies. Patient adherence to ACTs was assessed in 23 studies, non-artemisinin-based combinations in 12, and chloroquine and other monotherapies in 20. Only two studies involved the private sector. Adherence measurement methods included self-report with and without dose timing, pill counts and biological assays. Although some studies found very high adherence to ACTs, others endeavouring to capture “real life” situations reported adherence of 64-88%. Overall, adherence was higher in studies where consent was obtained at enrolment versus at follow-up, and in studies where patient consultations were observed by the study team. Comparison of results based on different measurement methods showed higher adherence when biological assays were used, but no other clear patterns. Multivariate models in 10 studies found 28 factors associated with adherence, but no factor was significant in more than one study. The suboptimal patient adherence to ACTs obtained in the public sector and the current dearth of data from the private sector represent significant challenges to ensuring ACTs are used appropriately and remain effective. To strengthen future studies, there is a clear need for awareness of the impact of study procedures on adherence outcomes, and the identification of improved measurement methods that are less dependent on self-report.

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**SONTOCCHIN AS A GUIDE TO DEVELOPMENT OF DRUGS AGAINST CHLOROQUINE RESISTANT MALARIA**

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Sontochin was the original chloroquine replacement drug, arising from research by Andersag two years after chloroquine (known as “Resochin” at the time) had been shelved due to the mistaken perception that it was too toxic for human use. We were surprised to find that sontochin, i.e., 3-methyl-chloroquine, retains significant activity against chloroquine-resistant strains of *Plasmodium falciparum in vitro*. We prepared derivatives of sontochin, “pharmacins”, with alkyl or aryl substituents at the 3-position and with alterations to the 4-position side chain to enhance activity against drug resistant strains. Modified with an aryl substituent in the 3-position of the 7-chloro-quinoline ring PH-203 exhibits low nanomolar IC₅₀ values against drug sensitive and multidrug resistant strains and in vivo efficacy against patent infections of *P. yoelii* in mice that is superior to chloroquine. Our findings suggest that novel 3-position aryl pharmacin derivatives have the potential for use in treating drug resistant malaria. A detailed structure-activity profile will be presented.

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**WEIGHT BASED DOSING CAUSES SIGNIFICANTLY LOWER CHLOROQUINE CONCENTRATIONS IN YOUNGER CHILDREN**

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Chloroquine (CQ) was previously the main drug for treatment of *Plasmodium falciparum* malaria but it is no longer recommended due to resistance. In Guinea-Bissau, West Africa double dose CQ (50mg/kg) split into 2 daily doses for 3 days was well tolerated, as efficacious as artemether-lumefantrine and eradicated 87% of *P. falciparum* with resistant genotypes in 2008. As part of 3 previous clinical trials, 100 µl of blood was collected on day 7 from children aged 6 months-15 years that had taken 25 or 50 mg/kg of CQ. Whole blood CQ concentrations were analysed using high performance liquid chromatography. CQ concentrations were obtained from 188 and 293 children after intake of 25 and 50 mg/kg of CQ, respectively. CQ concentrations after intake of 25 mg/kg and stratification by age were: 545 (10y) nmol/l. Using the same age groups, concentrations after 50mg/kg were 834, 1220, 1164, 1573, 1565 and 1546 nmol/l. The increases with age were significant, nonparametric test for trend P=0.008 and P=0.0001, respectively. Using the same age groups, the dose of CQ taken according to body surface area ranged from 489-702 and 978-1405 mg per square meter after intake of 25 and 50 mg/kg, respectively. The CQ concentration in children 10 years of age after intake of 25 and 50 mg/kg, respectively. CQ concentrations were only 21% higher in children <2 years taking 50mg/kg compared to children >10 years taking 25mg/kg. Dosing according to body weight rather than body surface area most probably accounts for the lower concentrations seen in younger children. Vomiting and spitting among the youngest children are unlikely explanations as treatment was repeated after vomiting and because it does not explain the trend throughout the age groups. Chloroquine should be dosed according to body surface area and the effect of dosing according to body weight should be assessed for other antimalarials.

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**RANDOMIZED, CONTROLLED TRIAL OF TREATMENT OF FEBRILE CHILDREN WITH A NEGATIVE MALARIA RAPID DIAGNOSTIC TEST WITH ARTEMETHER-LUMEFANTRINE VS. NO ANTIMALARIAL IN BAGAMOYO DISTRICT, TANZANIA**

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Until recently, most national malaria control programs recommended treating all febrile children less than five years of age with first-line antimalarial drugs to prevent severe malaria, disability and death. In 2010, WHO recommended uniform confirmation of malaria parasitemia by blood smear or rapid diagnostic test (RDT) prior to treatment. Unfortunately
little is known about the epidemiology of other common causes of non-malarial fevers in malaria endemic areas. Moreover, the effect of withholding malaria treatment from febrile children, even when they test negative, may have unintended public health consequences. From January 2010 to December 2011, we enrolled 1000 children aged 6 to 59 months with uncomplicated febrile illness and negative malaria RDTs from two health facilities in Tanzania. Subjects were randomized to receive either artemether-lumefantrine (AL) or no treatment and followed for 91 days to document symptom resolution, time to next malaria infection, and frequency of hospitalization or death. Subjects who missed more than two follow-up visits were not included in per protocol analyses. Preliminary results are available for 708 (70.8%) subjects. Among these 708 subjects, 353 (49.9%) were randomized to AL, 457 (64.6%) completed 91 days of follow-up per protocol without developing malaria, 15 (2.1%) were healthy to day 91 but missed more than two visits, 14 (2.0%) withdrew consent, 8 (1.1%) were given a non-study antimalarial, 118 (16.7%) were lost to follow-up, 93 (13.1%) developed malaria during follow-up, and 3 (0.4%) died of non-study related illness. Children randomized to receive AL had a lower risk of developing malaria during follow-up (RR=0.72, 95% confidence interval 0.49-1.04, p=0.09). The time to malaria infection by 10% of subjects in each arm was 56 days for the AL arm and 28 days for those who did not receive treatment, but the difference was not statistically significant (p=0.07). Data are preliminary. Study results will be used to improve the management of non-malarial febrile illness.

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AN INHIBITOR OF MULTIPLE CYTOCHROME P450S, 1-AMINOBENZOTRIAZOLE, ALTERS THE PHARMACOKINETICS OF PRIMAQUINE AND CHLOROQUINE IN A RHESUS MODEL OF MALARIA RADICAL CURE

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Rhodesian (P. cynomolgi) anti-relapse activity in mice. Subsequently, we attempted to use this inhibitor to explore if CYP450 metabolism is involved in PQ’s anti-relapse activity in P. cynomolgi-infected Rhesus monkeys. Infected monkeys were administered 1-ABT prior to treatment with a 7 day radical curative regimen of PQ plus chloroquine (CQ). Efficacy +/- 1-ABT administration was determined via daily parasitemia readings and safety was assessed using clinical laboratory results, including % methemoglobin (metHgb). The 7 doses of 1-ABT and primaquine planned were halted after the second dose because some monkeys had elevated alanine aminotransferase levels, which returned to baseline after stopping dosing. Increases in metHgb occurred only in monkeys treated with PQ plus CQ. Infected PQ decreased daily in animals pre-dosed with 1-ABT, suggesting 1-ABT blocks PQ-induced methHgb formation. Blood draws were included to assess plasma pharmacokinetics (PK) of PQ and CQ +/- 1-ABT. Pre-treatment with 1-ABT decreased PQ and CQ levels and prolonged half-lives. Animals pre-dosed with 1-ABT had a 4 to 7 day delay in onset of malaria relapse, relative to controls given CQ only, presumably because 1-ABT inhibits metabolism of the antimalarial active CQ parent drug. Besides PK interactions of 1-ABT with PQ and CQ, we noted a link between PQ and CQ metabolism. Animals given PQ plus CQ had a two-fold greater plasma exposure to CQ’s major CYP450 metabolite (desethyl-CQ) after 1 dose and 8-fold higher levels after 7 daily doses relative to animals given CQ alone. In contrast, pre-dosing with 1-ABT precluded formation of desethyl-CQ. We report that the CYP450 inhibitor, 1-ABT, alters PK properties of PQ and CQ, and that PQ potentially induces CQ metabolism. Our results also suggest PQ metabolism is linked to metHgb generation.

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EXPLORING THE PERFORMANCE OF RETAIL SECTOR PROVIDERS EXPOSED TO A PILOT INTERVENTION TO PROVIDE SUBSIDIZED ARTEMETHER-LUMEFANTRINE THROUGH THE PRIVATE SECTOR IN WESTERN KENYA

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To improve the quality of care received for presumptive malaria from the highly accessed private retail sector in Western Kenya, subsidised pre-packaged artemether-lumefantrine (AL) was provided on a pilot basis to private retailers, together with a one day training for retail staff on malaria diagnosis and treatment. The pilot was assessed using a cluster randomised, controlled design with nine control and nine intervention sublocations, equally distributed across three districts. Provider and mystery shopper cross-sectional surveys were conducted at baseline and eight months post intervention to assess the impact of the intervention on retailer performance. Data were analysed based on cluster-level summaries, comparing control and intervention arms, while adjusting for covariates, including baseline values where a difference of 5% or more was observed between the arms at baseline. On average 564 retail outlets were interviewed per year. At follow up, 43% and 1% of respondents reported that at least one staff member had attended the intervention training, in the control and intervention arm, respectively. The intervention significantly increased the percentage of providers knowing the first line treatment for uncomplicated malaria by 24.2% points (confidence interval (CI):14.8%, 33.6%; adjusted p=0.0001); the percentage of outlets stocking AL by 24.2% points (CI: 22.0%, 41.3%; adjusted p=0.0001), and the percentage of providers prescribing AL for presumptive malaria by 23.6% (CI:18.7%, 28.6%; adjusted p=0.0001). No significant difference was observed between the arms at baseline in the percentage asking for specified danger signs that determine need for referral to a health facility, and there remained substantial room for improvement in provision of appropriate dispensing advice to those who bought AL. Overall, subsidising ACTs and retailer training can significantly increase the percentage of outlets stocking and selling AL for the presumptive treatment of malaria, but further research is needed on strategies to improve the provision of counselling advice to retail customers.
CHLOROQUINE: PHARMACOKINETIC AND TRANSPORTER INHIBITION STUDIES

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The primary objective was to compare the corrected Acceptable Clinical and Parasitological Responses (ACPR) on Day 28 of artemether-lumefantrine (AL) and difludorartemisinin-piperaquine (DP) in children with uncomplicated falciparum malaria. This open-label, comparative trial study in Western Kenya randomized 454 children with uncomplicated falciparum malaria of age 6-59 months to receive either AL (n=227) or DP (n=227). Children were hospitalized for 3 days for observed treatment and 72-hour parasite kinetic monitoring, and actively followed up at scheduled visits after discharge from hospital on Days 7, 14, 28 and 42. Genotyping for determining treatment outcome was performed on Day 0 and any other day the study participant had a recurrence of parasitemia. No significant differences were observed for the corrected ACPR rates on Day 3, 14, 28 and 42 for AL (99.1%, 100%, 97.8%, 96.8%) and DP (100%, 100%, 99.1%, 98.7%). Similarly, for the uncorrected ACPR rates no significant differences were seen on Day 3, 14, 28 and 42 for AL (99.1%; 98.7%; 81.1%; 67.8%) and DP (100%; 100%; 87.7%; 70.5%), (p>0.05 for all comparisons). Both AL and DP are efficacious treatments for uncomplicated falciparum malaria in Kenyan children. No signs of P. falciparum resistance to artemisinins were noted.

INHIBITORS OF PRIMAQUINE METABOLISM AS MODULATORS OF EFFICACY AND HEMOLYTIC TOXICITY

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The 8-aminoquinoline drug primaquine (PQ) is the only drug approved for the treatment of relapsing relapsing malaria. However, PQ is known to cause hemolytic toxicity in G6PD deficient individuals. Proposed mechanisms of both efficacy and toxicity suggest a role for transient reactive oxygen species formed as a byproduct of metabolism. We previously showed that CYP 2D6 plays a major role in the production of the redox active metabolites most likely to produce oxidative stress, however the relevance of this role in vivo was not clear. To this end, the effects of Paroxetine (PX), a potent selective inhibitor of CYP 2D6 metabolism, co-administration was tested in models of both in vivo liver stage efficacy and G6PD deficient hemolytic toxicity. In C57BL/6 mice, co-administration of PX at 25 mg/kg with PQ at 2.5 mg/kg showed a reduction in liver stage potency at both 28 and 48 hr post infection with P. berghei sporozoites. Co-administration of PQ with the MAO-A inhibitor Clorgiline (CG) however, resulted in enhanced liver stage efficacy. Further, significant mitigation of the hemolytic toxicity associated with PQ dosing in a G6PD deficient strain of C3H mice was also observed after co-administration of PX. These data suggest that CYP 2D6 plays an integral role in the metabolic pathways necessary for PQ’s efficacy and hemolytic toxicity. While the effects of MAO-A inhibition on toxicity remain unknown, metabolic compensation may account for increased efficacy as a result of decreased primaquine clearance.

REPORTED ADVERSE EVENTS ASSOCIATED WITH ARTEMISININ COMBINATION THERAPIES (ACTS) IN NORTHERN GHANA

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Many African countries have adopted artemisinin derivative based combination therapy (ACT) as treatment for uncomplicated malaria, offering an opportunity to assess the safety of these drugs when in real life setting. Knowledge of side effects of these drugs is important for improved management of malaria. This study was conducted to document adverse events associated with Artesunate Amodiaquine (ASAQ), Artemether Lufenatrine(AL) and Dihydroartemisin Piperaquine (DHP) through comprehensive pharmacovigilance in the Kassena Nankana districts of Northern Ghana. As part of INDEPTH Effectiveness and Safety studies, a cohort event monitoring study was conducted at selected public and private health facilities after administration of artemisinin combination therapy to participants to treat uncomplicated malaria during visits to the hospital. Participants were recruited if they were prescribed an ACT. Each participant was followed up between the 3rd and 7th day after enrolment to document adverse events. A total of 4951 participants with uncomplicated malaria prescribed ASAQ, AL and DHP were recruited across all age groups from August 2010 to June 2011. Out of the 4951 participants recruited, 26.0 % reported at least one adverse event; none had a serious adverse event. Of the 1288 participants reporting an adverse event 78.0%, 19.4% and 2.6% took ASAQ, AL and DHP respectively. 27.1% of females reported an adverse event compared to 19.8% of males recruited. Participants in the 15-49 years age group reported 43.3% of adverse events. The most reported adverse events were dizziness (24.2%) and weakness (23.5%) and these were more associated with ASAQ. The unadjusted odds ratio for participants who took AL were 2.3 (95% CI: 1.9-
EVALUATION OF TWO QUALITY ASSURANCE APPROACHES FOR MALARIA RAPID DIAGNOSTIC TESTS IN PERIPHERAL HEALTH FACILITIES IN RURAL TANZANIA

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WHO recommendations for malaria diagnosis and management are based on the use of microscopy. However, microscopy is not widely accessible in malaria-endemic areas. Rapid diagnostic tests (mRDTs), but appropriate quality assurance (QA) systems for mRDTs remain a concern. Tanzania has begun a national scale-up of mRDTs at all government health facilities. We evaluated mRDT performance in two districts of Tanzania with low malaria transmission using two QA methods: a) reference microscopy and b) detection of parasite DNA by real-time quantitative polymerase chain reaction (qPCR). Blood samples were collected from patients undergoing mRDT during two to three consecutive days each month in 12 health facilities between January and August 2010. Thick blood films were examined at the district headquarters and the Ifakara Health Institute (IHI) Bagamoyo Laboratory. A third blood film reader was consulted for discordant results. Molecular analysis involved extraction of parasite DNA from dried blood spots for presence of Plasmodium falciparum DNA with a pilot real-time assay targeting the tubulin gene. The assay was performed at IHI and about 40% of the DNA aliquots were sent to CDC for validation of IHI results. Samples from 1,837 patients were analyzed. Malaria positivity rates were 6.5%, 3.4%, and 2.7% for mRDT, qPCR, and microscopy, respectively. When qPCR was a gold-standard, mRDTs had higher sensitivity (68.6%; 95% CI: 55.0-79.7) than microscopy (53.7%; 95% CI: 38.7-68.0), but the difference was not significant. When microscopy was the gold standard, mRDT sensitivity was the highest (85.3%; 95% CI: 70.0-93.6). With qPCR as a gold standard, positive predictive values were significantly different between the two tests: microscopy vs qPCR-IHI (75.9%; 95% CI: 58.0-78.8), and mRDTs vs. qPCR-IHI (36.5%; 95% CI: 27.5-46.4). Higher inter-observer agreement (kappa=0.75) was seen amongst the microbiologists. We identified many technical problems with qPCR analysis. QPCR may not be an appropriate QA tool to assess mRDT performance for routine care in this setting. A microscopy-based QA system may be a more suitable option.
robust supply chain management system, and implementation of QA/QC procedures. Areas that were performed well include laboratory registry documentation, generally consistent power supply, and patient safety procedures. The effectiveness of malaria microscopy training will be significantly augmented if the laboratory infrastructure enables microscopists to apply their training in routine practice. To this end there is an immediate need to improve the quality of laboratory equipment, supplies and improved standardized methodology to consistently prepare good quality stained malaria slides. The dual implementation of strengthened laboratory infrastructure and training will increase both malaria diagnostic capacity and competency that will directly lead to increasing accurate diagnosis. The dual focus on training and infrastructure strengthening will be the focus of ongoing WRAIR contributions to the PMI efforts in Tanzania and will directly improve the effectiveness of treatment and prevent over- or misuse of antimalarials.

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BASELINE ASSESSMENTS ON THE USE OF MALARIA RAPID DIAGNOSTIC TESTS (mRDT) IN HOSPITALS AND DISPENSARIES IN TANZANIA

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A key Tanzanian National Malaria Control Program (NMCP) objective is to increase the percentage of malaria microscopy and malaria rapid diagnostic test (mRDT) confirmed cases of malaria in public health facilities from 20% to 80% by 2012. Malaria diagnosis by microscopy requires well trained technicians and quality equipment, supplies and procedures. Increasingly Tanzania is relying on mRDTs for point-of-care malaria diagnosis in hospitals, health centres and peripheral dispensaries. Similar to microscopy, mRDTs can suffer from logistical supply chain problems, lack of quality assurance/quality control (QA/QC) procedures, and infrastructure deficiencies. In 20xx we conducted baseline assessments of healthcare workers’ performance of mRDT in 6 district hospitals in the Coastal Region, plus 7 regional/district hospitals and 13 health centers/dispensaries in the Kagera Region. Parameters assessed included testing procedures and performance, supply chain management, QA/QC, staff training, documentation, and storage and waste management. Significantly 44% (7/16) of health facilities scored ≤60% for testing performance and only one of 16 health facilities achieved 90%. Our overall analysis of the baseline assessments indicate need for focused improvement in the support provided to testing staff, including job aids, timers and adequate ambient lighting; increased supervision of testing performance; increased availability of training; strengthened training in test interpretation; and the implementation of QA/QC procedures. Improving mRDT testing and supply management will directly lead to increasing accurate diagnosis to improve the effectiveness of treatment in Tanzania. Implementing these changes will be the focus of ongoing efforts to strengthen malaria diagnostic services in Tanzania.

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MISCLASSIFICATION OF PLASMODIUM SPECIES BY CONVENTIONAL MICROSCOPY

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Microscopic differentiation of Plasmodium species relies on morphological characteristics manifested in stained blood films. Proficiency levels of microscopists as well as morphological variations within and between Plasmodium species may lead to misclassifications. Ten- day microscopy workshops were conducted from 2009 to 2010. Proficiency of the participants was assessed at the start and the end of each workshop using mono infection slide sets of P. falciparum, P. malariae, P. ovale and P. vivax. Each slide with densities between 1,000 to 30,000 parasites/µL was examined for 5 minutes. Errors observed on each of the Plasmodium species were false negatives, positive results with no species indicated, inability to differentiate between Plasmodium species and reports of mixed infections. Pre- workshop misclassification of P. falciparum as positive was significantly higher (p < 0.05) than all other reported misclassifications except P. malariae. Misclassification of P. malariae as negative was significantly higher than all other reported misclassifications. Misclassification of P. ovale and P. vivax as mixed infections was significantly lower than all the reported misclassifications. Post workshop misclassification of P. falciparum as mixed infections was significantly higher than all other reported misclassifications and there was no clear misclassification of P. malariae. P. ovale was highly misclassified as P. vivax and P. vivax equally misclassified as P. ovale. Microscopy workshops can minimize observed errors and improve reliability of both clinical and epidemiological data. Confirmation of results by expert microscopy in addition to molecular characterization of species is highly recommended.

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MALARIA MICROSCOPY QUALITY ASSURANCE USING A SMALL NUMBER OF SLIDES

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WHO’s “Universal Access to Malaria Diagnostic Testing, an Operational Manual” released in 2011 recommends “cross-checking of 10 to 20 slides if time (during a supervisory visit) allows.” Weak infrastructure in most Sub-Saharan African countries’ will hinder adherence to the previous WHO’s Malaria Microscopy Quality Assurance (MMQA) protocol released in 2009 of selecting five negatives and five weak positives per lab and per month and sending them to a certified microscopist at a referral lab for cross-checking. For health facilities in many African countries, there are not enough slides available and no fuel or transportation to reference labs. Even if slides can be sent, there are not enough skilled microscopists at the reference labs to read all the slides received. The backlog of unread slides contributes to long delays feedback for their work. The Improving Malaria Diagnostic (ImDaD) project tested if slide cross-checking during Outreach Training and Support Supervision (OTSS) visits could be used to identify underperforming labs and engage in on-the-spot problem-solving to address any deficiencies in slide preparation, staining or examination. As lab supervisors stayed in the lab for only one or two days, the number of slides cross-checked during the supervisory visit was on average 9.15 (standard deviation 1.96 slides) 76% of facility visits saving slides of QA. In Benin, 78% of laboratories visited for OTSS cumulated 12-20 slides in two consecutive visits. A sample size of 12 slides per health facility identified facilities under the decision rule for 90% parasite detection, and/or below average parasite detection. If OTSS is done quarterly, the minimum annual
aggregated number of slides would be 24, out of a target number of 40 slides (ten/visit), as opposed to 120 done by following the standard WHO MMQA 2009 protocol. Forty slides cumulated in a year selected randomly as per the LQAS stratified random sampling -with 50% slide positivity ratio- give a sufficiently precise estimate of parasite detection at laboratory level, allowing calculation of the % of laboratories attaining 90% agreement, and aggregate measurements at health zone level to focus MMQA efforts where they are needed the most. Resource-poor countries would be better served by considering a smaller sample size for MMQA selected with LQAS as opposed to not doing MMQA at all or doing MMQA in a way that fails to deliver feedback to participating labs.

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COMPARATIVE LABORATORY-BASED EVALUATION OF DIAGNOSTIC TESTS FOR G6PD

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Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common human enzyme deficiency in the world, affecting over 400 million people worldwide. It is characterized by abnormally low levels of G6PD, an enzyme involved in red blood cell metabolism. Individuals with diminished G6PD activity are susceptible to cellular oxidative damage, and can exhibit symptoms including hemolytic anemia and jaundice in response to a number of causes, most commonly infection or exposure to certain medications. In particular, treatment with anti-malarial drugs such as those in the 8-aminoquinolone group (e.g. Primaquine, Pamaquine and Tafenoquine) can cause acute hemolysis in people with G6PD deficiency. Because of this risk it is imperative to identify individuals with G6PD deficiency prior to administering these anti-malarial agents. As such, there is a need for a test that is appropriate for G6PD deficiency screening in areas of the developing world where malarial treatments are frequently administered. To explore the suitability of G6PD tests for use in conjunction with malarial management we conducted a laboratory-based evaluation to assess the performance and operational characteristics of several existing G6PD diagnostic tests. Tests evaluated included both qualitative and quantitative tests, utilizing a variety of test formats (fluorescent spot test, rapid point-of-care tests, dye reduction tests, and spectrophotometry-based tests). Our findings indicate that most of the currently available diagnostic tests for G6PD appear to have technical or operational shortcomings that may limit their applicability to low-resource malaria management settings. Further adaptation and/or modification of existing tests or development of new tests to better meet the needs of clinicians and laboratory staff involved in malaria-case management in the developing world may be needed. We present data from this evaluation and critical design inputs to guide development of new diagnostic tests for G6PD testing.

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DEVELOPMENT OF A READY-TO-USE GELIFIED REAL-TIME PCR ASSAY FOR SIMULTANEOUS SPECIFIC IDENTIFICATION OF PLASMODIUM FALCI PARUM, P. MALARIAE, P. VIVAX, AND P. OVALE IN CLINICAL SAMPLES

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The use of PCR for identification Plasmodium spp. represents an attractive alternative for diagnosis of malaria. Some robust PCR techniques exist for this purpose, but they still based on complex procedures. This is not only time consuming, but increases the cost of PCR applications limiting its usefulness for laboratories in developing countries. Nevertheless, this can be drastically changed with PCR techniques designed to be executed under minimal quality control standards. We selected a previously published TaqMan assay and converted into a gelified format for robust, specific and simplified multiplex identification of P. falciparum, P. malariae, P. vivax, and P. ovale. Gelification consists in a process where the components of enzymatic reactions are stabilized by addition of different agents. In order to execute the procedure the laboratorian simply needs to add water and the DNA sample to the reaction tubes coated with all chemicals required for PCR amplification. Next, the vessels containing the re-solubilized mixture and the template are inserted into the real-time PCR thermal cycler for DNA amplification. The preliminary evaluation assay’s liquid format indicated that it was very specific compared to the nested PCR, since it did not produce any cross-amplification with samples containing other Plasmodium species such as P. cynomolgi, P. hylobatii, P. inui and P. knowlesi (N=14); nested PCR primers for P. vivax cross-amplified P. cynomolgi (N=4). No false negative or false positive results were verified when this assay was compared to the nested PCR using approximately 100 blood specimens sent to CDC for confirmatory diagnosis of malaria. This evaluation showed that the gelified assay had more efficient amplification profiles in addition to being simple to execute and providing results within 2 hours, including preparation time. Also, the gelified format of the assay was stable for 7 days at room temperature and for 2 months at 4 C. We believe that the gelified assay format can streamline the use of real-time PCR for confirmatory diagnosis of malaria.

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A METHOD FOR IMPROVING THICK BLOOD FILM SLIDE ADHERENCE FOR MALARIA DIAGNOSIS

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The gold standard for malaria diagnosis remains the examination of thick and thin blood films. The thick film contains a greater amount of blood than the thin film and thus provides the greatest sensitivity for screening. Unfortunately, the larger quantity of blood may not adequately adhere to the thick film and some or all of the droplet may separate from the slide during staining. The possibility of sample loss has led to recommendations that thick films be allowed to dry from 3 hours to overnight to improve the blood droplet adherence to the slide. This delay in preparation of the thick film can delay diagnosis and treatment decisions if parasitemia is not evident on the thin film. Here we describe a simple and inexpensive ‘scratch method’ for improving thick blood film adherence, ameliorating the need for extended drying times. Standardized blood droplets (35 microliters) from twenty-six previously examined EDTA whole blood specimens (22 positive and 4 negative) were used to prepare Giemsa-stained thick films either by a traditional or scratch method. By the traditional method, the droplet was gently spread to an approximate nickel-sized area (22 mm diameter) on the slide using the edge of a second glass slide. Using the scratch method, the droplet was smeared while forcefully grinding or ‘scratching’ it into the slide with the point of a second glass slide. Using the scratch method, the droplet was smeared while forcefully grinding or ‘scratching’ it into the slide with the point of a second glass slide. All slides were dried for 1 hour in a laminar flow hood and Giemsa-stained using established protocols. Slides were then examined in a blinded manner for parasite identification, determination of percent parasitemia, and degree of blood droplet adherence by 4 independent trained examiners. There was no difference in detection of parasites or parasite morphology between the two methods, but blood droplet adherence was significantly improved by the scratch method. The scratch method is a simple and effective way to improve thick film adherence and thus facilitate rapid screening. This method does not require additional equipment or significant changes in sample preparation methods.

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HRP2 in a chronic infection. The impact that host anti-
Parasites on the first day of symptoms in malaria naïve individuals, and 
PfHRP2-based RDTs should be able to detect.

predicted that good quality treatment. Combining the model output and RDT sensitivity data, it is
heavily dependent on the duration and density of parasitemia prior to the actual duration of positive test results following curative treatment. However, PfHRP2 is predicted to cause
low detection threshold and long-half life of minimum detection thresholds were between 6.9 and 27.8 ng/mL. The

in malaria naïve hosts allowed the amount of HRP2 produced per
P. falciparum to mimic the kinetics of antigenemia during human infections. Fitting this
HRP2 dynamics model to data from human infection studies

Rapid diagnostic tests (RDTs) based on Plasmodium falciparum histidine-rich protein 2 (PhHRP2) are popular for diagnosis of this most virulent malaria infection. However, concerns have been raised about the longevity of the PhHRP2 antigenemia following curative treatment in endemic regions. It is also unclear how early in an infection the PhHRP2-detecting RDTs become positive. These are important questions for the widespread use of malaria RDTs in diagnosing malaria in clinical settings. We developed a mathematical model of PhHRP2 production and decay to mimic the kinetics of antigenemia during human infections. Fitting this PhHRP2 dynamics model to data from human infection studies in malaria naïve hosts allowed the amount of PhHRP2 produced per parasite to be estimated at g per replication cycle. The limit of detection for four commercial malaria RDTs was assessed in the laboratory; the

mothers were positive with the test Optimal-IT (78.2%), was due to false detection of
species with Optimal-IT, 15% of discrepancies were found between TDRs and the thick smear. Our study aimed at analyzing these discordant results using the nested PCR, for amplification of the gene representing the small under-unit of the ARN 18S. Out of the 415 analyzed samples (307 differences and 108 correspondences), 28.4% (38/171) were positive with the PCR. The Plasmodium falciparum infection was detected in 22.2% (38/171) of the positive samples with the Acon test, corresponding to 77.8% of false positive results and to more than 80% of bands with low intensity. The proportion of false negatives was 25.6%. The proportion of false positive with the test Optimal-IT (78.2%), was due to false detection of non falciparum species; that of the false negatives was lower (33%). Sensitivity, specificity and negative predictive value of both RDTs with the
thick smear corrected by the PCR considered as the reference exceeded 90%, except for the detection of the non falciparum species with Optimal-IT. Acon® and Optimal-IT remain of good interest for the biological diagnosis of malaria in areas where thick smears and well trained microscopists are not available.

The rapid and accurate diagnosis of malaria is key to the central to clinical management and the prevention of drug-overuse, which may lead to resistance development, toxicity and economic loss. So far, microscopy of Giemsa-stained thin or thick blood smears is the gold standard. Rapid diagnosis tests provide an alternative, although they cost more and give qualitative instead of quantitative results. A fluorescence (FL) dye- incorporated immunochromatographic assay (ICA) might offer a higher sensitivity than rapid device which can be used at the point of care (POCT). The fluorescence immunoassay was employed to detect parasite densities as low as 10 parasites/µL of both Plasmodium spp. and P. falciparum. In addition, the reaction can be duplexed to detect both Plasmodium spp. and P. falciparum in a single reaction. Further validation of this technique in field settings will help to assess its utility for large scale screening for malaria parasitemia, potentially important for control and elimination programs.

PhHRP2-based RDTs should be able to detect parasites on the first day of symptoms in malaria naïve individuals, and that as few as 8 parasites/µL may be required to maintain a positive RDT in a chronic P. falciparum infection. The impact that host anti-PhHRP2 antibodies may have on RDT positivity in endemic regions is currently being investigated.

The rapid and accurate diagnosis of malaria is key to the central to clinical management and the prevention of drug-overuse, which may lead to resistance development, toxicity and economic loss. So far, microscopy of Giemsa-stained thin or thick blood smears is the gold standard. Rapid diagnosis tests provide an alternative, although they cost more and give qualitative instead of quantitative results. A fluorescence (FL) dye- incorporated immunochromatographic assay (ICA) might offer a higher sensitivity than rapid device which can be used at the point of care (POCT). The fluorescence immunoassay was employed to detect parasite densities as low as 10 parasites/µL of both Plasmodium spp. and P. falciparum. In addition, the reaction can be duplexed to detect both Plasmodium spp. and P. falciparum in a single reaction. Further validation of this technique in field settings will help to assess its utility for large scale screening for malaria parasitemia, potentially important for control and elimination programs.
quantitative test was 95%, the new fluorescence immunoassay may be used as a POCT diagnostic tool and has potentials as a fast, accurate, reliable, easy, and suitable tool for the semi-quantitation analysis for Pf and Pv malaria diagnosis.

MODELING HEALTH SYSTEMS BARRIERS TO SUCCESSFUL MALARIA MANAGEMENT
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A functioning and efficient health system is required to maintain reductions in malaria disease and transmission. Few models demonstrate how to deliver a proven intervention most effectively through an existing system. The “systems effectiveness framework” has previously been used to describe how a cascade of interacting health systems barriers may sequentially reduce the effectiveness of treatment interventions. We contrasted this approach with a decision analysis model of malaria treatment in the public sector. A common set of parameters for malaria management in Africa including access to care, diagnosis and treatment were obtained from the literature. The decision analysis model more accurately reflected reported levels of appropriate management of fever (malarial and non-malarial) in the public sector (>50% attendees) compared with a systems effectiveness approach (<15%). Modeling increases in availability and usage of rapid diagnostic tests (RDTs) improved overall management of fever (upto 80% attendees) and reduced over-treatment of non-malarial fevers with anti-malarials (<12%), but had less impact on the proportion of malaria cases treated (<57%). In contrast, reducing stockouts of first-line anti-malarials had a substantial impact on the proportion of malaria cases treated (68%) even without increased RDT use. Improving adherence to test results was not predicted to substantially improve appropriate treatment rates for malaria since the risk of under-treatment is low, and baseline utilisation of RDTs was assumed to be only 40% as per the literature. Under conditions of perfect availability and use of RDTs, test adherence and drug availability, appropriate treatment rates were predicted to rise to 95%. Simple decision analysis models can provide insight into which aspects of delivering care are most likely to impact on care quality and treatment effectiveness, and at different transmission intensities. Further work into the amenability of health systems to change is required to explore the most cost-effective targets in expanding the delivery of anti-malarials.

RAPID DIAGNOSTIC TEST PERFORMANCE IN THE SETTING OF DIFFERING TRANSMISSION INTENSITIES: THE MALAWI ICECMR EXPERIENCE
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In Malawi, like many malaria-endemic countries, rapid diagnostic tests (RDTs) are used to confirm the diagnosis of malaria because access to microscopy is limited. Much attention has been given to the sensitivity of RDTs, but their positive predictive value has not been explored, especially in areas such as Malawi, where malaria continues to be endemic. RDTs may be positive for weeks following successful treatment. In cases of false positive RDTs evaluation and treatment of alternative diagnoses might be neglected because of the presumed malaria diagnosis. Through the Malawi International Center of Excellence in Malaria Research, we are conducting surveillance for malaria in three transmission settings using RDTs (Paracheck®), microscopy and molecular detection of malaria infection. Among all surveillance sites during the rainy season, 25-30% of people with symptoms compatible with malaria had had a positive RDT. We conducted a preliminary evaluation comparing RDT results to expert microscopy. Overall, the positive predictive value (PPV) of a RDT compared to microscopy was 76.1%. The RDT PPV was inversely related to transmission intensity. In the moderate transmission intensity regions of the rural highlands and urban highlands, PPV was 91.7% and 72.3%, while in the lowland area with intense malaria transmission the PPV was 66.7%. In the areas of moderate transmission, the PPV was higher in adults compared to children under five years of age (97.9% vs. 84.2% and 80.5% vs. 55.6% in the highlands and urban setting respectively). In contrast, in the most intense transmission region, PPV was slightly lower in adults compared to children (64.2% vs. 73.5%). Microscopy is being conducted on additional slides collected from patients with positive RDTs in both the rainy and dry seasons. Sensitivity and specificity compared to molecular diagnosis will also be reported. The rate of false positive RDTs is high and is related to transmission intensity. This raises the concern that alternative causes of illness will not be pursued in patients with a positive RDT.
PFMDR1 IS ASSOCIATED WITH RECRUDESCENCE AFTER TREATMENT WITH ARTMETHER-LUMEFANTRINE IN WESTERN KENYA

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Single Nucleotide Polymorphisms (SNPs) in PFMDR1 and PFCRT have been associated with *Plasmodium falciparum* resistance to drugs including chloroquine (CQ), amodiaquine (AQ), lumefantrine (LU) and mefloquine (MQ). Artmether-Lumefantrine (AL) is currently the first line antimalarial used in Kenya with artesunate-amodiaquine (ASAQ) and dihydroartesinin piperperide (DHA-PPQ) being readily available from private retailers. During an open-label randomized clinical study evaluating the efficacy of AL in Ombeyi, a malarial endemic district in Western-Kenya, we investigated the role of PFMDR1 and PFCRT in modulating tolerance to artesinin partner drugs. All recurrent samples were genotyped for MSP1, MSP2 and GLURP at day 0 and day of recurrence. Additionally all samples were assayed for SNPs in PFMDR1 codon 86 and PFCRT codon 72-76 as well as copy numbers in PFMDR1. All day 0 samples were assayed for drug susceptibility using the SYBR Green method. Among the 454 subjects enrolled in the study, there were 162 recurrent cases of which 134 were re-infections while 17 were recrudescences and 11 underestimated. PFMDR1 N86 was significantly associated with recrudescence compared to both day 0 and re-infection. There was no significant association between PICRT and recurrent infections and amplification of PFMDR1 gene was not observed. Significant positive correlation was observed between LU and MQ (r=0.5, r²=0.27, p<0.05). This data demonstrates an association not observed. Significant positive correlation was observed between LU and recrudescence after treatment with AL in Western Kenya. Co-resistance between LU and MQ indicates that LU pressure may lead to MQ resistance, an important prophylaxis for malaria naïve visitors to Kenya.

ANTI *PLASMODIUM FALCIPARUM* MO15-RELATED PROTEIN KINASE (PFMRK) AND P. FALCIPARUM PROTEIN KINASE 5 (PPFK5) ACTIVITIES OF NATURAL PRODUCTS FROM PLANTS

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There is urgent need to develop new chemotherapeutic anti-*Plasmodium falciparum* to replenish loses to resistance. Natural products, including flavonoids and quinones, currently being explored as anticancer agents inhibiting cyclin dependent kinases (CDKs) are also antiplasmodial, but information on their mechanism of action is scanty. This study assessed the in-vitro flavonoids and quinones inhibition of *Plasmodium falciparum* MO15-related protein kinase (PFmrk) and *Plasmodium falciparum* protein kinase 5 (PPFK5) using luciferase-coupled kinase assay. These compounds were obtained from six East African plants. Compounds coded as BA-4E, 0.26µM and BA-6S 0.22µM were the most active against PFmrk and PPFK5 respectively while BA-6U (0.4µM) and BA-4C (1.03µM) showed specificity against PFmrk. Flavonoids of the subclass flavanones were the most active compounds. Flavonones having two prenyl substituents (diprenylated compounds) on ring B with a hydroxyl or methoxy group at position 4’ had highest activity regardless of these groups’ position. These findings suggest that inhibition of PFmrk and/or PPFK5 may be among ways that flavonoids inhibit Pf replication.
CHLOROQUINE-RESISTANT PLASMODIUM FALCIPARUM MALARIA IN TRAVELERS FROM HAITI AFTER THE 2010 EARTHQUAKE

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Chloroquine (CQ) associated with primaquine is recommended as first-line treatment for uncomplicated malaria in Haiti. CQ in vitro and molecular surveillance data collected over the past two decades suggest continued Plasmodium falciparum sensitivity. However, a 2006-2007 study showed around 6% (5/79) of P. falciparum isolates had the CQ resistance-associated pfcrt76T genotype. The January 2010 earthquake and flooding followed by the “Tomas” in the same year may have created conditions for increased malaria infections. We have investigated the CQ sensitivity of P. falciparum parasites isolated from travellers recently returned from Haiti to France and Canada, using genotypic and phenotypic methods.

Retrospective data was collected from the French National Malaria Reference Centre (CNR) and the Public Health Ontario, 1988-2010 and 2007-2010, respectively. The definition of an infection probably acquired in Haiti was recent travel to the country prior to diagnosis with P. falciparum positive thin and thick blood smear. Basic demographic and epidemiologic data, clinic and parasitologic information, treatment, history of travel and malaria infection were collected systematically. Prior to the earthquake, all isolates (n=29) had the wild-type pfcrt76 allele, analysed by PCR-RFLP. The mean of the 50% growth inhibition (IC50) of CQ of the isolates (n=24) was 27nM (95% confidence interval[CI], 23 to 31). After the earthquake, two of ten isolates showed CQ resistance in vitro after culture adaptation. Both isolates had high CQ IC50 (506nM and 708nM) and high CQ IC50 isolate Pf3D7 (CQ susceptible clone) ratio (20 and 27). Resistance was confirmed by the molecular analysis demonstrating the presence of the CQ-resistant associated pfcrt76T allele (mixed K76T) only in these two isolates. Our data confirm the presence of CQ-resistant strains in Haiti. They highlight the importance of implementing a therapeutic efficacy study for assessing in vivo CQ-sensitivity, essential for informing rational control strategies and guiding prophylaxis recommendations in Haiti.

TRAVELER’S SURVEILLANCE: A TOOL FOR DETECTING EMERGENCE OF ANTIMALARIAL DRUG RESISTANCE IN ENDEMIC COUNTRIES

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There is growing concern about the emergence of resistance in Southeast Asia to artemisinin-based combination therapy (ACT), the first-line treatment for malaria. In the time since the widespread adoption of ACTs, a decrease in the systematic surveillance of antimalarial drug resistance has been observed in many endemic countries. Furthermore, high levels of host immunity complicate the identification of treatment failures associated with resistance. The aim of this project was to validate the use of travelers returning from Africa with malaria as an additional surveillance system for the emergence of drug resistance. We compared data collected between 1998-2011, from the French Malaria Reference Centre for traveler’s data versus field data from the literature and within the WWARN database. We compared temporal trends of the proportion of wildtype-genotype isolates for CRTL7 and DHFR108 molecular markers, as well as the in vitro response to chloroquine (CQ) of isolates using generalized linear models. Three countries were selected for the analysis: Senegal (SN), Mali (ML) and Cameroon (CM) based on a required sample size of 600 isolates per group. For CRTL7, no significant (NS) difference is shown between travelers and field studies in CM (slope = 0.03, respectively, p=NS), SN (β1=0.17, β2=0.21, respectively, p=NS) and ML (β1=0.19, β2=0.17, respectively, p=NS). These results are supported by in vitro analysis in SN (β1=0.03, β2=0.05, respectively, p=NS). An increase of CQ-sensitive isolates is observed, except for ML where data up to 2004 was included. For DHFR108, no significant difference is shown between travelers and field studies in CM (β1=-0.24, β2=0.10, respectively, p=NS), ML (β1=-0.17, β2=-0.11, respectively, p=NS) and SN (β1=-0.09, β2=-0.06, respectively, p=NS). A decrease of wildtype-genotype isolates is observed. Our results show similar trends in resistance extracted from the Demographic Health Surveys (DHS) and Multiple Indicator Cluster Surveys (MICS). Logistic regression models were used to detect trends in the susceptible isolates proportions. From 2000 to 2011, around 700 isolates were genotyped for each country. The frequency of the pfcrt76 wild-type significantly increased for Cameroon (CM) (from 10% to 41%, Slope=0.09, p<0.003, Cote d’Ivoire (CI) (from 37% to 69%, Slope = 0.14, p<0.003, and Senegal (SN) (from 22% to 53%, Slope=0.17, p<0.003). The mean of the 50% growth inhibition (IC50) of CQ decreased from 314nM (95% confidence interval, 102-526) to 101nM (71-131) in CM, from 109nM (70-148) to 47nM (28-66) in CI and from 144nM (91-196) to 75nM (36-115) in SN. Meanwhile, CQ use among children with fever significantly decreased during this period. An increase of CQ susceptibility following official withdrawal is observed in travelers returning from Cameroon, Cote d’Ivoire and Senegal. The length of time between policy changes and their subsequent implementation, as well as the cross resistance between antimalarial drugs, may affect the time for a significant recovery of CQ sensitivity. This information should be compared to country level CQ efficacy data.

RETURN OF CHLOROQUINE SUSCEPTIBILITY OF PLASMODIUM FALCIPARUM STRAINS IN TRAVELERS RETURNING FROM WEST AFRICA

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Following WHO recommendations, most African countries have discontinued chloroquine (CQ), and now promote artesimin-based combination therapy (ACT), as the first-line treatment for uncomplicated malaria. The policy changed in West Africa during the last decade (2002 in Cameroon; 2003 in Senegal and Cote d’Ivoire; 2004 in Mali). The aim of this study was to describe the evolution of CQ resistance in West Africa, through travelers returning from this region. The study was conducted by the Malaria National Reference Centre, France. The database collated in vitro response of reference and clinical isolates for CQ and the pfcr76 K76 molecular marker for CQ susceptible Pf malaria. As a proxy of drug pressure, CQ intake for children under five years of age with fever was
between travelers and field studies. This work highlights the value of an international traveler’s database to assess and monitor the emergence of drug resistance in endemic areas where information is limited.

MULTIPLE INSECTICIDE RESISTANCE IN WESTERN KENYA: IMPEDEMENT TO INSECTICIDE-BASED MALARIA VECTOR CONTROL PROGRAMS IN KENYA

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Indoor Residual Spraying (IRS) and long-lasting insecticidal nets (LLINs) have been extensively used for malaria prevention and control in Kenya. However, the development of resistance by mosquitoes to recommended insecticides for IRS and/or ITNs/LLINs would affect insecticide-based malaria vector control. We assessed the effect of extensive use of IRS and LLINs on development of resistance in Anopheles gambiae from western Kenya. Wall bioassays were performed on artificial walls and filter papers sprayed with ICON and deltamethrin using mosquitoes collected from different sites from western Kenya and Kisumu strain as a control. Net cone bioassays were also performed on nets collected from the fields using mosquitoes from two sites and Kisumu susceptible strain as a control. Chemical analysis of the netting material was also done using HPLC to determine the concentration of insecticides on the net. Kisumu strain was susceptible to all the insecticides with 100% mortality. Mosquitoes from Chulaumbo, Ahero, Chulambo, Emakaka and Kisan shows susceptibility to both deltamethrin and ICON with the mortality rates ranging between 80% - 85% but mosquitoes from Bungoma and Emutele shows resistance to both ICON and deltamethrin with mortality rates ranging from 69%- 74%. Sprayed artificial walls shows lower mortality rates compared to sprayed filter papers. ICON had high mortality rates on the mosquitoes compared to Deltamethrin. Mosquitoes from Bungoma and Emutele showed resistance in Net bioassays with the mortality rates ranging between 60% -75%, but the control strain was highly susceptible to the nets with 100% mortality. HPLC results indicated that the nets still had a high concentration if insecticides from 0.06 wt.% - 0.19 wt.% positive, the control net had the concentration of 0.14 wt%. The observed resistance to insecticides used for IRS and LLINs in An. gambiae Populations from western Kenya could affect the malaria vector control programmes in Kenya; therefore there is need urgent implementation of resistance management strategies and interrupted vector control intervention.

MECHANISM OF ARTELINIC ACID RESISTANCE IN PLASMODIUM FALCIPARUM IN VITRO

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The emergence of Plasmodium falciparum parasites with decreased in vivo sensitivity in several South East Asian countries has raised the urgent need to understand the underlying biological mechanism. We investigated the processes involved in the development of artelanic acid resistance using laboratory generated resistant P. falciparum lines in vitro. Our results demonstrate that resistance to artelanic acid has two major characteristics: 1) resistance affecting early asexual stage parasites demonstrated by the insensitivity of ring-stage parasites to the induction of dormancy, and a faster recovery from dormancy when it is induced with higher drug concentrations. 2) resistance of late stage parasites which allows continuous growth and multiplication of parasites under continued drug pressure. These results demonstrate that changes in the dormancy profile of parasites are part of the resistance phenotype and suggest that the development of artelanicin resistance may involve two steps. The molecular events important in each step are currently being investigated to determine whether full artelanicin resistance develops as a stepwise process or whether the two stages arise independently of each other.

PHARMACODYNAMICS OF ARTEMISININ COMBINATIONS FOR DRUG SENSITIVE AND RESISTANT PLASMODIUM BERGHEI IN VIVO

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Artemisinin combination therapies (ACTs) combine a very potent, yet short-lived artesiminin (ART) derivative with a partner drug that usually has a longer half-life, yet established resistance in the field. This strategy enhances therapeutic efficacy and theoretically delays the emergence of ART resistant Plasmodium falciparum. Although ACTs are the first-line treatment globally, pharmacodynamic (PD) properties of the combinations are not well studied. In particular the impact of existing resistance to the partner drug on emerging resistance and reduced clinical efficacy is poorly understood. To examine the PD properties of ACTs we used drug sensitive and resistant P. berghei in standard mouse efficacy models. In these studies we examined the PD properties of artemether-lumefantrin (ATM-LMF), artesunate-mefloquine (AS-MFQ), AS-amodiaquine (AS-ADQ), dihydroartemisinin-piperaquine (DHA-PIP), and AS-pyronaridine (AS-PND). First we used drug-sensitive P. berghei and identified minimal curative drug concentrations that rapidly clear parasite infections, prevent parasite recrudescence, and are not antagonistic. Secondly we evaluated the impact of the following partner drugs by using the MFQ-resistant N1100, ADQ- resistant NAM, and PND resistant-NPN-10 lines of P. berghei. The data obtained thus far demonstrate that partner drug resistance significantly erodes the PD properties of the ACTs in current clinical use. For example with MFQ resistance, the minimal effective regimen of AS-MFA for sensitive parasites was poorly efficacious versus MFQ resistant parasites. These data demonstrate the utility of the rodent model to estimate PD properties of ACT combinations and to determine the most effective ACT regimens to delay emergence of ART resistance.

HIGH THROUGHPUT ANALYSIS OF IN VITRO ANTIMALARIAL SENSITIVITY DATA IN THE ERA OF ARTEMISININ COMBINATION THERAPY: THE WWARN IN VITRO ANALYSIS AND REPORT TOOL (IVART)

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In vitro assessment of antimalarial drug sensitivity remains an important tool in the era of artemisinin combination therapy, providing a way to assess parasite susceptibility to a range of drugs that is largely independent of clinical factors. In addition, investigation of molecular mechanisms of resistance via transfection depends absolutely on accurate and relevant in vitro phenotyping. WWARN and its collaborators have established a repository of raw, in vitro data derived from a wide range of locations and readout methods. Analysis of this large and varied dataset has now been undertaken using WWARN’s In Vitro Analysis and Report Tool (IVART), an application that performs high throughput data analysis with calculation of standard IC50 parameters via non-linear regression. Here we describe the development and validation of IVART, and report the evidence base for its design features, including methods for curve fitting and quality assessment that, until now, have relied on expert opinion.

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The data also reveal differences in efficacy between artemisinins and their partner drugs in several forms of assay readout, highlighting the importance of matching in vitro assay readouts to in vivo properties in areas of emerging drug resistance. Improvements in the standardization of in vitro assays are critically important and the development of a free, adapted software tool like IVART addresses the heterogeneity of analytical in vitro output. Such standardized in vitro outputs could play a major role in the validation of potential molecular markers of resistance to antimalarials including artemisinin.

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TRENDS OF THE FREQUENCY OF PLASMODIUM FALCIPARUM DRUG-RESISTANCE MOLECULAR MARKERS IN ISOLATES FROM PREGNANT WOMEN SIX YEARS AFTER INTRODUCTION OF INTERMITTENT PREVENTIVE TREATMENT WITH SULFADOXINE-PYRIMETHAMINE (IPTP-SP) IN GABON

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Following WHO recommendations for malaria control, gabonese Ministry of Health adopted ACTs, insecticide-treated nets and intermittent preventive treatment with sulfadoxine-pyrimethamine (IPTP-SP) in 2003. Prevalence of triple dhfr and quintuple dhfr/dhps mutation were respectively of 86% and 22% in 2005. Six years after their implementation, the frequency of dhfr and dhps point mutations was assessed in Plasmodium falciparum isolates from Gabonese pregnant women according to the number of SP doses. Polymorphic codons of dhfr gene (51, 59, 108 and 164) and dhps gene (437, 540 and 581) were analysed using PCR-restriction fragment length polymorphism. Blood samples from 89 women were analyzed, 35 received 2 doses, 16 received 3 doses and 18 none dose of SP. Among patients with 3 SP doses, 11 had submicroscopic infection. None sample had a quadruple dhfr mutation but the frequency of triple mutation (51-59-108) was 98%. All parasites carried a wild-type allele at codon 164. The same was true for the codon 581 of dhps gene. These preliminary data indicate an increase in the frequency of multiple resistance markers to SP independently of the number of doses received during pregnancy. There is an urgent need to assess the in vitro susceptibility of P. falciparum isolates to SP, to study other factors associated with the presence of SP resistant parasites and to evaluate an alternative drug for IPTP for pregnant women.

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STUDIES ON AP2 ADAPTOR µ-CHAIN, A NEW CANDIDATE MOLECULAR MARKER FOR ARTEMISININ RESISTANCE IN PLASMODIUM FALCIPARUM

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There is evidence of reduced susceptibility of the malaria parasite Plasmodium falciparum to artemisinin derivatives, expressed by delayed parasite clearance times in vivo. If artemisinin resistance spreads, it would threaten global malaria control. We lack validated molecular markers for monitoring these phenotypes. Using whole genome sequencing in the rodent malaria parasite Plasmodium chabaudi, we identified a mutation in the μ chain of the AP2 adaptor protein complex (pcap2-μ) that arose along with the experimental evolution of artemisinin resistance. We screened several field isolates of P. falciparum from an ACT clinical trial in Burkina Faso, that were tested in vitro for their response to artemisinin derivatives and other drugs, and in pre- and post- treatment samples from an in vivo ACT trial carried out in Kenya, for genetic polymorphisms in the pfp2-μ orthologue. Genetic polymorphisms in pfp2-μ were analysed for association with several endpoints in both trials that might indicate a drug resistant parasite phenotype. Preliminary results indicate that polymorphisms in this adaptor protein subunit may be associated with in vitro and in vivo responses to artemisinin derivatives, quinine and lumefantrine. Further evaluation of pfp2-μ as a potential molecular marker of artemisinin resistance is now needed.

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DHFR AND DHPS SELECTIVE SWEEPS IN MALAWI AT A TIME OF HIGH SULFADOXINE-PYRIMETHAMINE USE

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Malawi and most other African nations have stopped using both chloroquine and sulfadoxine-pyrimethamine (SP) due to expansion of drug-resistant Plasmodium falciparum parasites. Directional selection of chloroquine resistance alleles in the form of a selective sweep has been shown, by analysing variation in microsatellites flanking the chloroquine resistance gene, pfcrt. Similar selective sweeps of regions flanking dhfr and dhps, the genes that cause resistance to SP, have been identified in Africa, Southeast Asia, and South America. Here we report evidence of positive directional selection of dhfr and dhps resistance haplotypes and describe characteristics of the associated selective sweeps, at a time of high SP drug pressure in Malawi. Resistance alleles and flanking microsatellites were genotyped on 689 filter paper samples from children aged 6 months-12 years in Blantyre, Malawi from 1999-2001 when SP was the first-line treatment for malaria. All but one of the genotyped samples carried one or more SP resistance alleles. Dhfr triple-mutants conferring strong SP resistance predominated (51I/59R/108N), forming a quadruple mutant with dhps 540E. Dhfr/dhps quintuple mutants (dhfr 51I/59R/108N+dhps 437G/540E) were also observed. A reduction in microsatellite heterozygosity was identified in the regions flanking both dhfr and dhps. The sweep flanking dhfr extended from 10kb upstream to 20kb downstream of dhfr. The sweep flanking dhps extended from approximately 10kb upstream to at least 9kb downstream. Extended Haplotype Homozygosity was estimated, and showed increased linkage disequilibrium (LD) in regions flanking both genes relative to genomic levels of LD. Selective sweeps of resistant dhfr and dhps indicate that these alleles were under recent positive directional selection. The characteristics of the selective sweeps reported here, which were detected during a period of high SP drug pressure, will be compared to those detected after removal of SP as the first line therapy and in settings with different levels of malaria transmission.

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EVOLUTION OF DRUG RESISTANCE IN MALARIA PARASITES

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Efforts to relieve the burden caused by malaria rely critically on the availability of drugs targeting Plasmodium falciparum. The efficiency of these treatments is however seriously compromised by the appearance and spread of drug resistance. Resistance is observed today to some
IDENTIFYING DRUG RESISTANCE GENOTYPES IN ECUADORIAN MALARIA PARASITES

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Approximately 40% of the world population lives in malaria-endemic areas and recent estimates indicate that there are several hundred million cases and about 1.2 million deaths each year caused by this disease. Severe disease and resistance to antimalarials has been documented for Plasmodium falciparum and P. vivax and efforts to control malaria have become more challenging in recent years due to widespread drug resistance. Today, the vast majority of P. falciparum isolates in Latin America are resistant to chloroquine (CQ) and other drugs and resistance to CQ has been reported for P. vivax. It is also widely recognized that drug resistance has played a role in the reemergence of malaria in the Amazon basin at the end of the 20th century. The antimalarial resistance situation in Ecuador is not well known and genotypes for drug resistance from different parts of the country have not been studied. In order to identify and analyze genotypic markers for drug resistance in Ecuador we are doing PCR-RFLP from confirmed malaria blood samples spotted in filter paper using specific primers for Pfcrt, Pfmdfr and Pfbdhps (P. falciparum) and Pvdhfr, Pvdhps (P. vivax). Our results so far show that the tested Ecuadorian P. falciparum isolates have a mutant Pfcrt. In addition, we will present P. falciparum and P. vivax genotype data from different resistance markers. The study of the prevalence of drug resistance in Ecuadorian P. falciparum and P. vivax will enhance our knowledge of drug resistance in Latin America, a necessary task to improve the way malaria is treated in this region of the world.
of asymptomatic malaria (aOR 1.21, p=0.4). This study shows that the effect of malaria in pregnancy on childhood malaria extends to age five years, and is the first report of an association between helminth infections in pregnancy and malaria in the offspring.

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VALIDITY OF SELF-REPORTED USE OF SULFADOXINE-PYRIMETHAMINE INTERMITTENT PRESCRIPTIVE TREATMENT DURING PREGNANCY (IPTp): A CROSS-SECTIONAL STUDY

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Malaria in pregnancy is a major health problem that can cause maternal anaemia, stillbirth, spontaneous abortion, low birth-weight and intra-uterine stunting. The WHO recommends use of Sulphadoxine-Pyrimethamine (SP) for Intermittent Preventive Treatment of malaria during pregnancy (IPTp) in endemic areas. Towards monitoring and assessing IPTp coverage in the population, the Roll Back Malaria program recommends use of self reported data. In this study, we assessed the validity of self reported use of IPTp by testing for sulfadoxine in maternal blood at delivery. Two hundred and four pregnant women were consented and enrolled in a cross-sectional study. We excluded participants who reported a history of taking sulfa containing drugs, those who were not sure of dates relating to last menstrual period or who took IPTp before 20 weeks of gestation. Data on demographic characteristics, obstetric history, and delivery outcome were collected. At delivery of the baby, we took the mother's venous blood, carried out blood smear microscopy for parasites and tested the plasma for sulfadoxine using High Performance Liquid Chromatography (HPLC). We found that 17.2% of participants reported to have used IPTp and indeed tested positive by HPLC while 30.4% reported not to have used IPTp and indeed tested negative for sulfadoxine. Participants possessing post primary education were more likely to have reported using IPTp. The low agreement between self report and actual presence of the drug in the blood casts doubt on the validity of self reported data in estimating IPTp coverage. We recommend further research of self reported data towards improving the accuracy of such information which is vital for guiding policy for malaria control in pregnancy since routine blood drug assays would be too expensive and impractical for population based studies.

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A SPATIO-TEMPORAL ANALYSIS OF PLASMODIUM FALCIPARUM AND P. VIVAX INFECTIONS AND TREATMENT SEEKING BEHAVIOR IN THA SONG YANG DISTRICT, TAK PROVINCE, THAILAND: 2008-2011

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There is considerable interest in the potential of private sector subsidies to increase availability and affordability of artemisinin-based combination therapies (ACTs) for malaria treatment. A cluster randomized trial of such subsidies was conducted in 3 districts in Kenya, comprising provision of subsidized packs of paediatric ACT to retail outlets, training of retail staff, and community awareness activities. The results demonstrated a substantial increase in ACT availability and coverage, though patient counselling and adherence were suboptimal. We conducted a qualitative study in order to understand why these successes and limitations occurred. Eighteen focus group discussions were conducted, 9 with retailers and 9 with caregivers, to document experiences with the intervention. Respondents were positive about intervention components, praising the focused retailer training, affordable pricing, strong promotional activities, dispensing job aids, and consumer friendly packaging, which are likely to have contributed to the positive access and coverage outcomes observed. However, many retailers still did not stock ACT, due to insufficient supplies, lack of capital and staff turnover. Advice to caregivers was poor due to insufficient time, and poor recall of instructions. Adherence by caregivers to dosage guidelines was sub-optimal, because of a wish to save tablets for other episodes, doses being required at night, stopping treatment when the child felt better, and the number and bitter taste of the tablets. Caregivers used a number of strategies to obtain paediatric ACT for older age groups. In conclusion, this study has highlighted that important components of a successful ACT subsidy intervention are regular retailer training, affordable pricing, a reliable supply chain and community mobilization emphasizing patient adherence and when to seek further care.

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UNDERSTANDING THE IMPACT OF SUBSIDIZING ARTEMISININ-BASED COMBINATION THERAPIES (ACTS) IN THE RETAIL SECTOR – RESULTS FROM FOCUS GROUP DISCUSSIONS IN RURAL KENYA

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LOW PREVALENCE OF PLACENTAL MALARIA INFECTION AMONG PREGNANT WOMEN IN ZANZIBAR: POLICY IMPLICATIONS FOR IPTp

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Efforts by the Zanzibar Ministry of Health to scale-up malaria prevention and treatment strategies, including intermittent preventive treatment for pregnant women (IPTp), have brought Zanzibar to the pre-elimination phase of malaria control. *P. falciparum* prevalence in the general population has been below 1% since 2008 and the diagnostic positivity rate among febrile patients was 1.2% in 2011. Zanzibar implemented IPTp using sulfadoxine-pyrimethamine (SP) in 2004 when malaria prevalence exceeded 20%. While coverage among pregnant women is low (47% received two doses SP), the value of this intervention in low transmission settings remains uncertain. Few countries in Africa have confronted policy questions regarding timing of IPTp scale-down. We designed a prospective observational study to estimate prevalence of placental malaria among pregnant women with no evidence of receiving any dose of SP for IPTp during pregnancy. From September 2011 to April 2012 we enrolled a convenience sample of pregnant women on day of delivery at six hospitals in Zanzibar (three in both Pemba and Unguja). Dried blood spots (DBS) on filter paper were prepared from placental blood specimens. DBS were analyzed via polymerase chain reaction indicating active *Plasmodium* infection (all species). To date, over 1,200 deliveries were enrolled at the six research sites (approximately 12% of total, range: 8-26%). Two (0.19%; 95% CI, 0.05-0.69%) of 1,046 DBS specimens analyzed to date showed evidence of *P. falciparum* infection. Both were from HIV uninfected, multigravid women in Unguja. Birth weights for both deliveries were normal (>2500 g). Data collection will continue through the peak transmission season of May-July 2012. The very low prevalence of placental infection among women who received no IPTp raises policy questions regarding continuation of IPTp in Zanzibar. Alternative efforts to control malaria in pregnancy in Zanzibar, such as active case detection via regular screening and treatment during antenatal visits, should be evaluated.
spatial heterogeneity of transmission intensity and secular trends in transmission intensity. Our results indicate fine scale spatial variation in the epidemiology of malaria in a 150 sq km area that was comparable with the variation seen on more coarse spatial scales across regions or countries.

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ESTIMATING TRANSMISSION INTENSITY FROM PLASMODIUM FALCIPARUM SEROLOGICAL DATA USING ANTIBODY DENSITY MODELS

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Sero logical data are increasingly being used to monitor malaria transmission intensity and have been demonstrated to be particularly useful in areas of low transmission where traditional measures such as EIR and parasite prevalence are limited. The seroconversion rate is usually estimated using catalytic models in which the measured antibody levels are used to categorise individuals as seropositive or seronegative. One limitation of this approach is that the cut-off between positive and negative is arbitrary. Furthermore, the continuous variation in antibody levels is ignored thereby potentially reducing the precision of the estimate. To overcome these limitations we developed a series of age-specific density models which mimic antibody acquisition and loss. These were fitted to antibody titre data from multiple Plasmodium falciparum endemic settings to estimate the rate of acquisition of antibodies as an alternative measure of transmission intensity. Our results indicate that a model in which the boost in antibodies following exposure depends on the existing titre (with an exponential decline in the size of the antibody boost with higher levels of circulating antibodies) and that includes variation between individuals in the size of the response fits the data well. Furthermore our results show a consistent ordering of transmission intensities compared to those from a catalytic model. This approach, if validated across different epidemiological settings, could be a useful alternative model for measuring transmission intensity which avoids the need for an arbitrary cut-off value.

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PREVALENCE OF MALARIA AND ANEMIA AMONG PREGNANT WOMEN ATTENDING ANTENATAL CARE CLINICS IN THE EJISU-JUABEN AND SEKYERE-EAST DISTRICTS OF GHANA

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Malaria and anaemia (haemoglobin concentration < 11g/dl) in pregnancy continue to be of public health importance in Ghana with malaria contributing to 9.4% of maternal deaths. Strategies are being implemented through the antenatal care system to reduce their occurrence but asymptomatic malaria parasitaemia and anaemia prevalence at term stand at 12.1% and 45.0% respectively. In preparation for a cluster randomised control trial to determine the effect of an enhanced antenatal care package on malaria and anaemia in pregnancy, a cross-sectional study was conducted from December 2011 to April 2012 among pregnant women with gestation ≥23 weeks and prior to delivery. Trained research assistants determined malaria parasitaemia and haemoglobin concentration levels using the malaria rapid diagnostic test and the HemoCue 301. An interviewer guided questionnaire was also administered to determine the demography, bed net use, IPTp administration and self-reported adherence to iron and folate supplementation among the pregnant women. The prevalence of malaria parasitaemia and anaemia was 15.5% and 42.6% respectively. Parasitaemia occurred in a significantly younger age group (25.1 (6.21) yrs vs 27.4 (6.29) yrs; p=0.007) and these had a significantly lower haemoglobin concentration (10.5 (1.37) g/dl vs 11.2 (1.29) g/dl; p<0.0001). Although 61.2% of the pregnant women owned bed nets, only 39.3% slept under one during the night before the survey. A total of 81.2% received two or more doses of SP and 50.5% reported high adherence to iron and folate supplementation however these were not significantly associated with the prevalence of parasitaemia or anaemia. Malaria parasitaemia and anaemia are still prevalent in the study area despite the implementation of current strategies including ITN use, SP-IPTp, iron and folate supplementation and prompt diagnosis and effective treatment of malaria. Probably new ways of delivering these strategies to make them more effective need to be explored.

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REVITALIZING ROUTINE HEALTH FACILITY DATA FOR MALARIA CONTROL

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Successful scale up of proven malaria control interventions across sub-Saharan Africa since 2000 has resulted in rapid changes in malaria epidemiology. Because of these changes, national malaria control programs and their partners need effective tools to adequately monitor malaria burden for surveillance and program planning. Existing tools, most notably national-level household surveys such as the Malaria Indicator Survey and Demographic and Health Survey, do not provide information on longitudinal changes in malaria burden. Cross-sectional data do not reflect seasonal fluctuations in malaria burden nor typically provide district- or sub-district-level data useful for program monitoring and planning. In many countries, routine health facility data are of unknown validity due to reporting of clinical diagnosis without laboratory confirmation and incomplete or late reporting. Recognizing the need for improved approaches to measure longitudinal changes in malaria burden, the authors have undertaken a comprehensive examination of routine health facility data and are optimistic about its potential to complement existing data for malaria control. Using models from Benin, Ethiopia, Madagascar, and Uganda, the authors have critically assessed the strengths and weaknesses of different health facility-based surveillance systems and created a framework to assist countries in developing robust data collection systems that will meet country-specific data needs while taking into account resource limitations. The framework guides stakeholders in the decision-making process and is comprehensive in that it considers scope, indicators, data collection tools, supervision, and data use. The anticipated outcomes of providing a framework for strengthening facility-based data collection systems for malaria include increasing the quality of routine system data, improving country capacity for planning, and sustaining the progress made in malaria control over the past decade.

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MOLECULAR EPIDEMIOLOGY OF PLASMODIUM VIVAX RELAPSES IN THE PERUVIAN AMAZON

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Plasmodium vivax accounts for 15-20% of malaria cases in the Americas. To determine the magnitude of P. vivax relapsing malaria in rural Amazonia, we carried out a study from 2005-2008 in four health posts in the Amazonian Region of Loreto in northeast Peru where the majority of nationally reported malaria cases originate. PCR-restriction fragment length polymorphism of PvMSP-3α (enzymes HhaI and AluI) and PCR of nine tandem repeat markers were compared for their ability to distinguish relapse vs. reinfection. Of 1507 subjects with P. vivax malaria, 354
developed >1 episode during the study. 97/354 (27.4%) were defined as relapses using *P. vivax* alone. Adding tandem repeat polymorphism analysis significantly reduced the number of definitively-defined relapses to 26/354 (7.4%) (p<0.05), allowing for more new infections to be identified. Odds of another episode of *P. vivax* malaria, whether due to relapse or reinfection, were 2.6 times higher in the more remote village of Mazan than in villages closer to Iquitos city (p<0.001) (OR=2.6, 95%CI: 2.0,3.4). People in Mazan were 2.4 times more likely to develop a relapse (not reinfection) than people in other villages (OR=2.4, CI95%:1.1, 5.5; p=0.03). The proportion of multiple genotype infections was 16.1% by TR, 4.5% by MSP-3a, and 18.8% using both. The use of highly resolving molecular markers of *P. vivax* allowed for finding an unexpectedly high proportion of multiple genotype infections, remarkable considering the current knowledge of transmission intensity and entomological inoculation rates in the region. Highly discriminatory molecular epidemiological tools will allow us to gain critical knowledge of the micro-geography of malaria transmission in this area of low transmission

### TEMPORAL TRENDS IN SEVERE MALARIA IN CHITTAGONG, BANGLADESH

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Epidemiological data on malaria in Bangladesh are sparse, particularly on severe and fatal malaria. This hampers the allocation of healthcare provision in this resource-poor setting. Over 85% of the estimated 150,000-250,000 annual malaria cases in Bangladesh occur in Chittagong Division with 80% in the Chittagong Hill Tracts (CHT). Chittagong Medical College Hospital (CMCH) is the major tertiary referral hospital for severe malaria in Chittagong Division. Malaria screening data from 22,785 inpatients in CMCH from 1999-2011 were analysed to investigate the patterns of referral, temporal trends and geographical distribution of severe malaria in Chittagong Division. From 1999 till 2011, 2,394 malaria cases were admitted, of which 96% harboured *Plasmodium falciparum* (Pf) and 4% *P. vivax* (Pv). Infection was commonest in males (67%) between 15 and 34 years of age. Seasonality of malaria incidence was marked with a single peak in *P. falciparum* transmission from June to August coinciding with peak rainfall, whereas *P. vivax* showed an additional peak in February-March likely representing relapse infections. Since 2007 there has been a substantial decrease in the absolute number of admitted malaria cases. Case fatality in severe malaria was 18% from 2008-2011 remaining steady during this period. A travel history obtained in 220 malaria patients revealed only 34% had been to the CHT in the preceding 3 weeks. Of all admitted malaria patients, only 9% lived in the CHT, but none in the more remote malaria endemic regions near the Indian border. The overall decline in admitted malaria cases to CMCH suggests recent control measures are successful. However, there are no reliable data on the incidence of severe malaria in the CHT, the most endemic area of Bangladesh, and most of these patients do not reach tertiary health facilities. Improvement of early treatment and simple supportive care for severe malaria in remote areas and implementation of a referral system for cases requiring additional supportive care could be an important component of further reducing malaria-attributable disease and death in Bangladesh.

### SOCIO-DEMOGRAPHICS AND THE DEVELOPMENT OF MALARIA ELIMINATION STRATEGIES IN THE LOW TRANSMISSION SETTING

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This analysis presents a comprehensive description of malaria burden and risk factors in Peruvian Amazon villages where malaria transmission is hypoendemic. More than 9000 subjects were studied in contrasting village settings within the Department of Loreto, Peru, where most malaria occurs in the country. *Plasmodium vivax* is responsible for more than 75% of malaria cases; severe disease from any form of malaria is uncommon and death rare. The association between lifetime malaria episodes and individual and household covariates was studied using polychotomous logistic regression analysis, assessing effects on odds of some vs. no lifetime malaria episodes. Malaria morbidity during lifetime was strongly associated with age, logging, farming, travel history, and living with a logger or agriculturist. Select groups of adults, particularly loggers and agriculturists acquire multiple malaria infections in transmission settings outside of the main domicile, and may be mobile human reservoirs by which malaria parasites move within and between micro-regions within malaria endemic settings. For example, such individuals might well be reservoirs of transmission by introducing or reintroducing malaria into their home villages and their own households, depending on vector ecology and the local village setting. Therefore, socio-demographic studies can identify people with the epidemiological characteristic of transmission risk, and these individuals would be prime targets against which to deploy transmission blocking strategies along with insecticide treated bednets and chemoprophyaxis.

### FINE-SCALE SPATIAL HETEROGENEITY OF DRY SEASON PREVALENCE AND ENVIRONMENTAL RISK OF MALARIA AMONG CHILDREN IN RURAL MALAWI: A CASE-CONTROL STUDY

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Environmental risk factors for malaria during the dry season in rural Malawi remain relatively unstudied. We investigated the role of local environmental features, in particular active smallholder agricultural land, on malaria prevalence among children <5 years old living in villages in two neighboring rural Traditional Authority (TA) regions in southern Malawi during the dry season. Ten villages from TA Sitola and Msamala were randomly selected. All houses with children <5 were approached and informed consent was obtained from those who agreed to participate, after which the house location was recorded with GPS. At each participating house, a nurse administered a malaria rapid diagnostic test (RDT) to one child, and a questionnaire to parents. Environmental data were collected in and around each house, including land cover <50 meters. Environmental variables found to be significantly associated with RDT status (+/-) at p<0.10 in bivariate analysis (X² or Student’s t-test) were analyzed with multi-level multivariate logistic regression (MLLR). Geographic clustering of RDT status, environmental factors, and Pearson residuals from MLLR were calculated using the Getis-Ord Gi* statistic. A total of 390 children were enrolled from six villages in Sitola (n=162).
variants that demonstrate a greater propensity for early
Pvmsp1 may be reactivated at the time of relapse. Additionally, we have identified
reactivate in concert to cause relapse. At the same time, the common
all or a subset of the multiple clones found in an initial infection can
find a complex scheme of relapse in which hypnozoites representing
P. vivax in Cambodia, we
variants were associated with subsequent
compared, two specific
Pvmsp1 sharing at least half their variants. At the same time, novel variants
cohort (= 0.86), 86% (38/44) of paired isolates were highly related,
question is whether certain
variants are more likely to cause relapse,
world. Yet little is known about the nature of relapse. A key unanswered
major obstacles to malaria control and elimination in many regions of the
world. In other words, the importance of an early epidemic detection system.
understand and address deficiencies in reporting timeliness, perhaps the
years of implementation, additional efforts and resources are required to
implementation moved forward from 10 (7%) clinics in 2008 to 52
of submitted data. Completeness of reporting was defined as submission of all weekly data
elements submitted to the system, regardless of date. Timeliness of
records was assessed through a manual count of weekly case totals from the
routine health management information system (HMIS) registers compared to
totals in MEEDS registers. Representativeness improved as MEEDS
implementation moved forward from 10 (7%) clinics in 2008 to 52
(37%) in 2009, 69 (49%) in 2010, 90 (63%) in late 2010, and finally 142
(100%) clinics by late 2011. Completeness of submitted data was 100% each year except 2009 (84%) when technical problems prevented data
transmission from many clinics. Timeliness of weekly reports received by the
following Monday increased from 19% in 2009 to 43% in 2011
(p<0.001). The MEEDS data accuracy as compared to routine HMIS
increased from 89% in 2009 to 97% in the first-half of 2011 and fell to
93% in the second-half of 2011. Despite accomplishments in reporting
representativeness, completeness, and accuracy of the MEEDS over four
years of implementation, additional efforts and resources are required to
understand and address deficiencies in reporting timeliness, perhaps the
most important attribute of an early epidemic detection system.

DECREASE OF MALARIA INCIDENCE AMONG CONFIRMED
CASES OF MALARIA IN MARY IMMACULATA CENTRE
MUKURU KENYA IN 2007-2010
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The aim of the study was to assess annual incidence of microscopically
positive cases of malaria in urban area of Nairobi within last five years
(2007-2011) and to compare malaria occurrence before and after intermittent preventive treatment (IPT) was initiated in this area. Mary
Immaculata Centre is located in the slum area of Mukuru (Nairobi, Kenya) with about 40 000 inhabitants in height of 1900 meters above sea level.
Traveling from Nairobi to down country of Rift Valley and back is usual
1-2 times per year. Two experienced lab technicians investigated daily
30-50 slides a day (15-25 per person). In 2011 also rapid diagnostic tests
(RTD) were used to confirm positive malaria slide. Within five years (2007-2011), 56 668 samples were microscopically evaluated (8466 - 12333 per year) and 905 were positive for malaria (1,60%). Annual proportion decreased from 1,96% (2007), 2,54% (2008) and 2,11% (2009) to 1% in 2010 and 0,79% in 2011 (P<0,001). Severe cases of malaria were seen only exceptionally. Number of cerebral malaria cases was 1-5 patients/ year and severe anaemia (<80 g/l) was also exceptional (15-30 cases/ year). Decreasing proportion of microscopically positive malaria cases was probably due to major improvement in infrastructure (disinfection of surface water, canalization, waste water drainage) as well as due to IPT in all four schools in Mukuru since 2009/2010 in all children coming to first school year age and also for all mothers coming to maternity check since 2009. Seasonal variation has been observed as well with maximum in June - October (rainy season) and minimum in November - December. In conclusion, decrease of annual incidence of microscopically positive cases of malaria in 2010-2011 has been observed in slum area of Mukuru in Nairobi, Kenya. Sewage water drains and canalizations of surface water in this area as well as IPT in school children and pregnant women may play a role in this trend within last five years.

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ESTIMATES OF MALARIA MORBIDITY BEFORE AND AFTER THE IMPLEMENTATION OF A SENTINEL SITE INPATIENT MALARIA SURVEILLANCE SYSTEM IN UGANDA

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In Uganda, the National Malaria Control Program (NMCP) relies on Health Management Information System (HMIS) data for planning and monitoring trends in malaria burden yet majority of malaria cases reported are based on clinical case definition. For the past 2 years, we have implemented an in-patient malaria sentinel surveillance program at select district hospitals with emphasis on laboratory-based case definition. To better characterize the quality of HMIS-based malaria data, and understand the true burden of malaria in Uganda, we compare HMIS data to malaria sentinel surveillance site data at four public hospitals. These hospitals are situated in districts with varying malaria endemicity: Tororo and Apac (high transmission), Mubende (medium transmission) and Kambuga (low transmission). At the four sentinel hospitals, >95% of inpatient children less than 5 years were tested for malaria, and only those children with positive laboratory confirmation were recorded as malaria cases. Based on HMIS data, the proportion of hospitalized children under 5 with malaria was higher 12 months prior to start of the program compared to 12 months after: Tororo (94% vs. 85%), Kambuga (83% vs. 52%), Mubende (71% vs. 55%) and Apac (67% vs. 40%). Actual comparison of HMIS data to surveillance program data, 12 month after its start, showed that HMIS data overestimates the burden of malaria when compared to surveillance program data: 27 percent higher in Kambuga (25% vs. 52%), 24 percent higher in Tororo, (61% vs. 85%), 18 percent higher in Mubende (55% vs. 37%) and 7 percentage points higher in Apac (42% vs. 35%). Improved precision of HMIS estimates of malaria adopted after start of the program may have contributed, in addition to other factors, to the observed differences in disease burden determined by HMIS before and after the start of the program. Even then, HMIS overestimated the burden of malaria among hospitalized children after start of the program. In order to improve the quality of HMIS malaria data, a case definition based on laboratory confirmation should be adopted.

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MALARIA AND RESPIRATORY TRACT INFECTIONS WERE THE COMMONEST TROPICAL DISEASES AND THE COMMONEST INFECTIONS IN AREA OF LOW HIV PREVALENCE IN SOUTH UGANDA: ANALYSIS OF 43,551 PATIENTS

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The aim of this study was assess reasons for hospitalization in rural hospital in South Uganda in area of low HIV prevalence among population (2-3 % HIV positivity). Since January 2008 to December 2010 all 43 550 patients has been investigated, of them 6454 (14,8 %) inpatients and 37 096 (85,2 %) outpatients. Total 32 938 (75,6 %) were children under 5 years of age (2965 inpatients). Rapid diagnostic test plus microscopy has been used in the hospital laboratory with four florescent microscopes and six experienced laboratory technicians (analysis about 100-120 test/ samples per day). Total 20 421 (46,9 %) of malaria cases within 3 years (2008-2010) were diagnosed. Of all malaria patients, 17 321 (84,7 %) were treated on outpatients and 3100 (15,2 %) on inpatients basis. Altogether 31 960 blood smears were microscopically investigated and 16 205 (50,7 %) of those were positive. Respiratory tract infections were diagnosed in 9255 cases (21,3 %), of them 3422 (36,9 %) had pneumonia and 5833 (63,1 %) lower respiratory tract infections. Otitis media (862 cases) was observed only in children. Tuberculosis was confirmed in 102 patients and 30 of them were HIV co-infected. Other frequently diagnosed infections were skin and soft tissue infections in 3383 patients (7,8 %), urogenital tract infections in 3145 (7,2%), sexually transmitted infections 966 (2,2 %), of them 241 (24,9%) laboratory confirmed cases of syphilis. Totally 2126 patients (4,9%) have microscopically diagnosed geohelmints infections and 1387 (3,2%) had diarrhea. Together 4513 patients were tested on HIV and 1188 (2,7 %) of these were positive. Another diseases with low prevalence were ocular infections in 793 (1,8 %) patients, meningitis in 53 patients (0,1 %), measles in 14 (0,03%), schistosomiasis in 12 (0,03 %), sleeping sickness in 10 patients (0,02 %) and tetanus in 12 (0,03%) patients (4 of them neonates). In the area of Buikwe (Lugazi, Buikwe District, South Uganda), prevalence of HIV was surprisingly low (2,7%) as well as geohelmints infections (3,2 %) probably due to MDR with albendazol in all school children. Low HIV prevalence is probably result of outreach mobile HIV testing program. Other factors, to the observed differences in disease burden determined by HMIS from before and after the start of the program. Even then, HMIS...
MILITARY-TO-MILITARY ENGAGEMENT TO ENHANCE MALARIA PROGRAMS DURING PEACETIME AND DEPLOYMENT IN EAST AFRICA

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Malaria remains an important parasitic disease of public health concern, especially in Africa. Malaria is a problem for military forces because of its ability to cause sudden epidemics which can hinder or halt operations. During the Malaria Symposium hosted by the United States Africa Command in April 2011, representatives from several African militaries proposed formation of a multi-national Malaria Task Force to address common military malaria programmatic challenges. After assessing countries’ current malaria activities, willingness to participate, perceived needs and expected outcomes, five nations of the East Africa Community (Burundi, Kenya, Rwanda, Tanzania and Uganda) united to hold the first East Africa Malaria Task Force (E-AMTF) meeting in December 2011. The mission of the E-AMTF is to strengthen and expand effective malaria programs and provide support for military personnel, their families and communities. The E-AMTF intends to assist national and regional malaria programs in harnessing the full potential of the armed forces as behavioral and social change agents. In preparation for the second E-AMTF meeting in Tanzania, gap analyses of the various malaria program components (Prevention, Diagnosis, Treatment, Surveillance and Human Resources/Capacity Building) during both peacetime and deployment were conducted. The process of critically evaluating their programs helped identify, document, and evaluate program requirements against current capabilities. Based on the urgency and impact on partner nations’ military malaria programs, components were prioritized. Partner nations will take their accountability roadmaps and have a follow-up review meeting with key stakeholders to review, endorse and validate the roadmaps and define clear roles and responsibilities. This regional multi-lateral cooperation between the militaries of partner African nations, leveraging data-driven programmatic assessments of their malaria program needs, allow for the collaboration with US agencies’ assets to enhance and develop malaria programs.

AN ASSESSMENT OF THE MALARIA-RELATED KNOWLEDGE AND PRACTICES OF TANZANIA’S DRUG RETAILERS: EXPLORING THE IMPACT OF DRUG STORE ACCREDITATION

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In Tanzania drugs can be purchased from 2 types of retail outlets: Part I pharmacies and drug stores. Since 2005 Tanzania has been upgrading the approximately 7,000 drug stores to Accredited Drug Dispensing Outlets (ADDOs), involving dispenser training, introduction of record keeping and enhanced regulation. ADDOs are permitted to stock 49 prescription only medicines, including artemisinin-based combination therapies. Non-ADDO drug stores can officially stock over the counter medicines only, although many stock prescription only antimalarials. By the end of 2011 ADDO conversion was complete in 14 out of 21 regions, but limited information is available on their performance. Here we examine the malaria-related knowledge and practices of Tanzania’s drug retailers, exploring variation between the different types of drug retailers. The data were collected as part of the AMFm Phase 1 Independent Evaluation, commissioned by the Global Fund to Fight AIDS, Tuberculosis and Malaria, which draws on methods developed by the ACTwatch group. We conducted a nationally representative survey of antimalarial retail outlets in Oct-Dec 2011. We randomly selected 49 wards, and interviewed all outlets stocking antimalarials. As Part I pharmacies were relatively rare these were oversampled by including all pharmacies in the districts – larger administrative units in which the selected wards were located. Interviews were conducted in 334 Part I pharmacies, 148 drug stores in ADDO regions, and 261 drug stores in other regions. We will present findings on outlet characteristics (number of staff, staff education and qualifications); staff knowledge (of first line antimalarial drug and its dosing); and antimalarials and malaria diagnostics (availability, retail prices, markups, sales volumes and wholesale sources). ADDO conversion is frequently cited as a model for improving retail sector drug provision but there is concern that the impact may be constrained by staff turnover and inadequate regulatory supervision. This study will provide important information to inform future policy on drug retailers in Tanzania and elsewhere in the region.

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EXPLORING THE IMPACT OF DRUG STORE ACCREDITATION

AN ASSESSMENT OF THE MALARIA-RELATED KNOWLEDGE AND PRACTICES OF TANZANIA’S DRUG RETAILERS:

A model for improving retail sector drug provision but there is concern that the impact may be constrained by staff turnover and inadequate regulatory supervision. This study will provide important information to inform future policy on drug retailers in Tanzania and elsewhere in the region.

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EVALUATING THE COST-EFFECTIVENESS OF INTERMITTENT SCREENING AND TREATMENT (IST) COMPARED TO INTERMITTENT PREVENTATIVE THERAPY (IPTP) DURING PREGNANCY IN PREVENTING LOW BIRTH WEIGHT: A MODEL-BASED ANALYSIS

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Malaria during pregnancy is the leading preventable cause of low birth weight in many areas of sub-Saharan Africa. The current recommended intervention is to administer up to 3 doses of Sulphadoxine Pyrimethamine (SP) to pregnant women during antenatal clinic visits (IPT-SP) to clear any existing infection and protect against re-infection. However, with the emergence of SP resistance in many parts of Africa, alternative strategies to IPT-SP are currently being evaluated. One such alternative is intermittent screening and treatment (IST), whereby long-acting artemisinin combination therapy is administered to women with a positive rapid diagnostic test (RDT). By linking a model of the progression of Plasmodium falciparum malaria during pregnancy to the risk of low birth weight, we explored the impact and cost-effectiveness of IST and IPT-SP in areas with different transmission intensity and levels of SP resistance. Our results suggest that in areas where the parasite is still sensitive to SP, IPT will be more cost-effective than IST. This is due to the limited sensitivity of RDTs to detect low-grade infections and the additional cost of the RDT relative to SP. However, in areas of East Africa with high levels of SP resistance, our results suggest that a switch to IST would lead to a reduction in the burden of malaria-attributable low birth weight. Whether IST is also more cost-effective depends mainly on the difference in cost between SP and the chosen RDT and less on the level of transmission, the level of immunity acquired or the relative cost of the antimalarial provided to those with a positive test. For example we found that in areas where SP fails to clear infections in 35% of parasitaemic women, IST would be cost-effective provided costs associated with an RDT are below $1 per test. In summary, our results suggest, conditional on our model assumptions, that a switch of policy to IST would only be effective in reducing the burden of low birth weight in areas where there are moderate to high levels of SP resistance, with the degree of resistance necessary to make such a decision cost-effective depending primarily upon the cost of the RDT used.

DEFINING THE MALARIA BURDEN IN NCHELANGE DISTRICT USING THE WHO MALARIA INDICATORS SURVEY

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Malaria is considered as one of the major public health problems and among the diseases of poverty. In areas of stable and relatively high transmission, besides children under 5 years of age, pregnant women and their new born babies are among the higher risk groups. A multicentre trial on the safety and efficacy of several ACTs during pregnancy is currently on-going in 4 African countries, including Zambia, whose study site is in Nchelenge district. As the study outcomes may be influenced by the local malaria endemicity, this needs to be characterised. Therefore, in March-April 2012 we carried out a cross-sectional survey to determine the prevalence and intensity of malaria infection among <10 years old children in Nchelenge district, on the shores of Lake Mweru. The sampling unit was the household where all children < 10 were included in the survey. We used a simple random selection of households using the GPS coded list. Individual consent to participate was collected from parents/guardians. A blood sample for Hb measurement and the detection of malaria infection was collected as well as information on the use of preventive measures such as Long-Lasting Insecticidal Nets (LLIN). Three hundred twelve households were sampled and 358 children included in the survey. Malaria parasite prevalence was 31.3% (95% CI: 26.6-36.4%); anaemia prevalence (Hb <11g/dl) was 49.1% (95% CI: 43.8-54.6%), a higher value than those previously found in the province. Though malaria has declined substantially in Zambia, there are still pockets of high endemicity such as Nchelenge district. These areas should be targeted for achieving high coverage of preventive interventions such as LLIN and indoor residual spraying.
CHANGES IN MALARIA PREVALENCE AND HEALTH PROVIDER’S BEHAVIOR TOWARDS FEVER WITH THE INTRODUCTION OF ACT AND RDT AT PERIPHERAL HEALTH CENTRE LEVEL IN SOUTHWESTERN SENEGAL (2000-2011)

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During 2000-2011, the staggered introduction of ACT (artesunate-amodiaquine, ASAQ) and RDT in Mlomp (~6000 inhabitants), southwestern Senegal coincided with profound changes in health providers’ behaviour and malaria epidemiology. Through 2006 ASAQ and microscopy were rolled-out on experimental basis, and from 2007 ASAQ+RDT were policy and free of charge. Injectable quinine has been available throughout. The dispensary is the only health provider in the village. The dispensary registries recorded 67,015 consultations, of which 35,169 (52%) for fever. Fevers accounted for 62% of consultations in 2000 vs. 33% in 2011; fevers dropped -74%, consultations -51%. Of all fevers, 9147 (26%) were diagnosed clinically as non-malaria (from 10% in 2000 to 88% in 2011) and treated accordingly, and 26,022 were clinically-suspected malaria (from 5046 in 2000 to 176 in 2011, -97%). The number of confirmed malaria fevers dropped by -90% from 1365 in 2000 to 112 in 2011). Of these, 23,481 (90%) received an antimalarial treatment (-36% in 2000 vs. 2000)., of which 6893 (29%) were for parasitologically-proven malaria (P+), 10,122 (43%) for parasitologically-negative fevers (P-), and 6466 (28%) without a parasitologic diagnosis (P0). Overall, 18,859 clinically-suspected malaria underwent parasitologic confirmation (72%). No change was seen in any of the above. ASAQ accounted for 12% of antimalarial treatments overall (41% of treatments for P+, 7% P-, 9% P0). Comparing 2007-11 (ASAQ + RDT deployed) to 2000-06, the yearly number of fevers halved, non-malaria fevers doubled, malaria treatments dropped -86%. ASAQ increased from 17% to 30% of antimalarial treatments and from 57% to 94% of P+ cases. There was no difference in the proportion of fevers tested parasitologically (75% with microscopy during 2000-06, 70% with RDT during 2007-11), nor in the P.falciparum positive rate (29% vs. 31%). Case management of fever improved (better detection of non-malaria fevers, few malaria treatments). Practice compliance with malaria policy increased (almost all treatments are ASAQ), ca. three-quarters of fevers are tested parasitologically. However, introduction of RDTs did not boost testing significantly (presumably because of prior successful training in microscopy), and confidence in RDT is still limited (presumably because the proportion turning out positive is low compared to clinical suspicion - the established prior practice).

RAPID DIAGNOSTIC TESTS AS A TOOL FOR MOLECULAR SURVEILLANCE OF PLASMODIUM FALCIPARUM MALARIA

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Prompt and accurate parasitological confirmation of Plasmodium falciparum malaria is essential for effective disease management. WHO recommends the use of malaria Rapid Diagnostic Tests (RDTs) in settings where microscopy services are not available. Following improved global malaria control and regional elimination efforts, there is a critical need for novel surveillance tools and strategies. Used RDTs have shown to be a reliable source of parasite DNA. Together with highly sensitive molecular assays, wide scale collection of used RDTs may serve as a modern tool for improved malaria case detection and drug resistance surveillance. The aim of this study was to compare and evaluate different methods of DNA extraction from RDTs and to test the field applicability for the purpose of molecular epidemiological investigations. DNA was extracted from two RDT devices (Paracheck-Pf and SD Bioline Malaria Pf/Pan), seeded in vitro with ten-fold dilutions of cultured 3D7 P. falciparum parasites diluted in malaria negative whole blood. The level of P. falciparum detection was determined for each extraction method and RDT device with multiple nested-PCR and qPCR assays. The field applicability was tested on 875 paired RDT (Paracheck-Pf) and filter paper (Whatman 3MM) blood samples collected from febrile patients in Zanzibar 2010. Preliminary in vitro results show that DNA extraction efficiency varied with extraction method and RDT device. The method of P. falciparum detection influenced the detection limit by 1-2 log units. No apparent difference in quality of DNA extracted from RDTs and filter papers was observed, in terms of PCR results from both in vitro and field samples. The results support the field applicability of RDT-DNA extraction for the purpose of improved molecular surveillance of antimalarial drug resistance, malaria case detection and RDT quality control.

PLASMODIUM FALCIPARUM EXPOSURE SINCE BIRTH AND RISK OF SEVERE MALARIA: A NESTED CASE-CONTROL STUDY ON THE COAST OF KENYA

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Severe malaria affects mainly young children in Plasmodium falciparum endemic areas. The mechanisms by which immunity to severe malaria develops remain largely unclear, as does the number of infections needed to acquire protection. The aim of this study was to establish how exposure to P. falciparum infections during the first years of life affects the risk of severe malaria. A cohort of 5949 children born 2001-2008 in Kilifi District on the Kenyan Coast was followed with three-monthly visits from birth until 2 years of age. Infection patterns in children who subsequently developed severe malaria (according to strict criteria) were compared to three-monthly profiles of age-matched community controls in a 1:3 nested case-control design. Detection of P. falciparum by microscopy or PCR in at least one sample from birth conferred an increased risk of severe malaria and particularly if a multiclonal infection, as defined by genotyping of the polymorphic merozoite surface protein 2 gene, was ever detected. Antibodies to P. falciparum schizont extract were similarly prevalent in cases and controls, indicating the overall same level of exposure. In this area of moderate-low malaria transmission, parasite positivity and diversity since birth confer an increased risk of developing severe malaria. This study demonstrates for the first time with parasitological data differences in previous exposure between children who developed severe malaria and community matched controls.

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As some malaria control programs shift focus from disease control to transmission reduction, there is a need for transmission data to monitor progress. At lower levels of transmission, this becomes increasingly difficult to measure precisely, whether through entomological or other studies. Many programs conduct regular cross-sectional parasite prevalence surveys, and have access to malaria treatment data routinely collected by ministries of health, often in health management information systems. By themselves, these data are poor measures of transmission. We propose an approach for combining annual parasite incidence and treatment data with cross-sectional parasite prevalence and treatment seeking survey data to estimate the incidence of new infections in the human population, also known as the force of infection, with limited supplementary data. The approach is based on extension of a reversible catalytic model. The accuracy of the estimates from this model appears to be highly dependent on levels of detectability and treatment in the community, indicating the importance of information on private sector treatment seeking and access to effective treatment.

**SPATIAL AND TEMPORAL TRENDS IN MALARIA TRANSMISSION CAN BE CAPTURED BY THE DIAGNOSTIC POSITIVITY RATE REPORTED FROM SUMMARIES OF QUALITY ASSURED HEALTH FACILITY RECORDS RELAYED THROUGH MOBILE PHONES**

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Measurement of malaria incidence among humans is central to monitoring malaria control program implementation. Quality-assurance and rapid reporting systems are required to reliably measure malaria transmission through passive reporting systems as current health management information system reports are not sufficiently rapid or reliable. Weekly summaries of malaria Rapid Diagnostic Test (mRDT) results from 14 health facilities (HFs) in Luangwa and Nyimba districts of central and eastern Zambia were reported via mobile phone text message. Diagnostic positivity rates reported by this passive monitoring system were compared with both the detailed data from the facility patient registers and a longitudinal incidence cohort comprising clusters of approximately 1000 residents in the immediate catchment areas of each facility. While passive HF-based surveillance reported fewer cases of malaria (10345 versus 12267, P=0.0001), particularly non-febrile cases (33 versus 8311, P=0.0001), the diagnostic positivity rates obtained correlated well with geographic (P=0.002) and temporal (P=0.000) heterogeneity in rigorously measured incidence rates. The HF surveillance system described adequately captured malaria transmission trends in local HF catchment populations and offers a cost-effective method for fine-scale program monitoring that can be applied on large scales. In conclusion, rapid, accurate reporting of quality-assured HF records of mRDT diagnostic positivity could enable population-wide, continuous longitudinal monitoring of malaria transmission so that integrated vector management programmes can be effectively managed, optimized by both local and national malaria control programmes.
2010, Rwanda initiated a 3 year longitudinal study to track net efficacy in 3,000 LLINs (1500 polyethylene/permethrin; 1500 polyester/deltamethrin) at 6 sites. At one month post-distribution, and every 6-monthly interval, 10 LLINs are sampled from each site and tested for bio-efficacy (insecticidal effect) using WHO cone bioassay. A colorimetric field test (CFT) is used to assess surface deltamethrin levels. LLIN durability is assessed using a probability hole index (phi) and theoretical cutoff values to identify the percentage of LLINs in good or serviceable condition. Preliminary results show that at one month, 6 months, and 12 months following distribution 8.5%, 12.9%, and 17.8% of LLINs were missing. LLIN cone bio-efficacy decreased to an average of 84.3% (84.0-84.7) at 6 months and 83.8% (83.3-84.3) at 12 months. Deltamethrin surface levels show 50-80% depletion of insecticide after 6 months with little change at 12 months compared to the baseline. LLINs remain viable with effective insecticide surface concentration at least equivalent to10% of the baseline. The durability assessment indicates that in 5 out of 6 sites <10% (pHb>768 threshold: polyethylene: 3-10%; polyester: 7-30%) of LLINs would require replacement after 6 months and 32% after 12 months (pHb>768 threshold: polyethylene: 37%; polyester: 13-50%). The data suggest that LLINs remain effective after one year of use in Rwanda. However, projected CFT and durability trends indicate that approximately 50% may become ineffective in the next 6 months. These observations highlight the need to conduct LLIN efficacy and durability studies to guide strategies for LLIN replacement and ensure effective universal coverage.

**STUDY ON PERSISTENCE OF INSECTICIDES (BIOASSAY TEST) IMPREGNATED NET-JACKETS FOR MALARIA PREVENTION IN RUBBER TAPPER GROUP AT SURATHANI PROVINCE**

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This study aimed to find out persistence of difference insecticides that were taken to impregnate net-jackets and to compare persistence of used and unused impregnated net-jackets in laboratory and field trial. The net-jackets were impregnated by insecticides namely Permethrin 10% EC, Deltamethrin 1% SC and Alphacypermethrin 10% SC at dosage 300 mg/m², 30 mg/m² and 30mg/m², respectively. Impregnated net-jackets were tested at laboratory room temperature and some were given to rubber tapper volunteer group at Surathani Province in field trial. This volunteer group usually daily wore impregnated net-jackets while they had worked at night. Evaluation was conducted by bioassay test method that Anopheles dirus (laboratory stain) was tested to determine insecticide persistence. In laboratory trial, impregnated net-jackets were bioassay tested after impregnation 4, 8, 18 and 24 weeks. The result of persistence of three insecticides showed mortality rate of An. dirus that were more than 80% significantly at 24 weeks or 6 months. In field trial, impregnated net-jackets were bioassay tested after impregnation 2,4 and 8 weeks. The result of impregnated net-jackets could kill An. dirus effectively that were less than 4 weeks. Deltamethrin and Alphacypermethrin were higher effectiveness than Permethrin. Thus, persistence of unused impregnated net-jackets (Permethrin 300 mg/m², Deltamethrin 30 mg/m² and Alphacypermethrin 30 mg/m²) were more than 6 months at temperature room. The used impregnated net-jackets would have persistence at less than 4 weeks.

**STRENGTHENING COMMUNITY SYSTEM COMPONENTS FOR MALARIA CONTROL: FIVE YEARS INTERVENTIONS IN PASTORALIST COMMUNITIES IN AFAR REGION-EThIOPIA**

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A community based malaria prevention and control programme has been implementing in Afar Region since 2005. The goal of the programme was to contribute to the reduction of malaria related morbidity and mortality among pastoralist population in Afar region, by specifically targeting children under five years and pregnant women.Interventions focused on improving case management of malaria, increasing ITN coverage at community level, and enhancing behaviour and social change in support of all interventions. A baseline survey was done followed by a midline survey in 2007 and a final evaluation in 2010. Both quantitative and qualitative data collection methods were employed to collect data from respondents to assess the impact of this five years programme by comparing the key indicators before and after intervention. The proportion of community members who correctly identified the transmission methods of malaria had increased from 27.4% in 2005 to 88% in 2010. ITN coverage of at least one had significantly changed from 7.5% of base line year to 76% in year 2010. ITN usage of pregnant women and children under five who slept under ITN had also considerably increased from 27% to 79% and 17% to 82% respectively between the two periods. Furthermore, treatment seeking behavior was also improved and the percentage of children under five with fever who took antimalarial drugs within 24 hours increased from 9% at baseline to 53.4% at end of the evaluation period. Mortality rate at health facility level decreased dramatically from 25% in 2005 to 2% at the end of year 2010. The results indicate that strengthening community system in pastoralist populations and linking them to the health system improve the capacity of the community to own their health and contribute to reduce malaria mortality.

**MAN, ECOSYSTEM HEALTH AND MALARIA ON RUSINGA ISLAND, WESTERN KENYA**

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Malaria is a leading cause of morbidity and mortality in Kenya. Existing evidence indicates that prevalence of the disease is greatly influenced by human and ecological factors. While it is well known that certain alterations on natural ecosystems aggravate malaria there is a dearth of information about the relationship between malaria and livelihoods. This study seeks to find the relationship between livelihoods, malaria and ecosystem health. The underlying objective is to generate an evidence base for reviewing malaria health and control policy. The study will be conducted on Rusinga Island, Lake Victoria, western Kenya, where local residents rely on fishing and small-scale farming to support their livelihoods. By carrying out fishing activities outdoors and at night Rusinga fishermen are exposed to a higher risk of malaria because (i) transmitting mosquitoes mainly bite at night, and (ii) currently deployed vector control tools are designed for indoor mosquito control. The apparently high risk of malaria posed by fishery and certain small-scale farming activities will be investigated. This will be accomplished by determining malaria prevalence and screening for mosquito saliva antigens among individuals associated with different livelihood practices. The specific livelihood-related groups of people that will be recruited for studies include subsistence farmers, fish traders, boat owners, fishing crew and stakeholders involved in fishery activities namely net/boat makers/repairers, transporters, and fish bait miners/traders. We will also attempt to explain how actions directed towards supporting livelihoods modify ecosystems in ways that may aggravate malaria. Communication tools will be developed to share knowledge generated from these activities among the local residents. An outcome mapping model will be developed to measure changes in behaviour among collaborating boundary partners namely Kibisom women group and the Mbita district public health office (Mbita-DPHO).
SIMULATED COMMUNITY-LEVEL EFFECTS OF COMBINING LONG LASTING INSECTICIDAL NETS WITH INDOOR RESIDUAL SPRAYING FOR MALARIA CONTROL IN AFRICA

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It is common practice to combine indoor residual spraying (IRS) with long-lasting insecticide nets (LLINs) in highly endemic communities, but there is limited evidence to suggest that the strategy confers greater protection against malaria than either intervention alone. Experimental hut trials have demonstrated improved personal and household protection with certain LLINIRS combinations, but it remains unclear whether there are proportionately greater benefits at community level. A deterministic mathematical model of mosquito life cycle processes was adapted and used to estimate how malaria transmission might be affected if LLINs are combined with IRS, relative to use of either method alone. The model was modified to use data derived directly from experimental hut evaluations where untreated bed nets are used as experimental controls. We simulated a closed community where residents own cattle, and the main malaria vector is *Anopheles arabiensis*, an increasingly important vector species in Africa, which remains a major challenge even with high LLINs and IRS coverage. Considering situations with either LLINs or IRS as the pre-existing intervention, we calculated relative improvement in transmission control each time a complementary intervention was introduced.

Transmission control is improved when the common pyrethroid based LLINs are added onto toxic IRS treatments such as pirimiphos-methyl and lambda cyhalothrin, but not DDT, which is known to be less toxic against mosquitoes. On the other hand, the outcome remains unchanged when IRS with lambda cyhalothrin or DDT is added to communities already using LLINs. Addition of pirimiphos-methyl IRS provided the greatest improvement relative to the LLINs alone. This *in-silico* assessment shows that whereas introduction of LLINs into communities with pre-existing IRS will generally result in improved control of malaria transmission, introduction of IRS into communities with pre-existing LLIN use will most likely be redundant unless the IRS is highly toxic to malaria mosquitoes.

EVALUATING PROGRESS OF INTENSE IMPORTED MALARIA TRANSMISSION IN SOUTH AFRICAN PROVINCES: RETROSPECTIVE ANALYSIS

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For the past 50 years, because of national and global malaria strategies put in place vector transmission was low in South Africa. Successful early malaria control policies and strategies developed non-immunity to malaria amongst most South Africans. The results were an increased risk of complicated and severe infections from *Plasmodium falciparum* and other untreated vector species. Southern African populations consistently visit or migrate to and from malarious areas, including countries bordering South Africa. Exposure to mobile populations with malaria infections contributes to the burden of disease in South African Provinces. The most vulnerable are children under five, pregnant women and those with co-morbidities such as HIV and TB. Imported malaria is identified as a major concern within endemic and non endemic Provinces of South Africa in regional mapping and by the Republic of South Africa's National Malaria Programme Performance Review-2009. A geographical focus is used to identify high transmission areas in South African Provinces in low lying North East areas of: Limpopo, KwaZulu-Natal and Mpumalanga where malaria is endemic and seasonal. A literature review synthesized previous research from 1982-2012. The Study also analyzed trends in the understanding and knowledge of imported malaria in Southern Africa. Quantitative indicators are used to build on existing malaria control measures in South and Southern Africa while evaluating the progress of the intense burden of imported malaria in South Africa. This was achieved by analyzing: sentinel surveillance measures, malaria control interventions, and transmission rates based on data from mosquito breeding sites and climate. The Study emphasizes sustainable capacity building for: surveillance, quantification and local community participation. Cross border malaria initiatives from five countries bordering the Provinces of: Limpopo, KwaZulu-Natal and Mpumalanga were analyzed based on the quantitative indicators described. Studying imported malaria in South Africa is a regional and global contribution to: improving surveillance, human interaction with ecological systems that are breeding sites for mosquitoes, economic development, health outcomes and public health policies associated with malaria as a debilitating and potentially fatal infection.
field to acquire durability data. The objective of the present work is to
develop one or more laboratory tests that can be used to evaluate how
well LLINs withstand realistic physical challenges, using standard textile
testing equipment. The focus has been on measuring the susceptibility
of fabrics to deterioration after suffering initial damage by rodents or hot
surfaces. Modifications of standard bursting strength, tensile strength,
tear resistance, and abrasion resistance test methods have been evaluated
for reproducibility and consistency with the results of field studies. This
investigation has also provided insight into the mechanisms of LLIN
deterioration and possible strategies for improving durability.

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MODELING THE EFFECTS OF VECTOR CONTROL
INTERVENTIONS IN REDUCING MALARIA TRANSMISSION
AND DISEASE BURDEN

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Malaria interventions are usually prioritized using efficacy estimates
from intervention trials, without considering the context of existing
intervention packages or long term dynamics. Currently, long lasting
insecticidal nets (LLINs) and indoor residual spraying (IRS) form the
mainstay of most malaria control programs. However, in the face of
emerging resistance in mosquitoes and a push to elimination, extensions
and new combinations of these interventions are being considered, along
with the development of novel interventions, such as outdoor traps,
and a revival of older interventions such as larval source management.
We use numerical simulations of an ensemble of mathematical models
of malaria in humans and mosquitoes to provide robust quantitative
predictions of the effectiveness and cost-effectiveness of combinations of
these interventions, in reducing transmission, morbidity and mortality. We
estimate reductions in entomological inoculation rate, prevalence, clinical
cases, and malaria deaths from simulations of different coverage levels of
LLINs, IRS, larval control, and outdoor traps. We simulate scenarios with
various vector distributions, and transmission and health system settings.
Our results suggest that sustained coverage of one or two vector control
interventions reduces malaria prevalence through the first two or three
campaigns but does not lead to continually increasing gains beyond
that. However, in some settings, even with sustained coverage, clinical
incidence of malaria increases as the population loses its naturally acquired
immunity. In some low to medium transmission settings, our simulations
suggest that high coverage of both LLINs and IRS can lead to interruption
of transmission, however, larval control or outdoor traps are necessary
when a separate population of mostly outdoor biting mosquitoes exists.
We can simultaneously capture in mathematical models the dynamics
of mosquito ecology, malaria epidemiology, human demography, health
systems effects, and control interventions. Fitting an ensemble of models
to data leads to plausible quantitative predictions, with accompanying
uncertainty ranges, of the effects of a comprehensive set of different
interventions in reducing and potentially interrupting transmission.

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MARKET COMPETITION AND CUSTOMER DEMAND
DETERMINE STOCKING PATTERNS AND RETAIL PRICES OF
ACTS IN PRIVATE DRUG SHOPS IN TANZANIA

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Many sub-Saharan African households utilize the private sector as a
primary source of treatment for malaria episodes. Cost, however, is
reported to be a major impediment to shop level stocking of effective
anti-malarials such as ACTs. The Affordable Medicines Facility - malaria
(AMFm), an innovative financing mechanism hosted by the Global Fund,
is a unique supply side subsidy designed to increase availability of ACTs in
eight pilot countries including Tanzania. A series of shop level surveys
of private drug retailers in two regions of Tanzania aimed to discover supply
and demand side factors which determine availability and reported retail
price of subsidized ACTs in private shops. Accredited Drug Dispensing
Outlets (ADDOs) in the Rukwa and Mtwa regions were surveyed
between Feb 2011 and May 2012. Surveyors noted whether subsidized
ACTs were being sold and recorded retail prices. Shop attendants were
asked a battery of questions including malaria knowledge, source of
drug supply, and participation in training programs. Exit interviews with
customers at the ADDOs provided data on the type and price of the
antimalarial purchased. Trends and extent of ACT stocking over time and
space were described using statistical and spatial methods. Supply side
determinants of ACT stocking were assessed using distance to reported
medicine supplier. Competition from surrounding providers was assessed
using the location of retail shops and public reproductive health clinics
(RCH). GIS layers for surrounding population were used to estimate the
size of catchment populations. Remoteness and distance to wholesale
sources of medicines were not found to be associated with ACT stocking
patterns. Shop size and population were found to be highly associated
with ACT stocking. Proximity to other shops stocking ACTs (p=.04)
and increased number of competing shops (p=.006) were statistically
significantly associated with an increased likelihood of stocking ACTs
in Rukwa. Similarly, presence of RCH clinics determined stocking. High
numbers of proximal shops was also associated with increased prices
charged for ACTs. Stocking of ACTs and prices charged were both higher
in areas along Lake Rukwa than in other areas of similar distance from
urban areas and population size. The AMFm program appears to have
resulted in increased availability of ACTs, though shop level factors also
influence stocking and prices charged.

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INDUCIBLE INSULIN-LIKE PEPTIDE SYNTHESIS IN ANOPHELES
STEPHENSII: A MECHANISM FOR PLASMODIUM MEDIATED
IMMUNOSUPPRESSION

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The insulin-like peptides (ILPs) and their respective signaling and regulatory
pathways are highly conserved across diverse phyla. Previously, we
reported that infection with the human malaria parasite, Plasmodium
falciparum, induces ILP transcription in the midgut of Anopheles stephensi,
suggesting that the ILPs are produced in response to infection-associated
signals and modulate some aspects of sporogonic development.
In particular, our data revealed that soluble factors derived from
P. falciparum, but not from bacteria or fungi, can induce ILP transcription
and secretion in An. stephensi cells. This induction was dependent on
insulin/insulin-like growth factor signaling (IIS) through MEK-ERK and
PI3K-AKT activation. Additionally, knockdown of an infection-induced
ILP in vivo resulted in enhanced immune effector gene expression and
decreased parasite survival in P. falciparum infected mosquitoes. Together,
these data suggest that Plasmodium-specific factors signal through IIS to
induce immunosuppressive ILPs in the midgut, a critical tissue for parasite
development. The ILPs should be considered, therefore, important targets
in future efforts to engineer Plasmodium-resistant mosquitoes.
Despite scaled-up coverage of insecticide treated nets (ITNs) in malarious areas of sub-Saharan Africa, proper and regular ITN use remains inadequate. An understanding of what determines ITN use could help improve effectiveness. In early 2011, a household-level, questionnaire-based survey of ITN practices was conducted following a mass distribution program. The goal was to assess post-intervention Plasmodium infection, and whether households used ITNs to protect target groups such as pregnant women and children. Following a complete enumeration of all households (~3,340), each one was censused for all residents and surveyed. Pre-school children were tested for presence of parasites using PCR methods. Questions of household heads involved who slept under what ITN the previous night, as well as age and sex. Data analysis involved spatial methods and regression models tailored to account for non-linear patterns in age-related ITN use. GIS methodologies were used to determine spatial patterns of ITN use and malaria cases. Information on 12,095 individuals aged 90 years old was gathered, of which ~25% were <5 years of age. More than half (56%) of people reported not sleeping under an ITN the previous night. Age was an important determinant of ITN use. Adults over 30 and infants sleep under ITNs more than children and young adults. The distribution of age and ITN use followed a significant (p <0.001) nonlinear pattern, decreasing from birth to age 18, increasing to and remaining constant after age 30. This pattern was significant even when accounting for confounding factors. Differences in gender were not significant for any age group, but women between the ages of 15 and 30 tended to use ITNs more than males. Household-level clusters of Plasmodium infections were associated with fewer children sleeping under nets, and were geographically located in wet, low lying areas closer to the lake, despite high levels of net use and possession. Though ITNs were found to be effective in reducing Plasmodium infections, spatially, evidence suggests that net possession and use were highest in areas prone to nuisance mosquitoes and possible perception of high malaria risk. Results suggest that ITN use may be high among some members of higher-risk groups, however there is inadequate coverage among young adults. The distribution of age and ITN use followed a significant (p <0.001) nonlinear pattern, decreasing from birth to age 18, increasing to and remaining constant after age 30. This pattern was significant even when accounting for confounding factors. Differences in gender were not significant for any age group, but women between the ages of 15 and 30 tended to use ITNs more than males. Household-level clusters of Plasmodium infections were associated with fewer children sleeping under nets, and were geographically located in wet, low lying areas closer to the lake, despite high levels of net use and possession. Though ITNs were found to be effective in reducing Plasmodium infections, spatially, evidence suggests that net possession and use were highest in areas prone to nuisance mosquitoes and possible perception of high malaria risk. Results suggest that ITN use may be high among some members of higher-risk groups, however there is inadequate coverage among young and school-age children. Efforts to further scale up ITN possession and programs to focus messages regarding proper use remain necessary.
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IMPACT OF COMMUNITY SCREENING AND TREATMENT OF ASYMPTOMATIC CARRIERS OF PLASMODIUM FALCIPARUM WITH ARTEMETHER-LUMEFANTRINE ON ASYMPTOMATIC AND GAMETOCYTE CARRIAGE: A 12-MONTH, CLUSTER-RANDOMIZED STUDY IN SUB-SAHARAN AFRICA

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Human to mosquito transmission of Plasmodium falciparum depends on the presence of sexual stage parasites, gametocytes, in the peripheral blood. Interventions in asymptomatic carriers (ACs) aiming to reduce disease transmission should also be effective against gametocytes. This 12-month, controlled, parallel, cluster-randomized (18 clusters: 9 intervention, 9 control) study was conducted in Burkina Faso to evaluate the impact at the community level of systematic screening and artemether-lumefantrine (AL/LF) dispersible treatment of RDT-detected ACs during three community screening campaigns (CSCs 1-3). CSCs 1-3 occurred before the rainy season and CSC4 occurred after, marking the end of the study. Symptomatic malaria episodes were treated with AL or an alternative in both arms during the study. The prevalence of microscopy-confirmed ACs in the intervention and control arms was 42.8% vs. 47.5%; 4.1% vs. 35.7%; 2.8% vs. 32.2% and 34.4% vs. 37.8% at CSC1, 2, 3 and 4, respectively. The proportion of gametocyte carriers (GCs) was evaluated by microscopy in all subjects at CSCs 1-4 in the intervention arm and in a randomly selected 40% subset of the control arm, and by qRT-PCR at CSC4 in 1,999 randomly selected subjects across both arms. The overall proportion of GCs in the intervention and control arms was 9.5% vs. 10.2%, 0.6% vs. 5.5%, 0.4% vs. 5.8% and 4.8% vs. 5.1% at CSC1, 2, 3 and 4, respectively. The prevalence (least square mean [SE]) of microscopy-confirmed GCs at CSC4 in the intervention arm was 4.9 (0.41) vs. 5.1 (0.41) in the control arm (p=0.7208). Prevalence of GCs at CSC4 as assessed by qRT-PCR was around 8 times greater in both arms compared to microscopy (49.7% vs. 6.0% intervention; 47.3% vs. 5.4% control). In this community-setting study, the intervention arm showed greater reductions in the prevalence of ACs and GCs than the control arm at CSCs 2 and 3, relative to CSC1 (p<0.0001). However, AC and GC prevalence rose thereafter in the intervention arm to reach a level similar to the control arm at CSC4 (p=NS).

ONLINE INTERACTIVE PLATFORM FOR MAPPING REPORTS OF INSECTICIDE RESISTANCE IN MALARIA VECTORS

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Insecticide-based interventions including indoor residual spraying and treated bed nets have led to significant reductions in malaria morbidity and mortality. However, the emerging and rapid spread of resistance to all available classes of public health insecticides threatens current malaria vector control efforts. The Global Plan for Insecticide Resistance Management released by the WHO in May 2012 contained guidance on the rationale and implementation of strategies for preserving the efficacy of current tools, which included utilization of insecticide resistance data for informing vector control decisions. There has long been a need for a comprehensive global resistance database to aggregate data currently scattered across many sources in order to facilitate a coordinated response across the malaria-stakeholder community. IR Mapper was developed to address this need (www.irmapper.com); this free online resource consolidates published information from WHO susceptibility tests on Anopheles malaria vectors from 1959 to date. Information is provided via a user-friendly interface that allows users to project data on maps based on selected vector species, insecticide classes and types. Susceptibility data are viewable based on old WHO susceptibility categories or using the new categories as recommended from May 2012. Resistance mechanism and gametocyte carriage data are similarly presented, with links to original data sources provided along with other key study information. The utility of this resource will be demonstrated using examples from two large-scale malaria control programs in Africa.

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DETECTION OF EASTERN EQUINE ENCEPHALOMYELITIS VIRUS RNA IN NORTH AMERICAN SNAKES

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The role of non-avian vertebrates in the ecology of Eastern Equine Encephalitis virus (EEEV) is unresolved, but mounting evidence supports a potential role for snakes in the EEEV transmission cycle, especially as overwintering hosts. To determine rates of exposure and infection, we examined serum samples from wild snakes at a focus of EEEV in Alabama for viral RNA using RT-PCR. Two species of vipers, the Copperhead

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A LABORATORY CONFIRMED CASE OF JAMESTOWN CANYON VIRUS ENCEPHALITIS IN A QUEBEC RESIDENT WITH TRAVEL HISTORY TO MAINE AND NEW HAMPSHIRE

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Jamestown Canyon virus (JCV) is a mosquito-borne arbovirus belonging to the California serogroup (CSG) of bunyaviruses. JCV is widely distributed throughout North America, however, reports of human JCV infection with associated febrile and neurological disease are rare. We report a recent laboratory confirmed case of JCV encephalitis in a Montreal, Quebec resident with travel history to Maine (ME) and New Hampshire (NH). The patient was a 53 year old male who presented with symptoms of fever, headache and chills 10 days after returning from a camping trip in ME and NH in mid August, 2011. Several days later he was hospitalized and his illness progressed to an altered mental state comprising of confusion and difficulty speaking suggestive of encephalitis. He had trouble breathing and was intubated. He was hospitalized for approximately a month during which blood was collected and lumbar punctures performed. Cerebrospinal fluid (CSF) testing indicated normal protein and glucose with the presence of a low leukocyte count. Serological testing of acute and convalescent serum collected three weeks apart gave a 4-fold rise in specific neutralizing antibody to JCV (640 to 2560). Using a CDC-based IgM ELISA the acute and convalescent serum samples were positive for JCV IgM. Testing of acute and convalescent CSF for JCV antibodies also indicated a positive IgM result for JCV and a seroconversion by neutralization testing (range 0 -16). Significantly lower or negative cross reacting titers were observed for related CSG viruses such as snowshoe hare and La Crosse viruses and no antibodies were detected to other arboviruses such as West Nile, eastern equine encephalitis, or Powassan virus. Based on several laboratory case definition criteria our results indicate that the patient's febrile and neurological symptoms were associated with an infection of JCV. The incubation period of JCV is believed to range from 3 to 14 days and the travel history of the patient is consistent with exposure to JCV infected mosquitoes in ME or NH. It is also possible that he may have been infected in Quebec since symptom onset did occur several days after his return to Canada. Based on available CDC CSG virus case data no confirmed cases of JCV illness have been documented in ME or NH previously. Our findings underscore that JCV can cause serious neurinvasive disease such as encephalitis and should be considered when an arbovirus infection is suspected in this region of North America.

IDENTIFICATION OF NEUTRALIZING ANTIBODY EPITOPES ON CHIKUNGUNYA VIRUS ENVELOPE PROTEIN

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To obtain anti-Chikungunya (CHIKV) Envelope monoclonal antibody (MAb) epitope maps at the resolution of individual amino acids, we individually mutated 920 residues of CHIKV (S27 strain) Envelope protein (E2/E1) to alanine, expressed each mutant in human cells, and analyzed them for effects on antibody reactivity and viral infectivity. This ‘Shotgun Mutagenesis’ approach offers the capability of mapping both linear and conformational epitopes, even for structurally complex proteins including oligomeric and glycosylated Envelope proteins such as CHIKV E2/E1. The neutralizing human anti-CHIKV MAbs used in our studies were derived from infected patient B-cells using phage display library panning against purified CHIKV virus like particles (VLPs) and from B-cell cloning. Critical amino acids required for the binding of each MAb were identified and purified CHIKV virus like particles (VLPs) and from B-cell cloning. Critical amino acids required for the binding of each MAb were identified and purified CHIKV VLPs on a biosensor. Critical amino acids required for the binding of each MAb were identified and purified CHIKV VLPs on a biosensor. Critical amino acids required for the binding of each MAb were identified and purified CHIKV VLPs on a biosensor. Critical amino acids required for the binding of each MAb were identified and purified CHIKV VLPs on a biosensor. Critical amino acids required for the binding of each MAb were identified and purified CHIKV VLPs on a biosensor. Critical amino acids required for the binding of each MAb were identified and purified CHIKV VLPs on a biosensor.

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contribute to neutralization of infection, and how they relate to protein function. We expect that this approach will help define the range of immunodominant structures on CHIKV Env and identify novel neutralizing antibody epitopes that can be used for the development of improved therapeutics, diagnostics, and vaccine candidates.

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DEVELOPMENT OF AN IRES-BASED VACCINE FOR WESTERN EQUINE ENCEPHALITIS VIRUS

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Western equine encephalitis virus (WEEV), a member of the family Togaviridae, genus Alphavirus, has a single-stranded, positive-sense RNA genome, and is an important mosquito-transmitted human and veterinary pathogen in North and South America. Infection with WEEV can result in severe neurological sequelae in human survivors, with an economic impact ranging from 21,000 to 3 million dollars per case. WEEV is also considered a bioterrorism agent since aerosolized virus causes high primate mortality. Regrettably, there is no vaccine or antiviral therapies to aid in mitigating a natural outbreak, bioterrorist attack, or accidental lab exposure. The objective of this study was to develop a safe and efficacious WEEV vaccine. Two different live-attenuated WEEV vaccines were engineered via the introduction of an internal ribosomal entry sequence (IRES) from encephalomyocarditis virus (EMCV), to control translation of the structural (WEEV/IRESv1) or capsid (WEEV/IRESv2) protein(s). Previous research shows the IRES element from EMCV cannot initiate efficient translation in arthropod cells, making this vaccine unable to be propagated in its natural transmission cycle. Serial passaging in Vero cells showed no reversion to a wild-type-like phenotype; however, several mutations were observed in the structural genes that provided for higher titers in cell culture. WEEV/IRESv1 and WEEV/IRESv2 were tested for immunogenicity and attenuation in relevant murine models. Our results suggest that our IRES-dependent live-attenuated vaccine for WEEV merits further study and this vaccine could be used for the development of an emergency vaccine that can be used during a natural outbreak, bioterrorism attack, or accidental lab exposure.

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DISTRIBUTION OF KILLER CELL IMMUNOGLOBULIN-LIKE RECEPTORS (KIR) GENES IN AN ADMIXED PERUVIAN POPULATION

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KIR are glycoproteins located on the surface of NK cells. These receptors are classified into two groups according to their cytoplasmic domain, which transduces inhibitory or activating signals, and consequently modulates NK cell function and most likely the susceptibility to diseases or infections. We studied the distribution of KIR genes in 363 Peruvian HTLV-1-infected individuals using two ethnic classification methods: 1) a questionnaire, which defined the participants as Andean (both parents born in the Andes) or Mestizo (only one parent born in the Andes); and b) ancestry informative markers (AIM), which allowed classifying the whole population into three groups according to their ethnic admixture proportions. DNA was obtained from blood samples of each individual and KIR genotyping was carried out using PCR-SSP. No significant differences were observed in gender and age according to the Andean/Mestizo classification, whereas significant differences were found when the ethnic admixture proportion criterion was applied. The frequency of KIR2DS3, KIR2DS4 and KIR2DL3 were statistically different between Andeans and Mestizos. When using ethnic admixture proportion, significant differences were observed for KIR3DL1 and KIR2DS4s in addition to those genes, among the three groups defined. No significant differences were detected in haplotypes and inhibitory-activating KIR genes using either the questionnaire or AIM-based classification. AIM helps minimizing both the bias in ethnic group definition and the effects of population stratification, and therefore should be used in order to avoid false results when searching for gene-disease associations in admixed populations.

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NOROVIRUS INFECTION IN PERU

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Norovirus (NoV) is one of the most frequent causes of outbreaks and sporadic cases of gastroenteritis worldwide. Although rarely fatal, NoV transmission has important economic repercussions, including loss of work days and incurrence of costly medical care. The incidence of NoV gastroenteritis is usually highest in adults. Although well-studied in industrialized countries, few data are available on NoV in the developing world. We report NoV surveillance data from 3 distinct regions and populations in Peru. The first two surveillance populations and areas were healthy children <5 years old in rural communities near the town of Pisco (coastal desert) in 2009 and in Loreto District (Amazon forest) in 2010. The third target population and surveillance area was from a 9-year prospective cohort study of diarrheal illness among Peruvian military recruits at the Vargas-Guerra Army Training Base in the city of Iquitos, also in the Amazon. At each site, fecal samples were collected and sent to the U.S. Naval Medical Research Unit-6 laboratory in Lima for testing for NoV by real-time PCR. From Pisco, NoV was found in 27 (9%) of 294 samples. Five (19%) were genotype I and 22 (81%) genotype II. From Loreto, 32 (11%) of 290 samples were positive, 10 (31%) genotype I and 22 (69%) genotype II. From the Vargas-Guerra Training base, 49 (25%) of 200 samples were positive, 6 (12%) for genotype I, 38 (78%) for genotype II, and 5 (10%) co-infection with both genotypes. Our results indicate that NoV circulates in both pediatric and adult populations in Peru and that genotype II predominates. Interestingly, infection was common even in the healthy children. Epidemiologic studies are underway to explore the significance of the NoV infections in children and the ultimate incidence of disease. In addition, we are undertaking further molecular characterization and phylogenetic analysis of NoV strains in Peru.

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SEROPREVALENCE OF ALPHAVIRUSES AND FLAVIVIRUSES IN FREE-RANGING GAME ANIMALS AND NON-HUMAN PRIMATES IN THE CONGO BASIN

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Vector-borne and zoonotic pathogens have comprised a significant proportion of the emerging infectious diseases in humans in recent decades. The role of many wildlife species as reservoirs for arthropod-borne viral pathogens is poorly understood. We aimed to investigate the exposure history of various African wildlife species from the Congo Basin to mosquito-borne flaviviruses (Flaviviridae: Flavivirus) and alphaviruses (Togaviridae: Alphavirus) by testing previously-archived serum samples. In total, sera from 24 African forest buffalo (Syncerus caffer nanus), 34 African elephants (Loxodonta africana), 40 duikers (Cephalophus and Philantomba species), 25 mandrills (Mandrillus sphinx), 32 mountain gorillas (Gorilla beringei beringei), five Grauer’s gorillas (Gorilla beringei graueri), two L’hoest’s monkeys (Cercopithecus lhoestii), two golden monkeys (Cercopithecus kandti), and three chimpanzees (Pan troglodytes) sampled between 1991 and 2009 in the Congo basin were tested for antibodies against chikungunya virus (CHIKV) (Togaviridae: Alphavirus),
to explore the prevalence of human infection to these viruses among SFV and herpes viruses in captive NWPs in Peru. We plan follow-up studies they are human or NWP species. Our results show a high prevalence of identify the specific viruses and, in the case of the herpes viruses, whether herpes virus. Molecular characterization of the viruses is ongoing to Saimiri and Ateles, Callicebus, Callithrix, Cebus, Lagothrix, Pithecia, Saguinus Alouatta, Aotus, positive for SFV, including animals from the genera (antibody in retroviruses correlates with active virus infection), and for SFVs by enzyme immunoassay, with confirmatory Western blot analysis of infection of these zoonotic viruses to humans with exposure to these herpes virus and its reservoir species. Herpes viruses are easily transmitted that infect a variety of animals, with co-evolution between each unique. Herpes viruses are ubiquitous agents Callithrix these data are limited to small numbers of captive monkeys from genera Previous reports show genetically distinct SFV variants among NWPs but exposed to Old World monkeys and apes in captivity and in nature. Infection has occurred in humans exposed to CHIKV, WN, and DENV-2, while mandrills in Gabon were also seropositive for CHIKV, DENV-2, WN, and YFV. One hundred percent of elephants tested had a very strong neutralizing antibody response to WN. This study also represents the first arbovirus serosurvey of gorillas, of which 4/32 (12.5%) were seropositive for either an alphanavirus and/ or flavivirus. Our results demonstrate a high prevalence of neutralizing antibodies against these arboviruses in wildlife in the Congo basin.

SIMIAN FOAMY VIRUS AND HERPES VIRUS IN CAPTIVE NEW WORLD PRIMATES IN PERU

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Many species of New World primates (NWPs) exist in Peru and are frequently illegally captured for pet trade, traditional medicine, or consumption. Government confiscation and placement of these animals has led to extreme crowding in zoos and rescue and rehabilitation centers, providing ideal conditions for animal-human transmission of zoonotic pathogens. Simian foamy viruses (SFV) are retroviruses found in high prevalence in various simian species. Infection has occurred in humans exposed to Old World monkeys and apes in captivity and in nature. Previous reports show genetically distinct SFV variants among NWPs but these data are limited to small numbers of captive monkeys from genera Cebus, Saimiri, Ateles, and Callicebus. Herpes viruses are ubiquitous agents that infect a variety of animals, with co-evolution between each unique herpes virus and its reservoir species. Herpes viruses are easily transmitted from their reservoirs to accidental hosts through direct contact and fomites, often resulting in a fatal outcome. We assessed the prevalence of SFV and herpes virus infection in captive NWPs to help assess the risk of infection of these zoonotic viruses to humans with exposure to these animals. Serum samples were collected and tested for antibodies to SFVs by enzyme immunoassay, with confirmatory Western blot analysis (antibody in retroviruses correlates with active virus infection), and for herpes virus by PCR. Sixty (38%) of 157 tested NWPs were antibody positive for SFV, including animals from the genera Alouatta, Aotus, Ateles, Callicebus, Callithrix, Cebus, Lagothrix, Pithecia, Saguinus and Saimiri. Twenty-one (15%) of 144 tested NWPs were PCR positive for herpes virus. Molecular characterization of the viruses is ongoing to identify the specific viruses and, in the case of the herpes viruses, whether they are human or NWP species. Our results show a high prevalence of SFV and herpes viruses in captive NWPs in Peru. We plan follow-up studies to explore the prevalence of human infection to these viruses among Peruvians in contact with captive NWPs.
pathogens being Coxackievirus A16 (CA16) and Enterovirus 71 (EV71). Increasingly, outbreaks of HFMD, particularly those caused by EV71, have garnered the attention of the public, clinicians and national and international health agencies. Recently, there has been an alarming increase in the number of patients and an increase in the number of cases complicated by central nervous system and cardiopulmonary involvement and deaths in young children in countries across South East Asia. Epidemic patterns are complex, though large outbreaks tend to be cyclical, occurring every 2 to 3 years. With vaccine in development, the question remains whether HFMD caused by CA16 or EV71 is an immunizing infection and whether infection by one pathogen confers protection from infection by the other. Using over a decade of weekly infection case data from Japan and Singapore, we find that the case data are consistent with the pathogen acting as an immunizing infection with a strong signature of herd immunity. Preliminary simulations indicate that vaccinations could succeed at limiting epidemics. We validate these findings with multiannual infection case data from other countries in the region. Our finding that HFMD acts qualitatively as an immunizing infection is promising for the infection case data from other countries in the region. The success of future vaccination efforts for the control of this disease. Further surveillance and regional cooperation including analysis of epidemiological, clinical, and virological data sets for Hand Foot and Mouth Disease would help guide future control strategies and inform policy.

HANTAVIRUS RODENT RESERVOIRS IN BULGARIA

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Hantaviruses are a group of RNA viruses belonging to the Bunyaviridae family, genus Hantavirus, and their natural reservoirs are wild rodents. Hantaviruses are the global emerging diseases, with evolving strains detected throughout the world. Different serotypes as Seol (SEO), Dobrava (DOB), Pumuula (PUU) and Hantaan (HTN) were detected in the Balkan region. Human disease causes from DOBV in Bulgaria were recently confirmed. In a previous study on hospital-based acute febrile illness cases, we detected PUUV IgM using ELISA (Progen) and DOBV neutralizing antibody by RT-PCR. In this study we conducted rodent surveillance in areas with documented human cases to identify the circulating strains of Hantaviruses. Rodents were trapped starting May 2011 through March 2012 for two successive night patterns per month. Temperature and humidity were monitored at specific GPS coordinates recorded for trapping sites. A total of 705 rodents from two different sites (Plovdiv and Burgas) were trapped. Fourteen different rodent species were collected during the study period. Serum samples and organs (lung, spleen and kidneys) were collected. An organ pool, preserved with RNA-later and homogenized under BSL3 conditions, followed by RNA extraction using Qiagen products. Real-time RT-PCR for DOBV and PUUV testing was performed on rodents collected in May, June and October (n=224), of which three DOBV and no PUUV position samples were detected. All DOBV was found in male Apodemus flavicollis species (susceptible to DOBV) collected in June: two from Plovdiv and one from Burgas. Screening of the remaining rodent collection is still in progress, and analysis of temporal conditions and abundance of species may provide a potential outbreak prediction model.

EVALUATION OF VIRUS STRAINS AND THE EFFECT OF E1 MUTATIONS ON THE EXPRESSION OF CHIKUNGUNYA VIRUS-LIKE PARTICLES

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A challenge to the development and manufacture of affordable vaccines for a global market is reducing the cost of goods required for vaccine production. Due to the inverse relationship between the productivity of antigen expression and cost, increasing the efficiency of antigen production is a strategy used to reduce cost. In collaboration with the NIH, we are developing a chikungunya virus (CHIKV) vaccine based on transient expression of ORF2 in human cells to produce CHIKV virus-like particles (VLPs), as reported previously. In order to minimize the cost of goods, we have tested two strategies to increase the levels of CHIKV VLPs in culture supernatants. First, we evaluated the levels of CHIKV VLPs among 10 different CHIKV strains. Expression of strain 37997 ORF2 yielded the highest levels of CHIKV VLPs in the culture media, which was consistent with the study by Akahata, et al. Six strains including LR2006 OPV-1 and S27 exhibited low levels of VLPs, while two strains, ALSA-1 and Nagpur, failed to produce any detectable VLPs. Western blotting demonstrated a defect in p62 processing and low levels of E1 compared to strain 37997. As an alternate strategy for increasing VLP productivity, we tested the hypothesis that increasing the stability of the E2-E1 heterodimer by reducing the threshold pH for conformational changes in E1 that lead to membrane fusion may result in increased CHIKV VLP production. To test this hypothesis, we introduced 3 different E1 mutations that were shown to decrease the pH of fusion of another aliphavirus. We found that those mutations increased the levels of VLPs in culture media after transient transfection. Moreover, additional substitutions for each of those 3 residues have identified several other mutants with enhanced VLP productivity. Current efforts are underway to determine if combining the mutations will further increase CHIKV VLP productivity. Work is also in progress to better understand the effect of these mutations on the stability of the E2-E1 heterodimer.

MOLECULAR CHARACTERIZATION OF ANTIVIRAL SUSCEPTIBILITY OF INFLUENZA A ISOLATES OBTAINED IN KENYA FROM 2008 TO 2011

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Presently, there are two main classes of antivirals in use which function by inhibiting specific steps within the virus replication cycle: M2 inhibitors block the uncoupling of the virus through acidification of the interior of the virion. In neuraminidase inhibition, inhibitor molecules mimic NA’s substrate and bind to the active site, preventing NA from cleaving host cell receptors and releasing new virus. The study characterized antiviral susceptibility of the 2008-2011 influenza A strains using known molecular markers in neuraminidase (NA) protein. In the 2008-2009, 2009-2010 and 2010-2011 influenza seasons, a total of 836 viruses were isolated. 344 (41%) were influenza A/H3N2, 144 (17%) seasonal influenza A/H1N1 and 348 (42%) belonged to the pandemic influenza A/H1N1 strain. A total of 108 (13%) isolates were analyzed for susceptibility to NA inhibitors. In the year 2008, 33 influenza A/H3N2 and 11 seasonal influenza A/H1N1 were included in the genotypic characterization assay for neuraminidase inhibitor resistant mutations. Sequence assembly and alignment revealed absences of molecular markers of neuraminidase inhibitor drug resistance (Y225) in influenza A/H3N2. 64% (7) of the 2008 seasonal influenza A/
H1N1 isolates had resistant marker H275Y. 4 (36%) of the seasonal A/ H1N1 isolates, lacked the drug resistant marker depicting sensitivity to the class of drugs. Genetic analysis of the 48 pandemic influenza A/ H1N1 strains in 2009 showed that all were sensitive to oseltamivir through possession of histidine at position 274 of the neuraminidase protein sequence. The same pattern was duplicated in 2 of the pandemic influenza A/ H1N1 isolates analyzed in the year 2010. All the 2011, 14 isolates belonging to influenza A/H3N2 subtype lacked the H275Y substitution in the neuraminidase protein. Genotypic data obtained in this study demonstrate antiviral resistance in seasonal influenza A/H1N1 viruses isolated in Kenya in 2008-2009 through possession of H275Y (N1 numbering) marker in the neuraminidase protein.

APPLICATION OF IN VIVO IMAGING IN THE CHARACTERIZATION OF OLFATORY INFECTION OF MICE WITH WEEV

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Isolates of western equine encephalitis virus (WEEV) can cause severe disease in both humans and animals, and may serve as a model for other neurovirulent alphaviruses. Infection of the McMillan strain (McM) of WEEV leads to high mortality in an outbred CD-1 mouse model. An infectious recombinant WEEV:McM expressing firefly luciferase (FLUC) was developed to characterize CNS infection after intranasal exposure. Correlative relationships were determined between bioluminescence and both viral titer and immunological markers of WEEV:McM/FLUC. Histopathological examination of tissue was guided by corresponding images and revealed that neuroinvasion occurred primarily through the olfactory tract. Olfactory bulb neurons were initial targets and led to the infection of the anterior olfactory nucleus, basal ganglia, hypothalamus, amygdala, thalamus, hippocampus, and cerebrum. IHC staining showed intense neurotropism with very few supportive cells of infection and a respiratory sign or symptom) in Santa Rosa (Nov 2007-Mar 2012), Quetzaltenango (Feb 2009-Mar 2012), and Guatemala City (Nov 2009-Apr 2011). Nasopharyngeal swab specimens were tested for RSV using real-time reverse-transcription polymerase chain reaction. Among ARI cases, we measured associations between RSV-positivity and indicators of severity using linear and logistic regression, adjusted for age, gender, surveillance site, and year. To test for effect modification by age, we added an interaction term for RSV-positivity and age ≤5 years to the models. We enrolled and tested for RSV 7919 patients; 5626 met the ARI and 2293 the ILI case definitions. In persons <5 years of age (n=5009), the proportion of cases RSV-positive was highest among ARI (34%) than ILI (17%); in person ≥5 years of age (n=2910), the proportions were similar for ARI and ILI (7%). Among ARI, RSV-positivity was associated with lower oxygen saturation (-0.9; 95% CI: -0.4, -1.4) and lower odds of admission to intensive care unit (OR=0.7; 95% CI: 0.6, 0.8), mechanical ventilation use (OR=0.70; 95% CI: 0.54, 0.91), and death in hospital (OR=0.69; 95% CI: 0.48, 1.00). We found a lower OR for death associated with RSV in persons <5 years of age (p=0.017). RSV infection is more common among hospitalized ARI compared to ILI cases in young children and ARI patients present with lower oxygen saturation if they are RSV-positive, both which suggest RSV is associated with more severe disease. However, other findings suggest RSV-positive cases are less severe. Further analysis is required to understand whether RSV infection causes more severe disease than other pathogens.

SEVERITY OF ACUTE RESPIRATORY INFECTIONS ASSOCIATED WITH RESPIRATORY SYNCYTIAL VIRUS, GUATEMALA, 2008-2012

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Respiratory syncytial virus (RSV) is a major cause of acute respiratory infections. The epidemiology of RSV in all age groups has not been well described in Central America, particularly regarding disease severity. We aimed to address these knowledge gaps with surveillance data from Guatemala. We conducted active surveillance of ambulatory visits due to influenza-like illness (ILI: cough or sore throat and measured fever >38°C) and hospitalizations due to acute respiratory infections (ARI: sign of infection and a respiratory sign or symptom) in Santa Rosa (Nov 2007-Mar 2012), Quetzaltenango (Feb 2009-Mar 2012), and Guatemala City (Nov 2009-Apr 2011). Nasopharyngeal swab specimens were tested for RSV using real-time reverse-transcription polymerase chain reaction. Among ARI cases, we measured associations between RSV-positivity and indicators of severity using linear and logistic regression, adjusted for age, gender, surveillance site, and year. To test for effect modification by age, we added an interaction term for RSV-positivity and age ≤5 years to the models. We enrolled and tested for RSV 7919 patients; 5626 met the ARI and 2293 the ILI case definitions. In persons <5 years of age (n=5009), the proportion of cases RSV-positive was highest among ARI (34%) than ILI (17%); in person ≥5 years of age (n=2910), the proportions were similar for ARI and ILI (7%). Among ARI, RSV-positivity was associated with lower oxygen saturation (-0.9; 95% CI: -0.4, -1.4) and lower odds of admission to intensive care unit (OR=0.7; 95% CI: 0.6, 0.8), mechanical ventilation use (OR=0.70; 95% CI: 0.54, 0.91), and death in hospital (OR=0.69; 95% CI: 0.48, 1.00). We found a lower OR for death associated with RSV in persons <5 years of age (p=0.017). RSV infection is more common among hospitalized ARI compared to ILI cases in young children and ARI patients present with lower oxygen saturation if they are RSV-positive, both which suggest RSV is associated with more severe disease. However, other findings suggest RSV-positive cases are less severe. Further analysis is required to understand whether RSV infection causes more severe disease than other pathogens.

TACKLING A GLOBAL CHALLENGE ON DOMESTIC GROUND: GEOGRAPHIC AND DEMOGRAPHIC ANALYSES OF TB IN ORANGE COUNTY, CA

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Orange County, California carries one of the country’s highest burdens of tuberculosis (TB) at a rate of 6.4 cases per 100,000 population in 2009. In this generally affluent county marked by pockets of poverty, the sociodemographic makeup of the populace was discerned through the use of GIS technology. We carried out a retrospective cohort study of all TB cases diagnosed in Orange County, CA from 2005 to 2009 and of all TB hospitalizations from 2005 to 2008 and performed geographic and demographic analyses on the data. Based on global trends, we expected the burden to be highest in the poorest portions of the county as well as in those with high immigrant populations. We found the highest incidences of both TB cases and TB hospitalizations in the city

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of Santa Ana. As the poorest city in the county, Santa Ana (per capita income $16,891), had a case rate of 9.65 per 100,000 population. The highest case rate by city was found in Westminster (23.76 per 100,000 population). Foreign-born patients treated by the county represented 85.4% of all TB cases, placing the burden largely on the immigrant population of the county. There were clear relationships between relative risks & case rates and per capita income of the city (R = 0.423 and R = 0.434, respectively). Relative risk ratios indicated that males [1.49], Asians [8.55], and seniors (65 yrs+) [3.34] were at greatest risk for a TB infection. The relative risk for a TB infection in an Asian male aged 65 years or older was 21.6. At greatest risk for hospitalization for a TB infection: males [1.43], Asians [4.17], and seniors [4.52]. The relative risk of an Asian male aged 65 years or older in the county being hospitalized due to TB was 17.4. More than 71% of all TB hospitalizations were government-funded with total charges exceeding $29.4 million over 4 years. These data suggest that the burden of TB in Orange County warrants continued attention and additional resources and also demands a change in policy with regards to the domestic handling of global health issues.

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INDIVIDUAL LEVEL RISK FACTORS FOR SECONDARY TRANSMISSION OF INFLUENZA-LIKE ILLNESS: SECONDARY DATA ANALYSIS FROM THE BANGLADESH INTERRUPTION OF SECONDARY TRANSMISSION OF INFLUENZA STUDY (BISTIS)
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Respiratory infections are a leading cause of mortality worldwide. Understanding risk factors for secondary transmission to close contacts will facilitate development of interventions to prevent respiratory pathogen transmission. We describe index case-patient and susceptible contact risk factors for secondary transmission of influenza-like illness (ILI) in the control arm of a randomized controlled trial evaluating the impact of handwashing promotion on ILI in Bangladesh. We identified index case-patients with ILI (fever in persons < 5 years old, and fever with cough or sore throat in persons ≥5 years old). Susceptible contacts were persons without respiratory symptoms at enrollment living in household compounds of index case-patients. Compounds included index case-patient households and ≥1 secondary households. We recorded demographics and behaviors among all contacts, and frequency of interaction with index case-patient in a subset. We conducted daily ILI surveillance from the day after enrollment to 10 days after resolution of index case-patient symptoms. We used logistic regression to evaluate risk factors for secondary transmission to close contacts.

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A COMMUNITY RANDOMIZED CONTROLLED TRIAL OF AN INTEGRATED HOME-BASED INTERVENTION IMPROVING HOUSEHOLD-AIR POLLUTION, DRINKING WATER QUALITY AND HYGIENE IN RURAL PERU

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Diarrhoea and acute lower respiratory infections are leading causes of childhood mortality. Simple low-cost interventions have proven efficient in reducing diarrhoea and severe pneumonia; however, an integrated package provides opportunities for synergism. We conducted a community-randomised controlled trial in 51 rural communities in Peru to evaluate an environmental home-based intervention package (HIP) in reducing acute lower respiratory infections, diarrhoeal disease and preventing malnutrition in children under 36 months of age. All homes used open fires and 80% had access to piped, untreated water supplies. Ecot was found in drinking water in 66% of the households. The proportion of stunted children was 55%. In the intervention arm an improved stove (OPTIMA) was installed and members were trained in the correct use and proper maintenances; a solar disinfection of drinking water (SODIS) method was established; and a water faucet with a kitchen sink was installed and hardwashing practices were promoted. Diarrhoea, respiratory (weekly) and anthropometric (every two months) surveillance was done at home during a 12 months period. To reduce potential impact of non-blinding bias, the control arm received a psychomotor stimulation programme. We randomized 51 communities and enrolled 534 children. Baseline characteristics were balanced between study arms: The rate of diarrhoeal episodes in children in the intervention was 2.8 episodes per child per year as compared to 3.1 episodes in the control arm. The relative rate was 0.78 (95% CI: 0.58-1.05). Similarly, care takers in the intervention group reported fewer days of diarrhoea (mean 4.9 vs. 6.4 days per year; OR: 0.71, 95% CI: 0.47-1.06). No effect on acute lower respiratory infections or child’s growth rates was observed. In conclusion we found no evidence for synergistic effects associated with the intervention package. Introducing several interventions and messages simultaneously may have overwhelmed the households and compromised use, operation and maintenance of all components.

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ACTIVE HOUSEHOLD-BASED SURVEILLANCE AND REGIONAL VARIATION IN INCIDENCE OF INFLUENZA IN PERU

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Most estimates of the disease burden of influenza rely on passive sentinel surveillance at health clinics and hospitals. These estimates lack population denominators necessary for calculations of incidence, especially of milder disease, potentially leading to underestimates of the true burden. In 2009 we implemented active community-based household surveillance in 4 ecologically distinct regions of Peru: coastal desert (Lima), dry
forest (Tumbes), highlands (Cuzco) and rainforest (Puerto Maldonado). Approximately 7200 people in 1500 randomly selected households are visited 3 times a week. Nasopharyngeal swabs are taken from persons with influenza-like illness (ILI) and tested for influenza virus by RT-PCR. Here we report the incidence and seasonal patterns for the first 1.5 years (June 2009-December 2010) comprising 9344 person-years (py) of follow-up. The overall influenza incidence was 14/100py, with a 95% confidence interval (CI) of 13-15. Of these, 6.5/100py (CI 6-7) sought care, 1/100py (CI 0.5-2) required hospitalization, and one died. Although surveillance started after the peak of the pandemic, the observed incidence was still higher in 2009 (16/100py, CI 15-18) than 2010 (13/100py, CI 12-14). The highest incidence was observed in ages 6 months-12 years (31/100py, CI 29-33), followed by 13-17 years (17/100py, CI 15-20), <6 months (13/100py, CI 7-25), and >17 years (6/100py, CI 6-7). Tumbes consistently showed the highest incidence (19/100py, CI 17-20), followed by Lima (17/100 py, CI 15-18), Puerto Maldonado (12/100 py, CI 11-13), and Cuzco (9/100 py, CI 8-10). The proportion of ILI due to influenza was 18% in ages <5 years, 46% in ages 5-17 years, and 30% in persons >17 years. Peak incidence was June-December in Lima and Cuzco and September-January in Tumbes and Puerto Maldonado, with a second peak in Tumbes during June-July. We estimate that 3.8 million people in Peru had influenza in 2009-2010. Furthermore, incidence and temporal patterns vary significantly by region and will require region-focused prevention and control strategies.

HUMAN METAPNEUMOVIRUS (hMPV) is a suspected cause of acute respiratory tract infections, mostly in young children, the elderly and immunocompromised patients. We investigated the incidence of hMPV in Kenya using longitudinal, population-based surveillance in two sites; two villages (total population = 28,000) in Kibera urban slum located in Nairobi City together comprising one site and in thirty-three villages in a rural Asembo community (total population = 26,000) in western Kenya as the second site. Between 1st October 2006 and 24th March 2011, nasopharyngeal and oropharyngeal swabs collected from consenting patients meeting the case definition for either hospitalized severe acute respiratory infection or outpatient influenza like illness were tested for hMPV by real time Reverse Transcription polymerase chain reaction (RT-PCR). Incidence rates were calculated as the number of hMPV cases by person-years of observation (pyo) per site with adjustments for patients meeting the case definition at study clinics who were not swabbed and for participants who sought medical attention at non-study clinics. The HIV status was included in analysis for patients aged >18 years. Seventeen (n = 17) hMPV isolates were sequenced at the 347bp F-gene fragment for subtyping. Of 9000 cases tested from both sites, 614 (6.8%) were positive for hMPV, consisting of 345/4284 (8.1%) in Kibera and 269/4716 (5.7 %) in Asembo. In Kibera, the adjusted rates were highest in children < 12 months [99.6/1000 pyo (95% CI 80.9 - 122.6)] and lowest in those >50 years: [0.7/1000 pyo (95%CI 0.1 - 5.3)]; in Asembo, the adjusted rates were highest in children aged 12 - 23 months [62.7/1000 pyo (95%CI 62.6 - 62.8)] and lowest in those aged in 18 - 34 years [14.6/1000 pyo (95%CI 14.5 - 14.7)]. In Kibera, 33% (14/43) of the hMPV-positive patients ≥ 18 years were also positive for HIV whereas in Asembo, 40% (12/30) of hMPV positive cases were positive for HIV. The clinical symptoms associated with hMPV included fever, cough, and runny nose. On genetic analysis, 5 of 17 (29 %) Kenya viruses belonged to subgroup A, and 12 (71%) viruses to subgroup B. No A1 subgroup viruses were detected. Thus hMPV incidence was higher in children aged ≤5 years in both study sites and incidence rates decreased with increasing age.
RANDOMIZED, DOUBLE-BLINDED, PHASE 2 TRIAL OF WR 279,396 (PAROMOMYCIN AND GENTAMICIN) FOR THE TREATMENT OF CUTANEOUS LEISHMANIASIS CAUSED BY LEISHMANIA PANAMENSIS

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This randomized double-blinded Phase 2 trial included 30 patients (17 adults and 13 children ages 5 to 17 years) with Leishmania panamensis cutaneous leishmaniasis (CL). Patients were randomly allocated (1:1) to receive once daily topical treatment with WR 279,396 (15% paromomycin + 0.5% gentamicin) or Paromomycin Alone (15% paromomycin) 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 by Day 63 and 100% reepithelialization by Day 100 with no relapse. The index lesion cure rate after 6 months follow-up was 13/15 (87%) for WR 279,396 and 9/15 (60%) for Paromomycin Alone (p = 0.099). When all treated lesions were evaluated for cure, the final cure rate for WR 279,396-treated patients was the same, but the final cure rate for Paromomycin Alone-treated patients was lower at 8/15 (53.3%; p = 0.046). Both creams were well tolerated with mild application site reactions including erythema (20%), edema (13.3%), and pain (6.7%) being the most frequent adverse event in the WR 279,396 group, which were slightly higher in the Paromomycin Alone group. PK data showed that there is limited paromomycin and gentamicin systemic absorption thus avoiding drug accumulation and toxicity. The increased final cure rate in the WR 279,396 group in this small Phase 2 study suggests that the combination product may provide greater clinical benefit than paromomycin monotherapy against L. panamensis CL. The excellent tolerability and low systemic drug exposure suggests that WR 279,396 may offer an alternative to more toxic systemic therapies for CL.

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 the effectiveness of IPTp in an area of western Kenya where Plasmodium falciparum resistance to SP is high. From August 2008 to June 2009, women presenting to deliver at two district hospitals were enrolled in a cross-sectional survey. We collected information on obstetric history, use of IPTp, insecticide treated nets, 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) by number of IPTp doses received by self-report or as recorded in the antenatal card. Overall 977 HIV-negative women were enrolled. Of these 637 (65%) were gravidae 1 or 2, anemia prevalence in women who received no IPTp was 14%, 1 dose: 11%, 2 doses: 7% and 3+ doses: 2% (p<0.01). Peripheral parasitemia was 19% for no IPTp, 12% for 1 and 2 doses and 7% for 3+ doses (p=0.07). Placental parasitemia was 22% for no IPTp, 12% for 1 dose, 13% for 2 doses and 8% for 3+ doses (p=0.04). Among gravidae 1/2, we found no reduction in LBW by IPTp doses administered (p=0.73). Among multigravidae, significant trends by number of SP doses received were not observed for anemia, or peripheral or placental parasitemia but were associated with reduced LBW (p=0.02). Among gravidae 1 or 2, but not multigravidae, having received more doses of IPTp was significantly associated with lower prevalence of maternal anemia, peripheral parasitemia and placental parasitemia. In multigravidae, IPTp resulted in reduced prevalence of LBW. During this time period, IPTp remained beneficial in this area of western Kenya, despite high SP resistance.
INVASIVE STAPHYLOCOCCUS AUREUS INFECTIONS IN CHILDREN IN THE TROPICAL TOP END OF AUSTRALIA: CLINICAL AND MICROBIOLOGICAL EPIDEMIOLOGY

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Previous studies report very high incidence of *Staphylococcus aureus* bacteraemia in adult Aboriginal populations in tropical northern Australia. There are few studies describing incidence or outcomes in children. We aimed to describe the clinical and microbiological epidemiology of invasive *S. aureus* infections in children. We conducted a retrospective review for all cases of bacteraemia and sterile site infection, for children under 15 years, in the Top End of Australia over a four year period (2007–2010). Forty-four cases (9 neonatal, 35 paediatric) were identified. The annual incidence of invasive *S. aureus* was 27.9 cases per 100,000 population and was significantly higher in the Aboriginal population (incidence rate ratio [IRR] compared to non-Aboriginal population: 5.3 [95%CI 2.5-12.6]). Among non-neonatal cases, the annual incidence was 22.2 per 100,000 population (Aboriginal 46.6, non-Aboriginal 4.4, IRR: 10.6 [95%CI 3.8-41.4]). There were significant differences between the neonatal and paediatric groups. Neonatal cases were all born prematurely, typically with significant comorbidities and episodes were often intravascular catheter related and nosocomially acquired. There was one death. Of the 35 paediatric (non-neonatal) cases, 17% had pre-existing comorbidities, 14% were malnourished and 11% were nosocomial. Major foci of infection were bone and joint (57%) and pleuropulmonary (17%); endocarditis was uncommon (6%). The median length of stay was 23 days (mean 27, SD 16.6, range 2-68). 14% were readmitted within 1 year for related reasons. There were no deaths. There were 9 cases (26%) due to community-associated MRSA strains. Molecular genotyping results will be presented. There was no difference in severity or outcome between infections due to MRSA and MSSA. In conclusion, the annual incidence of invasive *S. aureus* infection in this study is the highest described in any paediatric population. Almost 1 in 2000 Aboriginal children develop invasive disease each year. Late onset sepsis in premature infants is the main neonatal cause. Most paediatric cases were community acquired and severe, yet rates of mortality, endocarditis and readmission were low.

TRYPANOSOMA CRUZI SURVIVAL FOLLOWING STORAGE: IMPLICATIONS FOR TISSUE TRANSPLANTATION-DERIVED TRANSMISSION

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*Trypanosoma cruzi*, the causative agent of human Chagas disease, is typically transmitted via the feces of a reduviid bug vector following a blood meal. Infection can also occur through infected blood and tissue products. An estimated 300,000 individuals in the United States are infected with *T. cruzi* and six cases of transmission through organ donation have been reported. The potential for transmission by tissue transplantation is considered herein. Tissue recovered for transplantation, in contrast to solid organ transplants, undergoes a range of storage and processing procedures depending on the tissue type. At the outset, tissues do not have to be recovered from a beating-heart or live donor, but instead may be removed from the donor up to 24 hours after asystole. Once removed, tissues are often stored and eventually undergo some degree of processing, which can include lyophilization, irradiation, and various sterilization techniques. As an example, heart valves are cryopreserved and undergo minimal processing, whereas other tissue may be stored as is at -80 C for as long as five years. The ability of infected tissue to transmit *T. cruzi* is likely related to the ability of *T. cruzi* to survive these processing and storage conditions. We examined the viability of *T. cruzi* parasites after room temperature and cold storage conditions. *T. cruzi* trypomastigotes were unaffected by 24 hours at room temperature, both in cell cultures and when spiked into whole blood to mimic decomposition following asystole. Parasite-infected cells stored up to 5 days at 4 C proved 100% viable after re-culture, whereas only 2 of 8 cultures stored 28 days at 4 C became culture-positive. A significant decrease in parasite viability was observed in samples stored up to 120 days at -80 C in the absence of cryopreservation, yet some live, infective parasites were recovered. These data demonstrate the heartiness of *T. cruzi* following cold storage. Studies are underway to examine the effects of more rigorous tissue processing procedures on *T. cruzi* viability.

A SYSTEMATIC REVIEW AND META-ANALYSIS OF MALARIA AND SEXUALLY TRANSMITTED AND REPRODUCTIVE TRACT INFECTIONS IN PREGNANCY IN SUB-SAHARAN AFRICA: OPPORTUNITIES FOR ANTENATAL INTERVENTION

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Malaria and sexually transmitted infections/reproductive tract infections (STIs/RTIs) in pregnancy are direct and indirect causes of stillbirth, prematurity, low birth weight, and maternal and neonatal morbidity and mortality. Novel use of diagnostic tools and/or drugs may improve birth outcomes with the impact depending on the prevalence of malaria and STI/RTI. PubMed, MEDLINE, EMBASE, the World Health Organization International Clinical Trials Registry, and reference lists were searched for studies reporting malaria, *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Trichomonas vaginalis*, or bacterial vaginosis among pregnant women attending antenatal care facilities in sub-Saharan Africa. Included studies were conducted in 1990-2011 with open enrollment. Studies from South Africa, where malaria is no longer endemic, were excluded. Point prevalence estimates were corrected for diagnostic precision. A random-effects model meta-analysis was then applied to produce pooled prevalence estimates. A total of 171 studies met inclusion criteria, providing 307 point prevalence estimates for malaria or STIs/RTIs. The pooled prevalence estimates (95% confidence intervals; n=positive diagnoses) among studies in 1990-2011 in East and Southern Africa were as follows: *syphilis* 4.5% (3.9,5.1; n=8,346), *N. gonorrhoeae* 3.7% (2.8, 4.6; n=626), *C. trachomatis* 6.9% (5.1, 8.6; n=350), *T. vaginalis* 29.1% (20.9, 37.2; n=5,502), bacterial vaginosis 50.8% (43.3, 58.4; n=4,280), peripheral malaria 32.0% (25.9, 38.0; n=11,688) and placental malaria 25.8% (19.7, 31.9; n=1,388). West and Central Africa prevalence estimates were as follows: *syphilis* 3.5% (1.8, 5.2; n=851), *N. gonorrhoeae* 2.7% (1.7, 3.7; n=73), *C. trachomatis* 6.1% (4.0, 8.3; n=357), *T. vaginalis* 17.8% (12.4, 23.1; n=822), bacterial vaginosis 37.6% (18.0, 57.2; n=1,208), peripheral malaria 36.2% (32.3, 44.1; n=12,242) and placental malaria 39.9% (34.2, 45.7; n=4,658). In conclusion, the dual prevalence of malaria and STIs/RTIs in pregnancy among women attending antenatal care facilities in sub-Saharan Africa is considerable, with the combined prevalence of curable STIs/RTIs being equal to, if not greater than, malaria.

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ANSWERING THE MAIL: USING A CASE-BASED MODEL TO TEACH TELECONSULTATION SKILLS TO INFECTIOUS DISEASE FELLOWS

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The United States Military has offered a vast array of teleconsultative services to assist health care providers (HCP) deployed in remote areas of the world via e-mail. The Infectious Diseases Teleconsult system (IDTS) has been an effective tool in optimizing care for Service Members overseas. Despite the continuing development of telemedicine in both civilian and military communities, we are unaware of any standardized education given to HCP prior to participating in this method of consultation. Giving medical recommendations via a teleconsult system requires unique skills not used in the standard clinical setting. Consultants provide advice without questioning or examining the patient and must understand system constraints unique to the patient’s location (i.e. isolated mountain top in Afghanistan or remote village in West Africa). Cases referred to the IDTS often involve diseases unique to tropical climates and the developing world that inexperienced providers may not recognize. To address this gap in medical education, the Infectious Diseases (ID) fellowship program at Walter Reed National Military Medical Center implemented a training program in 2011 that utilized a series of simulated patients based upon real teleconsults from the IDTS system. Ten cases were chosen to highlight classic infectious diseases that have been common consults such as cutaneous Leishmaniosis, Q fever and malaria. The simulated cases are administered via e-mail to an ID fellow who then has one hour to appropriately answer the teleconsult. The fellow is then given feedback on his/her ability to generate an accurate assessment and plan for the simulated case and the practicality of their advice considering the patient care setting. An overview of the case-based model, selected examples, and evaluation criteria will be reviewed. This educational process is an effective way to prepare ID fellows for the real-world experience of offering advice and medical recommendations via a teleconsult system and has improved their knowledge base of infectious diseases in the developing world.

MATERNAL HIV EXPOSURE OR MOTHER TO CHILD TRANSMISSION OF HIV AND RISK OF TUBERCULOSIS IN INFANTS

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Infants born to HIV infected mothers who test HIV negative (seroexposed) and those that become HIV infected (seroreactive) are at an increased risk of progression from latent tuberculosis (TB) infection to disease. However, TB diagnosis in this group is challenging due to low utility of scoring systems and low tuberculin skin test (TST) sensitivity. We sought to document the incidence rates of TB disease among seroexposed and seroreactive babies, among an infant cohort under follow up to determine TB incidence. Infants aged 0-6 weeks were recruited from Western Kenya. HIV DNA PCR testing was offered at six weeks to all infants. Through follow up and sick visits, TB suspects were identified based on signs and symptoms and investigated for TB through gastric aspirates, sputum induction, chest radiographs, HIV tests and TST. Definite cases were only culture confirmed and probable cases had Keith Edward(KS) Scores of ≥7 and/or suggestive chest radiographs. Of 2900 infants enrolled, 435 (15%) were seroexposed, including 75 (2.6%) seroreactive. Of 923 (32%) TB suspects, 174 (19%) out of 923 TB suspects were seroexposed, including 43 (25%) seroreactive. The mean TST induration among seroexposed and non exposed suspects was 5mm (SD=6) and 4mm (SD=5) p<0.01 respectively. Additionally, mean KE score was 2.3 (SD2.4) and 1.6 (SD2.0) p<0.01 for seroexposed and non-exposed respectively. There were 48 TB cases, (incidence rate 1.44 per 100 person years) [95% CI (1.09, 1.91)], sixteen of these (33%) were among seroexposed infants RR 2.1 [95% CI(1.1,4.0)]. Out of ten definite TB cases, five (50%) were seroexposed (p=0.05). HIV exposure and infection was associated with increased risk of being diagnosed with tuberculosis. HIV infected mothers have increased risk of developing TB and exposing their babies to TB infection. Rigorous antenatal screening for tuberculosis in HIV infected mothers is recommended to protect their infants from early latent infection and progression to TB disease.

INCREASED RATES OF RESPIRATORY AND DIARRHEAL ILLNESSES AMONG HIV-NEGATIVE INDIVIDUALS ≥5 LIVING WITH HIV-INFECTED INDIVIDUALS IN AN URBAN SLUM (KIBERA) OF NAIROBI, KENYA

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For some acute illnesses in HIV-infected patients, prolonged pathogen shedding, symptom duration, and other socioeconomic factors potentially contribute to increased incidence. Using data from a prospective, population-based infectious disease surveillance system of acute illnesses and a cross-sectional HIV serosurvey conducted in the urban slum of Kibera, Kenya, we calculated incidence of influenza-like illness (ILI), diarrhea, and non-specific febrile illness among 2,105 HIV-negative household contacts of HIV-infected participants and 13,747 participants living within exclusively HIV-negative households during 2008. Of the 4,285 household in which a test was performed, 83.8% had only HIV-negative tests, 13.5% had 1 HIV-positive test, and 2.7% had more than 1 HIV-positive test; untested adults were not included in the analysis. We stratified household contacts by number of HIV-infected individuals in households and modeled it as a continuous variable to determine a dose-dependent relationship. For children and adults ≥5 years old, incidence was significantly increased for ILI (incident rate ratio [IRR], 1.47; 95% confidence interval [CI], 1.07:1.99; p<0.05), and diarrhea (IRR, 1.41; CI, 1.11:1.77; p<0.05) in HIV-negative household contacts of HIV-infected residents and 13,747 participants living within exclusively HIV-negative households during 2008. Of the 4,285 household in which a test was performed, 83.8% had only HIV-negative tests, 13.5% had 1 HIV-positive test, and 2.7% had more than 1 HIV-positive test; untested adults were not included in the analysis. We stratified household contacts by number of HIV-infected individuals in households and modeled it as a continuous variable to determine a dose-dependent relationship. For children and adults ≥5 years old, incidence was significantly increased for ILI (incident rate ratio [IRR], 1.47; 95% confidence interval [CI], 1.07:1.99; p<0.05), and diarrhea (IRR, 1.41; CI, 1.11:1.77; p<0.05) in HIV-negative household contacts of HIV-infected residents when compared with rates in HIV-negative persons living in households without an HIV-infected resident. The risk for illness among HIV-negative persons was directly proportional to the number of HIV-infected people living in the home for ILI (IRR, 1.40; CI, 1.03:1.88; p<0.05), diarrhea (IRR, 1.36; CI, 1.11:1.67; p<0.01), and non-specific febrile illness (IRR, 1.15; CI, 1.02:1.29; p<0.05). There was no evidence for a similar pattern of increased incidence in a parallel analysis among children <5 years old. Since treatment and support for HIV-infected individuals reduces their incidence of infections, future interventions ensuring that HIV-infected persons are receiving appropriate care may help to reduce infections for all household members.
RUSH TO JUDGMENT: SOURCES OF CONFOUNDING IN STI-HIV PREVENTION TRIALS

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Substantial evidence indicates that sexually transmitted infections (STIs) promote HIV transmission and acquisition by producing genital ulcers, inflammation, and viral shedding. Ten randomized controlled trials in sub-Saharan Africa (SSA) examined effects of STI control on HIV incidence. One produced statistically significant results. Consequently, support for STI treatment for HIV prevention has faded. We conducted an intensive review of methods and outcomes of the 10 STI-control trials in SSA and subsequent analyses. All 10 trials reveal potentially serious confounding from multiple untreated genital morbidities. Some trials studied the impact of treating bacterial STIs on HIV incidence; others studied treatment of viral STIs. None studied both. None examined treatment of genital morbidities from other causes that could enhance HIV transmission or acquisition. The trials excluded consideration of fungal STIs. None considered genital ulceration and inflammation from non-sexually transmitted pathogens, such as Schistosomiasis hematobium (highly prevalent in SSA), and from ulcers caused by abrasions infected with streptococci or staphylococci, also common. Treating one type of genital morbidities may have little effect on HIV incidence when there is untreated genital morbidity from multiple sources. Furthermore, 8 trials reported the same or lower levels of risky sexual behavior in the control arm as in the treatment arm or reported the same or larger reductions in risky behaviors among controls. (Two trials did not report on sexual behavior.) That could have resulted from the trials’ successful interventions in the control arm or from spontaneous reductions in risky behavior prompted by the trial. Confounding by genital morbidity of multiple etiologies, behavioral change, and other factors in the 9 trials lacking statistically significant results renders those trials unable to inform HIV-prevention policy. Given abundant evidence that STIs promote HIV spread, STI treatment should be considered an important method for reducing HIV incidence in SSA and elsewhere.

HIV STIGMA AS A BARRIER TO RECEIVING HIV CARE AT A GENERAL HOSPITAL IN LIMA, PERU: A CASE-CONTROL STUDY

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Poor retention in care may increase the risk of morbidity, mortality, and community HIV transmission. The role of HIV stigma in poor retention has not been well studied. The objective of this case-control study was to evaluate the association between HIV stigma and retention in care among HIV patients in Lima, Peru. We evaluated HIV-positive patients who were diagnosed and/or initiated care at a general hospital between 2005-2010, with inclusion based on status of care by March 31, 2011. Those retained in care (n=150) had ≥2 documented medical care visits per year and were approached and interviewed privately in clinic during their appointment. Those not retained in care (n=55) had no documented visits for ≥1 year and home visits were used to locate them and conduct interviews. The Berger HIV stigma scale was used to quantify the 4 domains of stigma: enacted stigma (ES), disclosure concerns (DC), negative self-image (NSI), and concern with public attitudes (CPA). Each domain had 5 items with higher scores indicating higher stigma (score range 0-15). Multivariable logistic regression was used to calculate adjusted odds ratios (OR) and 95% confidence intervals (CI) for being out of care. Stigma was modeled as a continuous variable and linearity assumptions were assessed. Mean stigma scores were low for ES (6.1) and NSI (5.3) but high for DC (9.6) and CPA (9.0). ES and NSI had U-shaped associations with retention (odds of not being retained increased then decreased at higher stigma levels). DC and CPA showed linear associations. Patients who agreed to all items (score of 10) were more likely to not be retained than patients who disagreed to all items (score of 5) for ES (OR=2.36; 95% CI: 0.98, 5.67), DC (OR=2.72; 95% CI: 1.11, 6.67), NSI (OR=1.82; 95% CI: 0.50, 6.60), and CPA (OR=3.30; 95% CI: 1.37, 7.92). This study suggests that all aspects of HIV stigma, particularly concern with public attitudes, play a role in being out of care.

DO PEOPLE LIVING WITH HUMAN IMMUNODEFICIENCY VIRUS (PLHIVS) HAVE MORE RISKY SEXUAL BEHAVIORS THAN UNINFECTED PEOPLE IN BURKINA FASO? A CASE-CONTROL STUDY

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Antiretroviral therapy has reduced mortality and morbidity due to HIV/AIDS. Consequently, it is widely believed that PLHIV could keep new infections occurrence by their more risky sexual behaviors. This study aimed to compare PLHIVs’ sexual behaviors with those who are infected, and to determine factors that affect their condom use. We conducted a case-control study involving all the PLHIVs and not infected people of the second round in Burkina Faso’s Treatment Accelerating Program assessment, from July to December 2009. This assessment involved PLHIVs (with their households) and households without PLHIV. Chi-square test was used to compare proportions with p<0.05 as statistically significant. Logistic regression was employed to determine associated factors of condom use. Two hundred ninety one PLHIVs and as many controls were interviewed in the 590 households. Seventy four percent of PLHIVs versus 58.1% of controls were women (p=0.001). Among men, 78.7% of PLHIV and 84.6% of controls had sex in the last twelve months (p=0.05). Among women, we had 48.1% versus 56.4% of PLHIV (p=0.05). three percent of PLHIV versus 13% of controls were not aware of their last sexual partner’s HIV status (p<0.05). Among PLHIV, 27.1% versus 67% of the controls had not used condom during their last sexual intercourse (p<0.001). Both men’s and women’s group had presented the same difference (p=0.001). ART status was not associated with condom use (OR=1.2, 95%CI=0.6-2.5). But, voluntary testing (OR=3.4, 95%CI=1.1-10.7) and knowing that the partner has had sex with another person during the last twelve months (OR =5.2, 95%CI=1.6-16.7) were associated with PLHIV’s condoms use. PLHIVs use condom more than uninfected and in general, they don’t have more risky sexual behaviors. Besides, condom use is not significantly affected by ART, so their availability doesn’t increase risky behaviors and therefore must be improved. This argument could be considered to strengthen public awareness campaigns against stigma and discrimination against PLHIVs in Burkina Faso.
We and others have previously shown that Plasmodium falciparum-derived hemozoin (PHz) promotes dysregulation of CCR5 and CXCR4, and their cognate ligands in monocytes and CD4+ T cells, resulting in increased HIV-1 replication. To further explore these molecular interactions, we examined the relative expression of CCR5 and CXCR4 on CD14+ monocytes and CD4+ T cells through flow cytometric analyses on cells collected from children (age, 2.6-33.6 mos; n=67) from western Kenya categorized into the following groups: 1) P. falciparum negative and HIV-1 negative (mal(-)/HIV-1(-), n=11), 2) P. falciparum positive and HIV-1 negative (mal(+)/HIV-1(-), n=33), 3) P. falciparum positive and HIV-1 exposed (mal(+)/HIV-1exp, n=20); and 4) P. falciparum positive and HIV-1 positive (mal(+)/HIV-1(+), n=3). Proportions of CD14+CXCR4+ cells were elevated in mal(+)/HIV-1(+)) children compared to the mal(-)/HIV-1(-) group (P=0.048). However, proportions of CD14+CXCR4+ cells were reduced in the mal(-)/HIV-1(-) children relative to mal(+)/HIV-1(-) (P=0.039) and mal(+)/HIV-1(exp) (P=0.077) groups. In addition, expression of CD3+CXCR4+ and CD3+CD4+CCR5+ cell subsets were lower in the mal(+)/HIV-1(+) group compared to mal(+)/HIV-1(exp) children (P=0.05 for both). Further analyses in the combined population of malaria-infected children revealed that CD3+CXCR4+ cells were inversely correlated with the percentage of monocytes containing PHz (ρ=-0.883, P=0.020). Taken together, the preliminary results presented here suggest that CXCR4 and CCR5 are dysregulated in children co-infected with malaria and HIV-1, and that altered expression may be driven, at least in part, through acquisition of PHz by monocytes.

**RELATIVE EXPRESSION OF CCR5 AND CXCR4 BY CD14+ MONOCYTES AND CD4+ T CELLS IN HIV-1-EXPOSED AND -INFECTED CHILDREN WITH PLASMODIUM FALCIPARUM MALARIA**

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**AN EVALUATION OF TB AND HIV PROGRAM INTEGRATION AT PRESIDENT’S EMERGENCY PLAN FOR AIDS RELIEF (PEPFAR)-SUPPORTED MILITARY HOSPITALS IN NIGERIA**

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Tuberculosis (TB) presents an important health problem and comorbidity among patients seen at sites supported by the President’s Emergency Plan for AIDS Relief (PEPFAR) in Nigeria. The objective of this evaluation was to use the TB cohort review process indicators to assess TB and HIV program integration at PEPFAR sites within the Nigerian Ministry of Defense (NMOD). The cohort review methods used were modified from those described previously by the Centers for Disease Control and Prevention. The sites were all affiliated with the NMOD and included clinics in Abuja, Lagos (2 sites), Makurdi, and Kaduna. At each site, the study team reviewed each TB case in detail with the assistance of the TB case manager, verifying key clinical outcomes. TB case managers were interviewed for qualitative information about the processes involved in TB care at each facility. 175 cases were reviewed at the 5 PEPFAR-supported sites. The sites evaluated were found to have substantial variability in outcomes. Treatment completion seen in this study (72%) was lower than that reported nationally (83%) and was lower than the WHO 2015 goal of (85%). Of particular concern, HIV patients were less likely to complete therapy (58%) than HIV negative patients (85%). The lack of contact investigation and TB case finding supports the low case detection rate reported nationally (20%). Similarly, the lack of LTBI treatment is consistent with the low proportion of treatment among eligible HIV patients (<5%) seen nationally. This is the first report of the cohort review methodology being applied to assess TB program integration within a PEPFAR population. Assessments of TB program integration through the cohort review can benefit the PEPFAR program by increasing staff awareness and accountability for patient outcomes, improving case management and investigation of contacts, improving monitoring and evaluation practices, and revealing program strengths and weaknesses. This evaluation has provided data to fill some of the gaps related to integration of TB control in a PEPFAR population.

**THE JOINT EFFECTS OF EFFICACY AND COMPLIANCE: A STUDY OF HOUSEHOLD WATER TREATMENT EFFECTIVENESS**

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The effectiveness of interventions to control infectious disease is related to the intrinsic efficacy of the interventions in removing pathogens, and how people comply with the interventions. However, little is known about the quantitative relationship between compliance and effectiveness, which is particularly important for household water treatment (HWT). Although many HWT methods are highly efficacious at inactivating pathogens, their effectiveness within actual communities is decreased by imperfect compliance. To assess the effectiveness of HWT on childhood diarrhea incidence via drinking water for three pathogen types (bacterial, viral, and protozoan), we developed a quantitative microbial risk assessment (QMRA) model. We examined the relationship between log10 removal values (LRVs) and compliance with HWT for scenarios varying by: baseline incidence of diarrhea; etiologic fraction of diarrhea by pathogen type; pattern of compliance; and size of randomly scheduled contamination spikes in source water. The benefits of increasing LRVs are strongly linked to compliance. For perfect compliance, diarrheal incidence decreases as LRVs increase. However, when compliance is incomplete in the scenarios we considered, there are diminishing returns from increasing LRVs at the community level. Higher LRVs are more beneficial if: contamination spikes are large; contamination levels are high in general; or the pattern of compliance includes some people who comply perfectly. The effectiveness of an HWT intervention at the community level may be limited by low compliance, such that the benefits of high LRVs are not realized. Therefore, patterns of compliance with HWT should be measured during HWT field studies and HWT dissemination programs. Studies of pathogen concentrations in a variety of developing country source waters should also be conducted. Guidelines are needed for measuring and promoting compliance with HWT, in addition to the recently published WHO HWT efficacy recommendations.
SPATIOTEMPORAL DYNAMICS OF CHOLERA EPIDEMICS IN THE FAR NORTH REGION, CAMEROON

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In the year 2010, the Far North Region of Cameroon experienced its most severe cholera outbreaks in the last four decades with about 9,400 reported cases and 600 deaths. This study describes the spatiotemporal dynamics of cholera epidemics from 1996 to 2010 using epidemiological data from this region, including reported cholera cases from 28 health districts, and environmental parameters such as temperature, relative humidity, rainfall and access to clean water. The data were entered into a geographical information system for further analysis. Regression analysis methods were used to analyze the data. The spatiotemporal patterns of the incidence rate were analyzed and associated with environmental factors to explore the determinant factors of the dynamics of cholera epidemics. The results revealed that there were three major epidemic periods and specific hotspots during the last 15 years. The annual dynamics showed a seasonal pattern coinciding with the wet seasons and significant differences in both incidence and timing by health districts. The spatial pattern revealed higher incidence rate in health districts in proximity with water bodies and in periurban areas. The study also revealed a connection with outbreaks in the neighboring countries of Chad and Nigeria. This study presents information related to the epidemiology and spatiotemporal pattern of cholera epidemics that can be used to help public health services plan prevention and control strategies against the spread of this disease.

THE RELATIONSHIP BETWEEN DISTANCE TO HOUSEHOLD WATER SOURCE AND MODERATE-TO-SEVERE DIARRHEA IN YOUNG CHILDREN IN THE GLOBAL ENTERICS MULTI-CENTER STUDY (GEMS), KENYA, 2009-2011

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Water sources for rural households in the developing world are often located away from the home. Fetching water can be a substantial burden that can negatively affect household water quantity and quality, thereby increasing diarrheal disease risk. We visited households of 127 randomly selected pairs of case and age-, gender-, and village-matched controls enrolled in the GEMS study of moderate-to-severe diarrhea (MSD) in Kenya. We asked households to guide field teams from their home to their water source, and this path was captured as spatial data using GPS units. If no guide was available, we used GPS coordinates of the home and the water source to estimate distances. We compared GPS-sourced data to self-reported data in GEMS about round trip times spent to collect water, and evaluated each type of measurement as a predictor of MSD using conditional logistic regression. The paths recorded were a median of 1.18 (range 1.00 - 2.49) times the length of the straight line distances between their start and endpoints. Self-reported collection times were significantly correlated with the log-transformed distance measurements via GPS (Spearman correlation coefficient =0.80, p <0.01). The median recorded distance to water source was 196m (range: 1 - 1775m); 89 (35%) households were within 50m, all of which also reported the source to be in the household area. Collection times of 30-59 minutes were reported by households of 24 cases (median distance 561m, range 100 - 1775m) and 8 controls (median 562m, range 197 - 981m), and the odds of MSD were significantly higher vs. those with no travel (p <0.01). Collection times longer than 1 hour were reported for 12 cases (median distance 744m, range 148 - 1466m) and 3 controls (median distance 530m, range 460 - 1048m) and were significantly associated with MSD (p=0.02). These data suggest that distances traveled by households in rural Kenya to fetch water varied widely, that self-reported water collection times are correlated with measured distances, and that these may be useful in multivariate analyses of risk factors for MSD.

FOOD PREPARATION PROCESSES AND HYGIENE PRACTICES IN RURAL BANGLADESH: OPPORTUNITIES TO IMPROVE HANDWASHING INTERVENTIONS

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In rural Bangladesh handwashing with soap during food preparation is associated with reduced child diarrhea, but we have limited data characterizing food preparation and related handwashing to inform behavior change interventions. This study explored the steps of food preparation, related handwashing opportunities, community perceptions and current practices regarding handwashing during food preparation. In 3 rural Bangladeshi villages, we conducted 12 in-depth interviews, 12 unstructured observations and 12 video observations with caregivers, and 3 focus group discussions with household heads on handwashing related to food preparation. Eating, preparing and serving food with bare hands is associated with reduced diarrhea risk similar to that found for bottled water. 

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hands was common in the study communities. Varieties of mashed foods, salads and mixed dried foods that involved direct hand contact, which are not further cooked, were popular in rural Bangladesh. Mashed foods are prepared by boiling vegetables or dried fish, then peeling, mashing and mixing with raw ingredients using bare hands. Salad preparation involved cutting raw vegetables and mixing them by hand. For mixed dried foods puffed rice and dried snacks are hand-mixed with raw ingredients. Participants perceived that handwashing with soap was only necessary if hands were covered with visible dirt. Most respondents reported that they wash their hands with water during food preparation, but we observed that out of 54 opportunities to wash hands, participants washed hands with soap 2 times, with water alone 9 times, rinsed hands or hands came into contact with water 26 times, and did not wash hands 17 times. Food preparation was often interrupted by other tasks that could contaminate the preparer’s hands, after which they continued food preparation without washing hands. Participants cited that absence of soap in appropriate place is a potential barrier to wash hands with soap. Caregivers do not usually wash their hands with soap during food preparation in rural Bangladesh. Food preparation is a complex, multi-step, often interrupted process where villagers do not recognize moments of high risk of environmental contamination as a time to wash hands with soap. Identifying the highest risk food preparation steps and prioritizing those when handwashing with soap is important will help focus handwashing interventions. Bringing soap and water together in the food preparation area may make it easier to wash hands with soap during such high risk moments.

964 INACTIVATION OF HELMINTH IN A SOLAR CONCENTRATOR

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More than 1 billion people worldwide are infected with helminths. Typical pit latrines and composting conditions do not inactivate helminths in fecal matter effectively. By concentrating solar energy and reaching pathogen inactivation temperatures (50°C and higher), a solar concentrator, with projected capital costs of $0.30 per person per year, has the potential to inactivate helminths in fecal matter. The goal of this work was to evaluate the efficacy of a solar concentrator in inactivating helminth in fecal matter and meet World Health Organization (WHO) guidelines for safe disposal and reuse of fecal matter. Inactivation was assessed by evaluating the viability of *Toxocara canis* eggs. *T. canis* is a helminth in the same taxonomic order as *Ascaris lumbricoides*, which is a WHO indicator for safe fecal disposal and reuse of fecal matter. Inactivation was assessed by evaluating the viability of *Toxocara canis* eggs. *T. canis* eggs were isolated from canine fecal matter, concentrated, placed in semi-permeable tea bags (1,500 eggs each) and inoculated into 40 liters of fresh canine fecal matter. *T. canis* eggs were inoculated into two control conditions: indoors in the dark and in a mimicking pit latrine. At the end of each trial, eggs were incubated and classified as viable if they contained a motile larva. In all 3 trials, temperatures reached 60°C at the center and 70°C at the edges of the solar concentrator for at least 4 hours daily. During all three trials, after one day in the solar concentrator, the die-off of eggs was greater than 99%. In the third trial, the inactivation rate was 0.67 Log10 eggs/hr and there was 99% inactivation after 4 hours. These results suggest that a solar concentrating unit can be used to rapidly inactivate helminths in fecal matter, and therefore, fecal matter treated by a solar concentrator can be safely disposed and reused on edible crops.
analysis of the wild-type 1575N allele showed rapid decay of linkage disequilibrium (LD), in sharp contrast to the extended LD exhibited by 1575Y. A haplotype with long-range LD and high/increasing frequency is a classical sign of strong positive selection acting on a recent mutant. 1575Y occurs ubiquitously on a 1014F haplotype background, suggesting that the N1575Y mutation compensates for deleterious fitness effects of 1014F and/or confers additional resistance to insecticides. Haplotype tests of association suggest the latter: The 1014F-1575Y haplotype confers a significant additive benefit above 1014F-1575N with survival to death (M form P = 0.03) and permethrin (S form P = 0.003). DNA-based diagnostics are supplementing phenotypic bioassays as a proactive means of detecting resistance alleles at low frequency. The discovery of N1575Y at an early stage highlights the importance of continual monitoring for novel resistance mutations and its spread should be monitored closely.

THE EVOLUTION OF RESISTANCE TO CARBABATES AND ORGANOPHOSPHATE INSECTICIDES IN ANOPHELES GAMBIAE

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Determining the number of origins of insecticide resistance-associated mutations is important not only from an evolutionary perspective but also for modelling its spread, which is increasingly important for the implementation and monitoring of malaria vector control programmes. In the mosquito Anopheles gambiae s.s., the main African vector of malaria, organophosphate (OP) and carbamate resistance is strongly associated with a single amino-acid substitution (G119S) in the insecticide target site, acetylcholinesterase 1 (Ace-1). This mutation apparently incurs a high fitness cost in the absence of insecticidal pressure. Recently, a duplication of the Ace-1 locus was observed in An. gambiae, which results in a wildtype and resistant-associated allele occurring on the same haplotype (Ace-1F). It is proposed that the Ace-1F haplotype is in effect a “fixed” heterozygote that may confer similar levels of resistance but with reduced fitness effects. We have screened for diversity in and around the Ace-1 locus in mosquitoes from a large number of sites in West Africa. We present data showing a selective sweep centred on the Ace-1 locus and extending for approximately 1MB in both centromeric and telomeric regions. The duplication of the Ace-1 locus was observed in An. gambiae, which is a major malaria vector in Southern Africa. It remains unclear whether the many reports of pyrethroid resistance in this region have the same underlying mechanisms spreading between countries through gene flow or if different mechanisms occurred independently. To elucidate these questions we dissected the molecular basis of pyrethroid resistance in three countries, Mozambique, Malawi and Zambia. Microarray analysis using an Agilent chip identified three main P450 genes associated with permethrin resistance (CYP6P9a, CYP6P9b and CYP6M7) but with significant differences in expression patterns between countries. Other genes potentially implicated involved a short chain dehydrogenase but with significant differences in expression patterns between countries. The overall higher fold-change in Mozambique correlates with the higher level of resistance to pyrethroid in this country. In contrast to neighbouring Kenya where An. gambiae s.s. nearly disappeared after vector control despite high kdr frequency, An. gambiae s.s. remains predominant in Kagera even with high coverage of long lasting insecticidal nets (LLIN) was carried out in 2011. The emergence of resistance could threaten the future of these two interventions. As a component of a cluster randomized trial comparing the combination of LLIN and IRS versus LLIN alone the distribution of vectors and prevalence of insecticide resistance is being monitored. From April to December 2011, monthly Anopheles gambiae collection using CDC light traps was carried out across 40 villages in the area. Resistance monitoring was carried out on An. gambiae s.l. using WHO cylinder test. CDC bottle bioassays with synergists examined the involvement of metabolic resistance. Species identification and prevalence of knock down resistance (kdr) was confirmed using real time PCR TaqMan assay. A total of 5844 Anopheles mosquitoes were collected over seven months, of these 67% were collected in April, two months after spraying with pyrethroid. 81.8% were An.gambiae s.s. and 17.2% were An. arabiensis. East kdr mutation which is associated with pyrethroid and DDT resistance was present at high frequency in An.gambiae s.s. (97%) but only at 5% in An.arabiensis. Mortality in WHO resistance tests ranged from 0% to 38% for lambdacyhalothrin, 12% to 40% for DDT, and 84% to 100% for bendiocarb. Result from the CDC bottle assay suggested the presence of elevated level of oxidases and esterases. East kdr mutation seems to have reached fixation in the An.gambiae s.s population. High phenotypic resistance to pyrethroid was observed. In contrast to neighbouring Kenya where An.gambiae s.s. nearly disappeared after vector control despite high kdr frequency, An. gambiae s.s. remains predominant in Kagera even with high coverage of pyrethroid IRS and LLINs.

DISSECTING THE MOLECULAR BASIS OF PYRETHROID RESISTANCE IN FIELD POPULATIONS OF THE MAJOR MALARIA VECTOR ANOPHELES FUNESTUS IN SOUTHERN AFRICA

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Anopheles funestus is a major malaria vector in Southern Africa. It remains unclear whether the many reports of pyrethroid resistance in this region have the same underlying mechanisms spreading between countries through gene flow or if different mechanisms occurred independently. To elucidate these questions we dissected the molecular basis of pyrethroid resistance in three countries, Mozambique, Malawi and Zambia. Microarray analysis using an Agilent chip identified three main P450 genes associated with permethrin resistance (CYP6P9a, CYP6P9b and CYP6M7) but with significant differences in expression patterns between countries. Other genes potentially implicated involved a short chain dehydrogenase but with significant differences in expression patterns between countries. The overall higher fold-change in Mozambique correlates with the higher level of resistance to pyrethroid in this country. In contrast to neighbouring Kenya where An. gambiae s.s. nearly disappeared after vector control despite high kdr frequency, An. gambiae s.s. remains predominant in Kagera even with high coverage of long lasting insecticidal nets (LLIN) was carried out in 2011. The emergence of resistance could threaten the future of these two interventions. As a component of a cluster randomized trial comparing the combination of LLIN and IRS versus LLIN alone the distribution of vectors and prevalence of insecticide resistance is being monitored. From April to December 2011, monthly Anopheles gambiae collection using CDC light traps was carried out across 40 villages in the area. Resistance monitoring was carried out on An. gambiae s.l. using WHO cylinder test. CDC bottle bioassays with synergists examined the involvement of metabolic resistance. Species identification and prevalence of knock down resistance (kdr) was confirmed using real time PCR TaqMan assay. A total of 5844 Anopheles mosquitoes were collected over seven months, of these 67% were collected in April, two months after spraying with pyrethroid. 81.8% were An.gambiae s.s. and 17.2% were An. arabiensis. East kdr mutation which is associated with pyrethroid and DDT resistance was present at high frequency in An.gambiae s.s. (97%) but only at 5% in An.arabiensis. Mortality in WHO resistance tests ranged from 0% to 38% for lambdacyhalothrin, 12% to 40% for DDT, and 84% to 100% for bendiocarb. Result from the CDC bottle assay suggested the presence of elevated level of oxidases and esterases. East kdr mutation seems to have reached fixation in the An.gambiae s.s population. High phenotypic resistance to pyrethroid was observed. In contrast to neighbouring Kenya where An.gambiae s.s. nearly disappeared after vector control despite high kdr frequency, An. gambiae s.s. remains predominant in Kagera even with high coverage of pyrethroid IRS and LLINs.
that bites after dawn (06:00) was 26%. Bioassays showed no resistance to deltamethrin. In conclusion, this study provides evidence for a switch in malaria vectors biting behaviour following the implementation of LLIN at universal coverage. We show first evidence for a diurnal activity of a major malaria vector in Africa. These changes may reflect phenotypic plasticity or selection of genetically inherited traits and may have direct consequences on the burden of malaria in Africa. These findings highlighted the need for alternative strategies for better targeting outdoor malaria vectors.

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QUANTIFYING THE MOSQUITO’S SWEET TOOTH: MODELING ATTRACTIVE TOXIC SUGAR BAIT FOR VECTOR CONTROL

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Current vector control strategies focus largely on indoor measures, such as long-lasting insecticide treated nets (LLINs) and indoor residual spraying (IRS); however mosquitoes frequently feed on sugar sources outdoors, inviting the possibility of novel control strategies. Attractive toxic sugar bait (ATSB), either sprayed on vegetation or provided in outdoor bait stations, has been tested in Mali and Israel and has been shown to significantly reduce mosquito densities in these settings. We fitted models of mosquito population dynamics to data from these experiments to gain a better quantitative understanding of mosquito sugar feeding behavior and the potential of ATSB to control mosquito populations. In Mali, we estimate that 42% of female mosquitoes in the experimental setting fed on ATSB solution per day, dying within three hours of ingesting the toxin. A model incorporating the number of gonotrophic cycles completed by female mosquitoes found a higher feeding rate for younger mosquitoes and a slower rate for older mosquitoes. This model was extended to assess the role of ATSB as part of an integrated vector management (IVM) program. Our simulations suggest that an IVM program based on both ATS and LLINs/IRS is likely to cause substantial reductions in mosquito density as multiple stages of the mosquito’s lifecycle are targeted. In addition, ATS is expected to be particularly effective against Anopheles arabiensis, which is relatively exophilic and therefore less affected by IRS and LLINs. ATS has a benefit over larvicides in the sense that it skews the age distribution towards younger mosquitoes, which is beneficial for malaria control because only older mosquitoes have time to acquire, incubate and transmit the parasite. These encouraging results suggest that ATS should be seriously considered as a promising component in future IVM malaria control strategies.

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CHANGES IN ANOPHELES FUNESTUS BITING BEHAVIOR FOLLOWING UNIVERSAL COVERAGE OF LONG-LASTING INSECTICIDAL NETS IN BENIN

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Behavioural modification of malaria vectors in response to vector control methods is of great concern. We investigated whether full coverage of Long-Lasting Insecticide-treated mosquito Nets (LLIN) may induce a switch in biting behaviour in Anopheles funestus, a major malaria vector in Africa. Human landing collections were conducted indoor and outdoor in two villages (Lokohoué and Tokoli) in Southern Benin prior, 1 year and 3 years after implementation of universal LLIN coverage. Outdoor Biting Rates (OBR) and Median Catching Times (MCT, the hour for which 50% of An. funestus mosquitoes were collected) were compared. The resistance status of An. funestus to deltamethrin insecticide was monitored using bioassays. MCT of An. funestus switched from 02:00 in Lokohoué and 03:00 in Tokoli to 05:00 after 3 years (Mann-Whitney p-value<0.0001). In Tokoli, OBR increased from 45% to 68.1% (OR=2.55;95CI 1.72-3.78;p<0.0001) 1 year after the universal coverage whereas OBR was unchanged in Lokohoué. In this latter place, however, the proportion of An. funestus

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A POTENTIAL ROLE FOR URIC ACID IN ENDOTHELIUM ACTIVATION AND DAMAGE IN PLASMODIUM FALCIPARUM MALARIA

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Inflammatory cytokinemia and systemic endothelial activation are central to the pathogenesis of Plasmodium falciparum (Pf) malaria. Recently, Pf-derived uric acid (UA) - in both its soluble and precipitated forms - was shown to activate human immune cells in vitro, and elevated plasma UA levels were associated with inflammatory cytokinemia and disease severity in children with malaria. A role for Pf-derived UA in endothelial inflammation has not been investigated. Since UA elevations are associated with endothelial inflammation in a variety of non-malarial diseases, we hypothesized that elevated UA levels contribute to endothelial activation and damage in P. falciparum malaria. To test this, we measured levels of UA and soluble forms of intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), E-Selectin, thrombomodulin (TM), tissue factor (TF) and vascular endothelial growth factor (VEGF) in the plasma of 567 Malian children (aged 6 months - 17 years) with uncomplicated malaria (UM, N=489) and non-cerebral severe malaria (NCSM, N=68). In 69 of these children, we measured these same factors during their malaria episode and twice when they were healthy (before and after the transmission season). We found that levels of UA, sICAM-1, sVCAM-1, sE-Selectin and sTM increased significantly during a malaria episode, returning to ‘healthy’ levels at the end of the transmission season (p<0.0001). In children with UM, UA levels correlated significantly with those of sICAM-1 (r=0.255, p<0.0001) and sTM (r=0.175, p=0.0005). To test the possibility that Pf-derived UA precipitates activate EC, we co-cultured 3D7-infected red blood cells (PfRBCs) with primary microvascular endothelial cells (MVECs) with or without uricase, which degrades UA. Our preliminary results show that PfRBCs stimulate MVECs to release IL-6 and IL-8 in a dose- and time-dependent manner and that uricase abrogates the production of these cytokines. Our data suggest that parasite-induced elevations in UA levels contribute to malaria pathogenesis by causing endothelial activation and damage.
depleting agents. Serial microultrasonography revealed zones of decreased echogenicity in the periphery of egg granulomas in macrophage-depleted versus -replete mice, suggestive of relative hypocellularity. This was confirmed by histology, which revealed hypocellular cavitations in macrophage-depleted granulomas, less fibrosis, and fewer infiltrating leukocytes. None of the control vehicle-treated mice receiving eggs died, whereas 60% of macrophage-depleted mice receiving high doses of eggs died by day 11 post-egg injection, indicating a crucial role for macrophages in prevention of detrimental systemic effects of helminth exposure. Our results confirm a critical role for macrophages in schistosomal bladder pathogenesis, even in the setting of a single exposure to S. haematobium eggs. This suggests that macrophages may be a suitable therapeutic target for advanced schistosomal bladder pathogenesis.

DEVELOPMENTAL AND SPATIAL EXPRESSION OF ANTIGENIC GLYCANS BY LARVAL STAGES OF SCHISTOSOMA MANSONI

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Many glycans of schistosomes are differentially expressed during the parasite's lifecycle. In human infections, antibodies are produced against antigenic schistosome glycans, which might form promising novel vaccine targets. Schistosome larvae, up to several days old, appear most vulnerable to immune attack, but their glycosylation is poorly characterized. In this study we determined the structure and localization of the antigenic glycans expressed by early developmental stages of S. mansoni ranging from invading cercariae to transformed schistosomula and mature worms, with the objective of identifying antigens exposed to the immune system of the host. The protein-linked glycans from 14 different lifecycle stages were isolated and profiled using a mass spectrometry-based analysis strategy. Although N-glycans were continuously present during the whole lifecycle, our analysis indicated a gradually changing N-glycome during development. Expression of immunogenic glycan elements such as core-xylose and LeX-antennae motifs as N-glycans, strongly diminishes after transformation to schistosomula, but expression decreased after 3 days of maturation. On the other hand, glycans with LDN termini become abundant in cercariae and shortly after transformation to schistosomula, but expression decreased after 3 days of maturation. On the other hand, glycans with LDN termini become abundant in the adult stages. O-glycan expression, often with similar antennae motifs as N-glycans, strongly diminishes after transformation of the cercariae, but becomes abundant again in eggs. Using a glycanscan constructed of schistosome-derived glycans, we determined the fine-spectrum of a panel of anti-carbohydrate monoclonal antibodies obtained from schistosome-infected mice. Application of these mAbs in immunofluorescence microscopy assays of the infective cercariae and 1-3 day schistosomula stages of S. mansoni indicated that some glycans epibolize (e.g. ODN, F-LDN, F-LDN-F) identified in the structural studies are expressed at the surface throughout development, whereas others such as the LeX-motif appear at the surface only after transformation. These observations further underline the potential of specific glycans as targets for immune attack.

NEMATODE AUTOPHAGY REGULATES WOLBACHIA POPULATIONS AND IDENTIFIES A NOVEL MODE-OF-ACTION FOR ANTI-FILARIAL TREATMENT

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Filarial nematode parasites are amongst the most important neglected parasitic diseases of humans and animals. Over 150 million individuals are infected with lymphatic filariasis and onchocerciasis and heartworm is an important pathogen of dogs and cats. A new target for anti-filarial treatment is the obligate mutualistic endobacteria Wolbachia. Depletion of Wolbachia with antibiotics induces defects in nematode development, fertility and viability. In order to identify novel mechanisms to deplete Wolbachia as part of the A-WOL drug discovery and development programme, we investigated the role of activating host nematode autophagy to control bacterial populations. Our studies revealed that periods of rapid bacterial population growth and expansion were accompanied by activation of the autophagy pathway and that chemical and genetic manipulation of this pathway could directly regulate bacterial populations at an equivalent level to antibiotic treatment. The activation of the autophagy by using drugs or small-molecules resulted in Wolbachia reduction in both in vitro and in vivo treated Brugia malayi. The induction of the host nematode intracellular autophagy defence mechanism may therefore be considered as a novel mode-of-action, which delivers bactericidal activity that can be used to develop improved drugs and regimes for anti-filarial treatment.

FUNCTIONAL REDUNDANCY IN PLASMODIUM HEMOGLOBINASES AND PARASITE DEVELOPMENT INSIDE RETICULOCYTES WITHOUT HEMOGLOBIN DEGRADATION

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Clinical symptoms of malaria infection manifest when the Plasmodium parasites replicate in host red blood cell. During this intraerythrocytic cycle, the parasite ingests and catabolizes up to 75% of the host cell hemoglobin (Hb). The hemoglobin is broken down by a number of proteases in a semi-order cascade in an acidic, Plasmodium-specific, digestive food vacuole (DFV). Whether this degradation is essential for parasite survival has not been established. To characterize biological roles of various hemoglobinases residing in digestive food vacuole, we attempted targeted gene disruption of all the predicted hemoglobinases in the rodent model P. berghei and found that most of them are functionally redundant. We have also created a double gene-deletion mutant, ApmA4bp2, lacking expression of both plasmepsin-4 (equivalent to P. falciparum plasmepsin I, II, III and IV) and bergheipain-2 (equivalent to P. falciparum falcipain-2a, 2b and 3), which are thought to be involved in initial cleavage of Hb. Despite severe growth and virulence attenuation, the parasites are able to develop into mature schizonts in reticulocytes. These schizonts produce either no or vastly reduced levels of hemozoin, the crystallized product formed by detoxification of heme that is released early in Hb digestion. This was confirmed by examining hemozoin deposition in both liver and spleen of ApmA4bp2 infected mice, which was greatly reduced compared to wildtype infected mice. The cerebral complication (CM) sensitive C57BL/6 mice were able to clear the infection without visible CM manifestation and survive later wild-type challenge. Our results show that Hb digestion may not be essential for parasite growth in reticulocytes. These findings have implications for the design of drugs against DFV enzymes and for possible mechanisms that underlie Plasmodium resistance to drugs, the majority of which target Hb digestion and heme detoxification.

BIOCHEMICAL CHARACTERIZATION OF UFSP, THE UFM1 ASSOCIATED PEPTIDASE IN LEISHMANIA DONOVANI

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Leishmaniasis is a spectrum of diseases caused by protozoan parasites belonging to several different Leishmania species. There are no effective
vaccines against leishmaniasis. Currently available therapeutic regimens are often limited in effectiveness due to unwarranted side effects and rapidly emerging drug resistance. Therefore, the quest for a novel vaccine and therapeutic targets acquires urgency towards controlling leishmaniasis. Ubiquitin and ubiquitin-like protein modifiers (Ubls) regulate a variety of biological functions ranging from endocytosis, membrane trafficking, protein kinase activation, DNA repair and chromatin dynamics. Studies of Ubl functions in human parasitic organisms are limited. Recently, we described the existence of a novel Ubl named ubiquitin-fold modifier 1 (Ufm1) that conjugates to parasite proteins in Leishmania donovani. To elucidate the enzymatic activities associated with Ufm1 conjugation, we identified a putative Ufsp in the trypanosomatid genomes. Biochemical analysis of L. donovani Ufsp showed that this protein possesses the 3'-hydrolase activity necessary for processing the precursor Ufm1 into a conjugatable form. To examine the effects of abolition of Ufm1 processing activity, we generated a L. donovani knock out mutant lacking the Ufsp. Analysis of the Ufsp mutant revealed that lack of this protein results in the absence of processing of precursor Ufm1. We also showed that Ufsp null mutant results in reduced survival of L. donovani in infected human macrophages suggesting a role for this protein in Leishmania pathogenesis. This growth defect was reversed by re-expression of wild type but not the mutant of the catalytic cysteine (cys-ser) in the Ufsp indicating the essential nature of this protease for Ufm1 conjugation. Therefore, Leishmania Ufsp has the potential to be a novel drug target. Further, Ufsp/- parasites also provide an opportunity to explore such parasites as live attenuated vaccine candidates.

MOLECULAR AND FUNCTIONAL STUDIES OF THE SCHISTOSOMA MANSONI VENUS KINASE RECEPTORS SMVKR1 AND SMVKR2: POTENTIAL ROLES IN LARVAL DEVELOPMENT AND OOGENESIS

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Venus kinase receptors (VKRs) form a new family of receptor tyrosine kinases. Atypically, VKRs contain an extracellular Venus Flytrap (VFT) domain, a ligand-binding domain activated by small molecules such as aminoacids. Vkr genes are found in diverse eumetazoan genomes, from cnidarians to echinoderms and are particularly well conserved in protostomian species, as reported previously. In the platyhelminth Schistosoma mansoni, two VKRs have been previously described, SmVKR1 and SmVKR2. Quantitative RT-PCR as well as in situ hybridization indicated a large expression of both genes in larval stages and in female oocytes. RNA interference experiments performed on sporocysts and adult worms further confirmed the implication of SmVKRs in larval development and oogenesis. Using Xenopus laevis oocytes for protein expression, we demonstrated that SmVKR1 could bind and be activated by amino acids, mainly by L-Arginine, whereas SmVKR2 activation was triggered by calcium ions. In order to decipher the downstream signalling pathways of SmVKR1 and SmVKR2, we have started to identify binding partners of these receptors by the screening of an adult worm cDNA library using the yeast two-hybrid system. Our results suggest that both SmVKR1 and SmVKR2 participate in cytoskeleton rearrangement and in developmental mechanisms. Potential substrate/adapters for SmVKR1 have been identified and their function in the activation pathway of the receptor is under investigation.

DETECTING CRYPTIC GENETIC EXCHANGE IN TRYPANOSOMA CRUZI TCI

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Clonal propagation is considered to be the predominant mode of reproduction among many parasitic protozoa. However, this assumption may overlook unorthodox, infrequent or cryptic sexuality. Trypanosoma cruzi, the causative agent of Chagas disease, is known to undergo non-Mendelian recombination in vitro, while evidence of natural genetic exchange is more limited. T. cruzi displays remarkable genetic heterogeneity and is recognized as a complex of six discrete typing units (DTUs). Infection with T. cruzi is life-long and can lead to a spectrum of pathological complications. However, the relationship between specific clinical outcomes and parasite genotype remains elusive. The availability of whole genome sequences has driven advances in high-resolution genotyping techniques and re-invigorated interest in exploring the genetic diversity present within the various DTUs. We developed a highly resolutive mitochondrial multilocus sequence typing (mMLST) scheme, which was validated against current nuclear typing tools using isolates belonging to the oldest and most widely occurring lineage TcI. In parallel, we exploited read depth data, generated by illumina sequencing of the mitochondrial genome from the TcI reference strain to investigate the existence of mitochondrial heteroplasmy (heterogeneous mitochondrial genomes in an individual cell) in T. cruzi and resolve its role as a potential source of genotyping error. Comparison of nuclear and mitochondrial data uncovered multiple novel mitochondrial introgression events among disparate geographical populations as well as between major T. cruzi DTUs. Illumina sequencing data from the TcI genome strain revealed multiple different mitochondrial genomes within an individual parasite (heteroplasm) that were, however, not sufficiently divergent to represent a major source of typing error. mMLST provides a powerful approach to genotyping at the sub-DTU level. This strategy will facilitate attempts to resolve phenotypic variation in T. cruzi and to address epidemiologically important hypotheses in conjunction with intensive spatio-temporal sampling. The observations of gross nuclear mitochondrial phylogenetic incongruence indicate that genetic recombination is geographically widespread and continues to influence the natural population structure of TcI, a conclusion which challenges the traditional paradigm of clonality in T. cruzi.

A PLASMODIUM VIVAX GENETIC CROSS TO INVESTIGATE MOLECULAR DETERMINANTS OF CHLOROQUINE RESPONSE

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To investigate determinants of Plasmodium vivax chloroquine (CQ) resistance we have generated a genetic cross between two parasite lines with distinct CQ responses. A chimpanzee was infected with a mixture of these parental lines to produce infectious gametocytes for cross-fertilization in Anopheles mosquitoes. Recombinant sporozoites from the infected mosquitoes were purified and cryopreserved and subsequently used to re-inoculate the same chimpanzee after it was completely cured of the parental lines infection. When parasitemia was detected in the
re-inoculated chimpanzee, pools of mixed intraerythrocytic recombinant progeny were collected and inoculated into Aotus monkeys. Progeny in these pools showed responses spanning the range of the parental lines, including some parasites surviving a total CQ dose of 15 mg/kg (5 mg/kg/day x 3 days). Comparison of genetic markers in the mixed progeny before and after CQ treatment identifies regions of chromosomes that may be subject to linkage group selection and contain possible candidate genes. The P. vivax ortholog of the P. falciparum CQ resistance transporter gene (pf crt ortholog, pvcrt-c) resides in one of these chromosome regions.

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ARTEMISININ RESISTANCE IN PLASMODIUM FALCIPARUM IS ASSOCIATED WITH AN ALTERED PATTERN OF TRANSCRIPTION

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The emergence of artemisinin resistance in Western Cambodia and spread of resistance evidenced by the recent report of resistance in neighboring Thai-Myanmar border are major obstacles to the global containment and elimination of malaria disease. Although several genome-wide association studies on artemisinin resistance have been carried out proposing candidate genes, yet no definite molecular makers of artemisinin resistance have been commonly identified by the various research groups and none validated so far. Using DNA microarrays, we characterized the transcriptional profile of the ex-vivo intra-erythrocytic stage of total 36 parasite isolates from patients collected from 2008 to 2010 from Laos, Pailin, Western Cambodia and Thai-Myanmar border, of which 15 are resistant to artemisinin as reflected by increased parasite clearance half-lives. Features of the profile associated with artemisinin resistance include reduced expression of metabolic and cellular pathways such as glycolysis, pentose phosphate pathway, protein synthesis, DNA replication and redox metabolism in early stages. In contrast, protein synthesis related functions including cytoplasmic translation, transcription and chaperone-assisted protein folding genes have increased expression in the schizont stage. Hence, artemisinin resistance may be associated with lower metabolic activity of the ring stage that leads to decreased drug activation and simultaneously, increased protein synthesis, folding and turnover, that compensate the loss of proteins damage caused by the drug. In addition, we observed the differential expression of several key regulatory proteins that may underlie the observed transcription profile. The transcriptional profiles of a further 73 samples including 53 Pailin, 18 Lao and 2 Thai isolates from the ongoing TRAC (Tracking Resistance to Artemisinin Collaboration) study has been generated and analyzed and results will be discussed. In order to identify CNVs associated with resistance, we performed comparative genomic hybridizations using genomic DNA sequentially isolated from the same clinical samples and found several genes with copy number variations (CNV) associated with increased clearance half life. The involvement of these CNVs in resistance as well as their relation to the differential transcriptional profile associated with resistance phenotype will be reviewed and discussed.
vivo antimalarial drug susceptibility in W. Cambodia, and compare them with those in Northern and Eastern Cambodia, we obtained *Plasmodium falciparum* isolates directly from patients with uncomplicated malaria. Using a SYBR-Green I-based method, we measured the ex vivo susceptibility of 252 parasite isolates to 6 antimalarial drugs: chloroquine (CQ), MQ, quinine (QN), PPQ, ART and DHA. Data from 80% (203/252) of these assays were interpretable for ≥4 drugs. The proportions of parasite isolates showing reduced ex vivo susceptibility to CQ, MQ, QN and PPQ in W. Cambodia were 98%, 22%, 4% and 10%, respectively. The same proportions in N. Cambodia were 97%, 20%, 7% and 7% and in E. Cambodia were 84%, 5%, 0% and 10%. Reduced ex vivo susceptibility to ART and DHA was not observed in the 3 regions. The ex vivo mean IC50 values for CQ, MQ, QN, ART and DHA were significantly higher in W. and N. Cambodia than in E. Cambodia (p<0.001). However, there were no significant differences in the ex vivo GM IC50 values for PPQ between these regions. We detected significant positive correlations between MQ and ART (r=0.54, p<0.001), MQ and DHA (r=0.32, p<0.001), and ART and DHA (r=0.62, p<0.001) and MQ and DHA (r=0.42, p<0.001). Our data indicate that reduced *P. falciparum* susceptibility to MQ and PPQ is present in all 3 regions of Cambodia. In different regions of Cambodia, where either DHA-PPQ or ART+MQ are the recommended treatments, studies to monitor the clinical efficacy of these drugs is warranted.

### DEVELOPMENT OF ARTESUNATE RESISTANCE IN VIVO USING A *PLASMODIUM FALCIPARUM* HUMANIZED MURINE MODEL OF MALARIA

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Development of resistance against artemisinin-combination therapies (ACTs) is a major threat for the control and eradication of malaria. Humanized murine models of malaria allow the growth of *Plasmodium falciparum* in human erythrocytes engrafted into mice. In this work we show that the *P. falciparum* murine model can be used to analyze the development of resistance against antimalarials. Treatment failure with artesunate was observed after suboptimal therapy in thirteen serial passages whereas atovaquone required two, which is compatible with their corresponding propensity to generate resistance. The artesunate resistant strain showed a marked decrease in the parasite reduction ratio (PRR) whereas the atovaquone resistant strain showed almost complete resistance to treatment *in vivo*. None of the resistant strains showed measurable impairment of proliferative capacity *in vivo*. Interestingly, in contrast with atovaquone, the reduction of susceptibility to treatment with artesunate was not associated with reduced susceptibility *in vitro*. Therefore, these results suggest that the *P. falciparum* humanized murine model can be a valid model to study the development of resistance caused by sub-therapeutic treatment.
WHAT DETERMINES PARASITE CLEARANCE: A POOLED ANALYSIS OF FREQUENT PARASITE COUNTS AFTER TREATMENT WITH AN ARTEMISININ DERIVATIVE ALONE OR IN COMBINATION WITH OTHER ANTIMALARIALS

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Parasite clearance (PC) is considered to be the most robust measure of antimalarial effect and is a key component to characterize artemisinin resistance. The dynamics of PC following artemisinin treatment are influenced by several factors other than parasite susceptibility including host immunity, initial parasite biomass, and partner drug efficacy. It is critically important to control for such potential confounding factors to identify changes over time in PC due to reduced parasite drug susceptibility. We have pooled parasitaemia data collected at least every 12 hours from individual patients who participated in completed studies. The WWARN Parasite Clearance Estimator was used to produce standardized estimates of parasite half-life (HL). The effects of covariates such as artesunate dose, partner drug, transmission intensity, year and location of study, age and baseline characteristics on PC were examined in a regression model and the relationship between treatment outcome and HL was evaluated in a subset of patients with efficacy data available using Cox regression. Random effects or frailty were used to account for study effect. Fourteen studies with 4655 patients in Cambodia, Thailand, Laos, Bangladesh, Mali, Tanzania and Kenya were included in the analysis. Clinical outcome was evaluated in 8 studies with 702 patients during 42 (4 studies) or 63 days (4 studies) follow-up period. The median (range) of estimated HLs was 3.2h (0.6 - 21.4). Estimates varied significantly between study location and year (p<0.001), with median HLs ranging 1.9-6.9 h between studies. Among 696 patients with available efficacy outcomes, twenty four had PCR-confirmed recrudescence and slower PC (p<0.001) with a median (range) HL of 6.8h (2.5-11.1) compared to 3.3h (0.9-12.2) in cured patients. HL was not affected by initial parasite count or patient age but was longer in patients with gametocytes, low haematocrit or prolonged fever at enrollment. This analysis provides key reference baseline data to characterize antimalarial resistance and understand factors affecting measurement of PC.

DEFINING THE ELUSIVE ARTEMISININ RESISTANCE PHENOTYPE IN VITRO

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Artemisinin resistance has emerged in Cambodia and Thailand and is observed clinically as a reduced parasite clearance rate in vivo following treatment with an artemisinin derivative alone or in combination. Recent evidence suggests the in vivo phenotype is linked to heritable genetic trait(s), yet to date a clear artemisinin resistance phenotype in vitro has not been defined. This is in direct contrast to experience with other antimalarial drugs where in vitro drug resistance was clearly evident in cultured parasites either before or simultaneously with the advent of clinical resistance. Through a series of studies we have begun to define the elusive artemisinin resistance phenotype in vitro. First we generated stable artemisinin resistant lines of Plasmodium falciparum, cloned them, and used these clones to assess new in vitro phenotype assays. Secondly we applied the new assays to culture adapted isolates of P. falciparum from Cambodia and Thailand. Isolates with evidence of artemisinin resistance in vitro were immediately cloned and characterized. Results from these studies suggest that both the in vitro generated resistant lines and clones of Cambodian P. falciparum express stable resistance to artemisinin derivatives in vitro. Interestingly, the highest level of resistance in all resistant lines was to artelinic acid (AL), a compound that has never been used clinically. We found 4-8 fold resistance to AL in each of the resistant lines as compared to 3-5 fold resistance to artemisinin. A reduced level of resistance (2-3 fold) was consistently observed for dihydroartemisinin. In addition, each of the artemisinin resistant lines expressed the artemisinin-induced ring stage dormancy phenotype in which the resistant line recovered more rapidly from dormancy than artemisinin susceptible parasites. These new artemisinin resistance phenotypes can be used to monitor emerging resistance in the field and to accelerate the discovery of drug resistance mechanism(s) in stable, culturable, clonal lines.

ASSOCIATION BETWEEN ANTIBODIES TO Plasmodium falciparum AT DELIVERY AND IMPROVED PREGNANCY OUTCOMES AMONG WOMEN EXPOSED TO MALARIA

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Antibodies against VAR2CSA, the variant surface antigen binding to placental choroidin sulfate A, have been suggested to mediate protection against Plasmodium falciparum in pregnancy, although some studies have indicated that these antibodies at delivery are markers of exposure to P. falciparum. We hypothesized that variations in parasite exposure and HIV infection affect levels of antimalarial antibodies and also their associations with pregnancy outcomes. We measured IgGs against placental and pediatric isolates, VAR2CSA (DBL2X, DBL3X, DBL5c, DBL6c) and other blood stage antigens (DBLγ, DBLα, MSP1, AMA1, EBA175) in plasmas from 293 Mozambican pregnant women at delivery. The number of antigens recognized by IgG in plasma (breadth of recognition) was higher in women with placental infection (adjusted rate ratio [aRR]=1.59, 95%CI[1.44-1.77]), in women living close to the river (aRR=1.16, 95%CI[1.02-1.32]), in HIV-infected women pregnant during the rainy season (aRR=1.82, 95%CI[1.15-2.86]) and in HIV-infected women not receiving intermittent preventive treatment (IPTp: aRR=1.39, 95%CI[1.1-1.72]). HIV-infection attenuated the parity-dependent increase of IgGs against placental and pediatric isolates, DBLγ and AMA1 (p for interaction between HIV and parity=0.046). Among women who had a malaria episode during pregnancy, high antibody level against VAR2CSA (DBL3X and DBL6c), placental and paediatric isolates and AMA1 were associated with increased weight and gestational age of the newborns (p=0.036). Anti-parasite IgGs in women at delivery are sensitive to factors influencing malaria exposure and are affected by HIV infection, probably through its impact on the longevity of antibody responses. Reducing the variability of parasite exposure by including in the analysis only women with proven exposure during pregnancy allows the identification of IgGs against merozoite antigens, VAR2CSA and other variant surface antigens that may contribute to reduce the adverse effects of malaria in pregnancy.

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CD44 IS A FUNCTIONALLY RELEVANT RECEPTOR FOR ADHERENT PLASMODIUM FALCIPARUM IN THE PLACENTA

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Plasmodium falciparum infected red blood cells (iRBCs) accumulate in the maternal blood space of the placenta during malaria infection, culminating in pathological consequences deleterious to pregnancy success. The fetal cell in contact with maternal placental blood is a syncytialized epithelium called syncytiotrophoblast (ST). ST has a rich supply of low sulfated chondroitin sulfate A (CSA), a principle ligand for VAR2CSA parasite protein, present on the surface of placenta-adherent iRBCs. It is critical to examine the role CSA-bearing proteoglycans on ST play in anchoring iRBCs as well as their potential role as signaling molecules. Because it is known that STs are immunologically active in the presence of CSA-adherent iRBCs, here we examined the role of CD44 proteoglycan, a known CSA-bearing molecule with a transmembrane cytoplasmic domains adept at signaling functions. STs membrane proteins (SMPs) were extracted from cultured primary cells as well as whole placental preparations. SMPs were incubated with CSA-adherent and non-adherent iRBCs; binding of CD44 was specific to CSA-adherent iRBCs as observed by flow cytometry. CD44 from SMPs pre-treated with chondroitinase ABC lost significant iRBC binding activity. In vitro exposure of primary ST to CSA-adherent iRBCs promoted upregulated expression of CD44 as detected by ELISA, and immunohistochemical staining for CD44 antigen in placental tissue from Kenyan women showed a significant increase in expression of this adherence receptor coincident with active placental malaria. Current efforts are exploring the activation state of CD44 following exposure of ST to CSA-adherent iRBCs, as well as the impact of CD44 knockdown by RNA interference on malarial activation of ST. In summary, this work provides evidence that CD44 proteoglycan is an in vivo receptor for VAR2CSA-expressing iRBCs, the expression of which is modulated by malarial infection, and may additionally serve as a signaling molecule, promoting an ST response to placental malaria.

CHRONIC INFECTIOUS EXPOSURE DURING PREGNANCY AFFECTS NEONATAL B CELL SUBPOPULATIONS

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Chronic infections during pregnancy can expose the fetus to antigens that affect fetal B cell development. We hypothesize that resultant changes in B cell subpopulations may affect the infant's susceptibility to infection and disease. To investigate this, we examined cord blood B cell subpopulations and B cell responses to non-specific polyclonal activation in neonates with and without exposure to chronic prenatal infections. We developed six-color flow cytometry panels to differentiate subpopulations of B cells from cord blood mononuclear cells (CBMC) isolated from North American and Kenyan neonates. North American neonates had no prenatal chronic infectious exposures. Kenyan neonates examined had evidence of prenatal HIV, cytomegalovirus (CMV), P. falciparum malaria or no infectious exposures. Proportions of B cell subpopulations were compared between the exposure groups. Additionally, we examined the ability of B cells in each group to respond to polyclonal activation in culture. We found that neonates exposed to chronic prenatal infections (HIV, CMV and malaria) displayed higher levels of atypical (CD19+CD27+CD21-IgD-) and activated (CD19+CD27+CD21-IgD-) memory B cells compared to Kenyan non-exposed and North American neonates. Little differences were appreciated in naive B cell (CD19+CD21+CD27-CD10-) or classic isotype switched memory B cell (CD19+CD27+CD21+IgD-) populations. Neonates exposed to HIV had a lower proportion of CD5+ B cell compared to all other groups. Polyclonal activation of B cells resulted in subtle shifts in CD5 and TLR2 expression, which were similar among the exposure groups. The results of our study suggest that the presence of chronic infections during pregnancy affects B cell development, leading to increased levels of atypical and activated memory B cells. The functional effects of these differences will need to be further investigated.

THE SUPPRESSION OF MALARIA ANTIGEN-SPECIFIC RESPONSES BY REGULATORY T CELLS ACQUIRED IN UTERO PERSISTS INTO EARLY CHILDHOOD

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Prenatal exposure to malaria blood stage antigens has been associated with impaired malaria-Ag-specific Th1-type immune responses in early childhood as well as increased risk of malaria infection. Here we examined the hypotheses that tolerogenic fetal natural (CD25hi, Fox3+CD4+) and adaptive (high IL-10) regulatory T cells develop in utero, and that these cells persist into early childhood, impairing T cell-assisted production of protective antibody to malaria blood stage antigens. We show that depletion of CD25hiCD4+ T cells or neutralization of IL-10 in cord blood is associated with 2.3 to >10 fold increased IFNγ production by and/or proliferation of malaria blood stage-specific lymphocytes in samples from newborns of a subgroup of malaria-infected women; otherwise these newborns (classified as putatively tolerant, N=10) show weak or absent malaria Ag-driven proliferation or IFNγ production. By contrast, offspring of women uninfected with malaria (not exposed, N=24) or offspring who develop a malaria Ag-driven predominantly Th1-type response in the face of maternal prenatal malaria infection (exposed-sensitized, N=13) fail to show consistent augmentation of lymphocyte proliferation and/or IFNγ production with IL-25hiCD4+ depletion and/or IL-10 neutralization. Repeat examination of these same children at 12 to 24 months of age shows persistence of these phenotypes, with putatively tolerant offspring showing an overall increased lymphocyte proliferation with CD25hiCD4+ depletion and enhanced IFNγ production with IL-10 neutralization compared to children identified as exposed-sensitized or not exposed (p=0.006, 0.01 and p=0.02, 0.009 respectively). Thus, in utero exposure to malaria blood-stage antigens can induce a form of immune tolerance that is probably regulatory T cell-mediated and likely modulates malaria antigen-specific immune responses throughout early childhood.

INTERACTIONS BETWEEN THE GENETIC DIVERSITY OF PLASMODIUM FALCIPARUM INFECTIONS AND BREADTH OF ANTIBODY RESPONSES IN RELATION TO IMMUNITY TO MALARIA

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In areas of high malaria transmission, the presence of asymptomatic infections with genetically diverse Plasmodium falciparum clones is associated with reduced risk of malaria by a yet unknown mechanism. Asymptomatic P. falciparum parasitemia can modify the association between antibodies to both merozoite and variant red blood cell surface antigens and the risk of malaria. Antibody responses to merozoite antigens are short-lived in the absence of continued infection. Considering these
observations and the step-wise reduction in risk of malaria with increasing breadth of antibody responses to merozoite antigens, we hypothesize that the presence of genetically diverse *P. falciparum* infections interacts with antibody responses to enhance the acquisition of immunity to malaria. To test this hypothesis, we have studied a longitudinally followed population in an area of high malaria transmission in Tanzania. A cross-sectional survey was conducted in March and April of 1999, just before the rainy season in which whole blood was collected. All the participants were monitored in the subsequent 40 weeks and episodes of malaria were recorded by a passive case detection system. We assessed the genetic diversity of *P. falciparum* infections at baseline by genotyping the *P. falciparum* merozoite surface protein 2 (msp2) gene by fluorescent PCR and capillary electrophoresis. We measured antibody levels to four of the leading malaria vaccine candidate antigens; 2 alleles of MSP-2, two alleles of MSP-3, two alleles of apical merozoite antigen 1, and the 19-kilodalton fragment of MSP-1 using a multiplex platform. Increasing breadth of antibody responses and presence of increasing number of genetically distinct clones at baseline were associated with reduced risk of malaria both individually and when analyzed in combination. These findings suggest that in an area of high malaria transmission, genetic diversity and antibody responses are additive or synergistic in conferring protection from malaria.

**USE OF TETRAMER STAINING TO ENUMERATE AND CHARACTERIZE MALARIA ANTIGEN-SPECIFIC CD8+ T-CELLS INDUCED IN VOLUNTEERS IMMUNIZED WITH ADENOVIRUS SEROTYPE 5 PLASMODIUM FALCIPARUM MALARIA VACCINES**

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Protection against pre-erythrocytic stages of malaria is believed to be mediated in part by cytotoxic T-lymphocyte (CTL) responses against epitopes of protective antigens presented by HLA on the surface of infected liver cells. Because IFN-γ secreting CD8+ T cells have been implicated in protection against pre-erythrocytic stage malaria through IFN-γ-mediated induction of nitric oxide to kill intra-hepatic stage malaria parasites, it has become common practice to attempt to associate the magnitude of malaria vaccine-induced and CTL-mediated IFN-γ production with protective immunity, since antibody responses are usually poor. However, malaria antigen-specific CD8+ T cells might kill infected hepatocytes by non-IFN-γ mediated mechanisms, such as the direct killing of hepatocytes through release of perforin and granzyme. In order to establish the total magnitude of the vaccine-induced CD8+ T cell response, we have identified putative CD8+ T-cell epitopes on CSP and AMA1, using PBMC samples from volunteers immunized with a single dose of serotype 5 adenovirus expressing two malaria antigens, CSP and AMA1 (AdCA). We next prepared a series of tetramers, each consisting of one of seven HLA-A or five HLA-B class I molecules and each presenting one of 16 distinct CSP epitopes or one of 11 distinct AMA1 epitopes. We then used the tetramer staining technique to label and thereby enumerate the total number of malaria antigen-specific CD8+ T cells in immunized HLA-A*01:01 and HLA-B*18:01 volunteers. We have also demonstrated that malaria tetramer-positive CD8+ T cells obtained three weeks post immunization express high levels of the CD38 and HLA-DR activation markers, raising the possibility that these markers might serve as a surrogate to detect multiple antigen-specific CD8+ T cells induced by whole parasite vaccines where the majority of antigens have not yet been identified. Work is also ongoing to carry out tetramer staining, ELISPot, and intracellular staining assays using pre-vaccination and post vaccination PBMC samples to look for comparability of results generated from the different assays.

**GENERATION OF NOVEL “HUMAN-IMMUNE-SYSTEM” HUMANIZED MOUSE STRAINS EXPRESSING HLA CLASS I AND CLASS II MOLECULES IN NOD.RAGKO.II2RGGCKO BACKGROUND**

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The current animal models that are used to test approaches that target the immune system (i.e., vaccines) are imperfect and accounts for many failures when human vaccines are tested in clinical trials. Development of humanized mouse models able to generate a surrogate human immune system is a highly pursued goal for investigating human immunology and for testing human vaccines. We previously showed that humanized mice expressing HLA class II (DR4) molecules in NOD.RagKO.II2RgckO background and infused with HLA-DR-matched human hematopoietic stem cells, develop a functional human immune system and respond to vaccination (PLoS One 6:e19826, 2011). While the frequency of human CD4 T cells, B cells, and dendritic cells in blood and lymphoid organs of humanized DRAG mice was similar to that in humans, the frequency of human CD8 T cells was however lower. This was attributed to the lack of HLA class I expression in humanized DRAG mice, since HLA class I molecules are required for thymic selection and survival of human CD8 T cells. Herein we have generated a new humanized mouse strain co-expressing HLA class I (A2) and HLA class II (DR4) molecules in NOD.RagKO.II2RgckO background, and provide evidence for human immune cell reconstitution as well as function of human CD8 T cells upon infusion of HLA-matched human hematopoietic stem cells.

**QTL MAPPING OF PLASMODIUM FALCIPARUM GENES THAT ALLOW EVASION OF THE MOSQUITO IMMUNE SYSTEM**

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The mosquito *Anopheles gambiae* L3-5 strain is capable of eliminating some lines of *Plasmodium falciparum* but not others. This elimination involves the mosquito immune complement-like system. A Quantitative Trait Loci (QTL) mapping was carried out to identify the *P. falciparum* gene(s) that allow some parasite strains to evade the *A. gambiae* immune system. The gene mapping was done in a *P. falciparum* cross between 2 lines that differ in their survival in *A. gambiae* L3-5; GB4 successfully infects the mosquito while 7GB is mostly eliminated by melanotic encapsulation. Phenotyping of parental lines and progeny lines for survival/encapsulation in *A. gambiae* L3-5; GB4 successfully infects the mosquito while 7GB is mostly eliminated by melanotic encapsulation. Phenotyping of parental lines and progeny lines for survival/encapsulation in *A. gambiae* L3-5; GB4 successfully infects the mosquito while 7GB is mostly eliminated by melanotic encapsulation. Phenotyping of parental lines and progeny lines for survival/encapsulation in *A. gambiae* L3-5; GB4 successfully infects the mosquito while 7GB is mostly eliminated by melanotic encapsulation.
41 genes identified 15 genes with non-synonymous polymorphisms between GB4 and 7G8 lines. Based on the expression differences and sequence polymorphisms, 5 candidate genes were selected for detailed genetic analysis by testing phenotype changes between survival and encapsulation, after allele replacement in P. falciparum. Identification of P. falciparum gene(s) that allow evasion of the mosquito immune system may be important to understand malaria transmission and could be a target for transmission blocking strategies.

**EVIDENCE OF RECOMBINATION IN THE X-CHROMOSOME CENTROMERIC REGION IN ANOPHELES GAMBIAE MOLECULAR FORMS FROM AN AREA OF PUTATIVE SECONDARY CONTACT**

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Anopheles gambiae M and S molecular forms are typically strongly reproductively isolated and clearly identifiable based on a SNP in the multi-copy IGS rDNA region, co-segregating with the single-copy insertion of a SINE element located approximately 1 Mb apart from the X-centromeric IGS region. However, an area of putative secondary contact has been recently detected at the westernmost extreme of M and S range. Preliminary indications of discordant M and S genotypes at the two X-linked markers near the centromere in female samples suggest that introgression and inter-locus recombination may be occurring in this area. This hypothesis is intriguing because recombination is known to be highly reduced in centromeric regions, and this is believed to have played a significant role in the incipient speciation process ongoing within A. gambiae. Here we present data from M and S female (N=275) and male (N=392) samples collected in Safim village in Guinea Bissau. Notably, males provide the opportunity to separate recombination, as distinct from heterozygosity, along the hemizygous X-chromosome. Results from IGS and SINE PCR-genotyping show: i) a 22% frequency of SINE MS-heterozygotes in females (consistent with previous data) and an absence of heterozygotes in males (as expected for a single-copy X-linked marker); ii) a 34% and 9% frequency of IGS MS-heterozygous pattern in females and males, respectively, strongly supporting the occurrence of recombination within even the most centromere-proximal region of the X chromosome; iii) the occurrence of discordant SINE/IGS genotypes in 12% and 18% of SINE-M and SINE-S females, respectively, and in 10% of SINE-M and SINE-S males, showing that recombination is occurring in both molecular forms. Moreover, multilocus SNP analysis carried out on a subsample of males provides estimates of recombination along the whole X-chromosome and novel original insights on M and S form status in their putative secondary contact zone.

**GENOMES IN FLUX: ‘REAL-TIME’ DYNAMICS OF INCIPIENT SPECIATION IN ANOPHELES GAMBIAE**

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Anopheles gambiae s.s. is considered to be in the process of incipient speciation into two molecular forms (M and S), originally defined by a single pericentromeric rDNA-IGS marker on the X chromosome. The molecular forms are sympatric throughout much of west and central Africa, but show broad bionomic differences which can extend malaria transmission in time and space. Genetic differentiation between M and S forms tends to be concentrated into genomic islands, which are resilient to gene flow. However, this genomic heterogeneity varies markedly between areas in line with levels of contemporary gene flow, most notably in Guinea-Bissau where the highest levels of inter-form gene flow are observed. Using Illumina Goldengate genotyping and whole genome resequencing we investigated the stability of M/S differentiation in Guinean samples collected in 1993 and in 2010. In the older samples genomewide differentiation, though very heterogeneous, clearly partitioned the molecular forms suggesting at least partial reproductive isolation. In the recent samples general M-S differentiation has decreased and a more complex within-population structure has emerged, with groupings exhibiting different degrees of resemblance to ‘typical’ M and S forms. Instability in population structure is not unexpected in an area of such high gene flow, but we also show adaptation-driven changes in genomic differentiation between the molecular forms from low gene flow areas. These data highlight that the dynamics of genome divergence in Anopheles gambiae speciation are occurring in real time, making the speciation process of relevance to contemporary control programmes.

**THE GENETIC BASIS OF HUMAN HOST CHOICE IN THE MALARIA VECTOR ANOPHELES GAMBIAE**

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The predominant malaria vector Anopheles gambiae s.s preferentially takes its blood meals from human hosts, often at rates as high as 90% in natural populations. The adaptation of these mosquitoes to human hosts has a genetic basis in the olfaction system, which includes several key gene families - the odorant receptors (ORs), odorant binding proteins (OBPs) and ionotropic receptors (IRs). To identify An. gambiae genes responsible for human host preference, we conducted a quantitative trait loci (QTL) mapping experiment based on introgressive backcrosses between the anthropophilic An. gambiae and the zoophilic An. quadriannulatus, in which F1 females were backcrossed to An. quadriannulatus males. These backcross females were subjected to a host-choice experiment in an olfactometer in which they were presented with a human and cow odor. Only individuals that selected the same odor on three consecutive days were included in the experiment. A total of ~15,000 individual backcross females were run through host-choice experiments, resulting in two pools totaling 432 mosquitoes with divergent host preferences. We are using 24 microsatellite markers to genotype individuals from the two pools and performed QTL analysis using R/QTL. Preliminary results based on 13 markers identified one highly significant QTL that explains 16% of the phenotypic variance. This QTL region is estimated to span a 10 Mb region.
and contains several ORs, OBP s and IRs. These genes are candidates for being involved in the adaptation of An. gambiae to its human host and were sequenced in six anopheline species to identify those that show evidence of positive selection.

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DENGUE 2 INFECTION ALTERS MICRORNA EXPRESSION IN Aedes aegypti
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Emerging studies show that important avenues in the post-transcriptional regulation of gene expression occur via small RNA regulatory pathways. Non-coding RNAs (ncRNAs) are key features of these pathways, and critical molecules involved in their biogenesis and function are conserved in plants, insects and mammals. To identify products of anti-viral RNA interference in vector mosquitoes, deep sequencing small RNA (sRNA) libraries were prepared from DENV2-fed Ae. aegypti females (Rex0 strain) and matched un-infected controls at 2, 4, and 9 days post-infection (dpi). An earlier publication described DENV2-derived viral sRNAs (viRNAs) across three size classes: unusually small RNAs (usRNAs) (14-19nts), canonical sRNAs (20-24nts), and piRNAs (25-30nts) (Hess et al., BMC Microbiology, 2011). In the present analysis, these libraries were mined to determine whether substantive changes occur in mosquito microRNA (miRNA) levels during DENV2 infection. Reads were aligned to miRBase release 17 hairpin database (mirbase.org). MiRNAs with over 50 reads across treatment groups and showing ≥ 2 fold-changes were chosen for further study. Our analysis reveals that significant changes to specific miRNA levels occur in DENV2-infected mosquitoes compared to un-infected controls. Moreover, some miRNAs showed coordinated enrichment or depletion at both 2 and 4 dpi, substantiating the hypothesis that they are important to the establishment of virus infection, whether by being exploited by the virus as part of an anti-viral mechanism. Coordinate co-regulated miRNAs or *miRNAs include miR155, miR2755, miR281 and miR277, among others. miRNAs were classified by type: conserved (homologous to previously reported miRNAs), *miRNA (complementary to miRNA), non-canonical or unclassified. Although the precise part played by each differentially expressed miRNA remains to be elucidated, orthologous miRNAs in other animals are important effectors of cellular differentiation, neurogenesis, transcriptional regulation, nutritional metabolism and the regulation of apoptosis. Our results show an intriguing new way in which major cellular processes of mosquitoes respond to arbovirus infection.

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GENETIC REGULATION OF VECTOR MOSQUITO SALIVARY GLAND DEVELOPMENT
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Understanding mosquito salivary gland development is critical given the importance of this tissue in blood feeding and pathogen transmission. Our recent survey of the mosquito genomes indicated that mosquitoes have orthologs of many genes that regulate embryonic salivary gland development in Drosophila melanogaster, a well-characterized insect genetic model organism. The expression patterns of a large subset of these genes were assessed during development of Aedes aegypti, an emerging model for vector mosquito development. These studies revealed that the early stages of Ae. aegypti salivary gland development significantly differ from that of D. melanogaster. We are now using an RNAi knockdown strategy to investigate the roles of genes expressed in the developing Ae. aegypti salivary gland. Functional characterization of cyclic-AMP response element binding protein A (creBA) indicates that this gene encodes a key regulator of secretory function in the Ae. aegypti salivary gland. These studies highlight the need for further analysis of mosquito developmental genetics and may foster comparative studies of salivary gland development in additional vector insect species.

1003
MEDUSA: A NOVEL GENE DRIVE SYSTEM FOR CONFINED SUPPRESSION OF MOSQUITO POPULATIONS
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Following successful field trials of sterile GM mosquitoes designed to control dengue fever, interest is now growing in the use of gene drive systems, such as X-shredders, capable of inducing a population crash as they spread. These systems hold much promise for wide-scale disease control; however issues arise from their potential to spread across international borders. We propose a novel gene drive system, Medusa, capable of inducing a local population crash without spreading into neighboring populations. Medusa consists of four components - two at a locus on the X chromosome and two at a locus on the Y chromosome. A maternally-expressed, X-linked toxin and a zygotically-expressed, Y-linked antidote suppress the female population because only males can protect themselves against the effects of the toxin. A zygotically-expressed, Y-linked toxin and a zygotically-expressed, X-linked antidote ensure that the two constructs are always inherited together. We use simple population genetic models to explore the dynamics of the Medusa system. An all-male release is preferred since males don’t bite and, if released over two generations, Medusa is expected to induce a population crash within seven generations for modest release sizes. Re-invasion of wild mosquitoes can lead to the population eventually rebounding; however this can be prevented by small, regular releases of Medusa males. The Medusa system could serve as a proof of principle for invasive population suppression systems such as X-shredders. We describe molecular solutions to chromosomal anomalies that could interfere with Medusa dynamics.

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VACCINATION WITH EXCRETORY/SECRETORY PRODUCTS CONFER PARTIAL PROTECTION IN A MURINE MODEL OF FILARIASIS
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Infection with filarial worms can cause severe and debilitating diseases in both humans and animals. While many vaccine candidates have been studied in filariasis, our understanding of protective immune responses in a permissive model to filariasis is incomplete. In this study, we evaluated preparations of worm antigens for protection against challenge infection in the BALB/c Litomosoides sigmodontis model. The fractions included LS, soluble antigens of a homogenate of adult worms, and ES, excretory/secretory products of adult female worms. 6-8 week old female BALB/c mice were given 3 intraperitoneal injections of 10 micrograms LS or ES with CpG/Alum and subsequently challenged with 40 infectious larvae. Mice vaccinated with LS, soluble antigens of a homogenate of adult worms, and ES, excretory/secretory products of adult female worms. 6-8 week old female BALB/c mice were given 3 intraperitoneal injections of 10 micrograms LS or ES with CpG/Alum and subsequently challenged with 40 infectious larvae subcutaneously. 8 weeks after infection mice were euthanized and parasite burdens were determined. Mice that were vaccinated with LS antigen showed no significant protection against challenge infection compared to control mice. Mice vaccinated with ES product, however, harbored 60% fewer adult worms than control mice. While mice vaccinated with LS and ES produced similar levels of IgG antibodies to both antigen preparations, analysis by western blot demonstrated that ES vaccinated mice recognized different ES proteins than LS-vaccinated mice. Currently, we are in the process of conducting mass spectroscopy to identify a ~160kDa protein that was strongly preferentially recognized by ES-vaccinated mice. No substantially large differences were observed between the two vaccinated groups with regards to lymphocyte activities.