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BACKGROUND: Most of the currently available anti-malarial drugs act on blood stages of plasmodium and have limited impact on the sexual stages to block transmission. Therefore, search for drugs active against the sporogonic stages of the parasite would be a novel approach for the development of transmission blocking product.

OBJECTIVE: The study aimed to evaluate in vivo malaria transmission blocking activity of ethanol and water extract of Vernonia amygdalina leaves (VA) using a murine malaria model (Plasmodium berghei GFP / Anopheles stephensi / BALB/c mice) and assess in vitro inhibitory effect of the extracts on the development of P. berghei CTRP ookinetes.

METHOD: In the transmission blocking experiment, mice were infected by intra-peritoneal (i.p.) inoculation of one million P. berghei infected red blood cells. On day 3 and 4 post-infection, they were treated with solvent, aqueous extract (100mg/kg) and alcohol extract (500mg/kg) by i.p. route, and then exposed to the bites of about 150 female An. stephensi mosquitoes. On day 10 and 11 after the infective blood-meal, the midguts of mosquitoes were dissected and the oocyst number was recorded. Oocyst density and prevalence was determined. In the ookinete development assay, VA extracts in triplicates was dispensed in a 96-well microplate containing gametocyte-infected blood and ookinete medium. After 40 h at 19°C, ookinetes were counted under fluorescent microscope (X400).

RESULTS: The oocyst density of mosquitoes fed on alcohol extract of VA treated mice were 13 [95% confidence interval (CI), 7-21], which was lower than in the control group, 326 [95% CI, 243-438], by 96%. The prevalence of mosquito infection was also less in the group treated with alcohol extract of VA. Alcohol extract reduced the early sporogonic stages by about 90% than in the control group, 326 [95% CI, 243-438], by 96%. The prevalence was determined. In the ookinete development assay, VA extracts in triplicates was dispensed in a 96-well microplate containing gametocyte-infected blood and ookinete medium. After 40 h at 19°C, ookinetes were counted under fluorescent microscope (X400).

CONCLUSION: Ethanol extract of VA leaves inhibits the ookinete development, which may be translated to the reduction of oocyst density and prevalence of infection in exposed mosquitoes. Studies on fractions and molecules is ongoing.

P003: Statistical analysis of malaria cases in the Savelugu/Nanton district of the Northern region (2004-2008)

Abdul-Muktadir Abdul-Mumin

BACKGROUND OF THE STUDY: Despite considerable efforts throughout the country to eradicate or control malaria, it is still the most prevalent and most devastating disease in the tropics. The disease has a crippling effect on the economic growth and perpetuates vicious cycles of poverty. It cost Africa US$10-12 billion every year in lost domestic product even though it could be controlled for a fraction of that sum (UNICEF 2004). In Africa, malaria causes approximately 20% of cerebral conditions leading to coma and death. One important strategy to prevent people from the risk of malaria infection is the use of insecticide treated nets (ITNs). Recent studies have shown that the use of bed nets especially ITNs can reduce both transmission and mortality by at least 25% when used properly (Sagoe-Moses 2005).

GENERAL OBJECTIVES: 1. Identify the causes of the disease. 2. To make recommendations to the Government and NGOs. 3. To create the awareness of the people on how the disease spreads.

SPECIFIC OBJECTIVES: To find a suitable model to represent the infection of malaria in the district. To forecast into the future base on pass records (Malaria Case).

To reveal the trend of the disease in the district.

METHODOLOGY: This chapter deals with the methods employed in collecting and analyzing data. The data comprises of malaria cases recorded monthly from year 2004 – 2008. The five (5) year duration was used because of the difficulty in acquiring data before the year 2004. The monthly information about the disease can be used to achieve the research objectives.

CONCLUSIONS: The results showed that malaria cases in the Savelugu/Nanton District have an increasing trend. That is the number of malaria cases for year 2009, 2010, and 2011 are 18980 cases, 21447 cases and 23914 cases respectively.
P004: Malaria Prevention and Control in Nigeria: Current Recommendations and Perspectives

Aliyu Samuel Abdulmumuni,1 Abubakar Sa’idu,1 and Yusuf Abdullahi1
Departments of Pathology,1 and Medicine2 Federal Medical Centre, Gombe, Gombe State, Nigeria.

There is an increasing global interest in malaria eradication but the recent resurgence in many parts of Nigeria suggests that the principal strategies behind the global campaign to eliminate malaria are fundamentally flawed. To date Nigeria is far from achieving the Roll Back Malaria (RBM) partnership’s target as stated in the United Nations Millennium Development Goals (MDGs). Only 31% of children below the age of five and 36% of pregnant women sleep under bed nets. Only a third of small children received an anti-malaria drug when they last had fever and 13% of pregnant women had received at least two doses of intermittent preventive treatment. Drawing from the extant literature, we note that successful interventions exist in Nigeria but this has been negatively influenced by the complicated system of healthcare delivery. Against this background, this paper explores the current control strategies employed at eradicating malaria while giving broad yet detailed reasons why malaria control has largely failed. In order words, we argue for the increased realization that the success of any intervention entails a careful re-think of the ideas and guidelines underlying our current preventive and management strategies, and this must come at the intersection of an informed policy development.

P005: Haematological and Biochemical Studies of Co-administration of Artemether and Lumenfantrine in Non-Malaria Infected Rats

Abiodun Humphrey Adebayo, Chioma Uchechukwu Mbogu, Adebosayo Odunlami
Department of Biological Sciences, College of Science and Technology, Covenant University, Canaan land, Ota, Ogun State, Nigeria.

BACKGROUND : Artemisinin combination therapy (ACT) is recommended as first-line treatment regimen for P. falciparum malaria and for mixed plasmodial infections. Despite these official recommendations, ACT is used in many areas for the treatment of all forms of malaria including non-falciparum infections. In sub-Saharan countries, the in-take of ACT is becoming alarming, most of which are purchased without adequate prescription and laboratory analyses. Our study was conducted to investigate the effects of biochemical and haematological parameters of co-administration of artemether and lumenfantrine, one of the recommended ACTs in non-malaria infected rats.

METHOD: The effects of artemisinin derivatives (artemether and lumenfantrine) on the haematological and biochemical parameters in albino wistar rats were studied. Thirty two (32) albino wistar rats were distributed into four groups. Animals in Group A were administered with Tween 80 and served as the control group. Groups B, C, and D were administered artemether (8mg/kg bw), lumenfantrine (48mg/kg bw) and artemether/lumenfantrine (8/48mg/kg bw) through gastric intubation. Half of the animals in each group were sacrificed after 3 days of treatment and the remaining half after 6 days of administration. Animals were subsequently anaesthetized in diethyl ether, blood samples and liver organ were collected. The following biochemical and haematological parameters were assessed in blood and liver homogenate: glucose, glucose 6 phosphate dehydrogenase (G6PD), glutathione S-transferase (GST), superoxide dismutase (SOD), malonyl dialdehyde (MDA), catalase (CAT), packed cell volume (PCV), haemoglobin (Hb), white blood cell count (WBC), neutrophil, leukocyte, eosinophil, monocyte and basophil.

RESULTS: The results after 3 days of treatment showed significant decreases (p < 0.05) in glucose, GST, MDA, CAT, PCV, Hb and WBC levels. Furthermore, after 6 days of treatment, there was significant reduction (p < 0.05) in the activity of CAT and GST.

CONCLUSION: It could be concluded that the administration of artemether and lumenfantrine could induce hypoglycaemia, stimulate the generation of free radical systems, reduce haematological indices, while increasing G6PD activity in non-malaria infected rats.

P006: Quantitative Whole Genome Resequencing and Genetic Linkage Analyses To Identify Genes Controlling Virulence in Malaria Parasites

Megumi Inoue1,2, Hussein Abkall1, Phonepadith Xangsayarat1, Paul Hunt1, Augustin Zounggrana1, Hayato Mitaka1, Osamu Kaneko1, Ville Mustonen1, Andrej Fischer2, Chris Illingworth3, Axel Martinelli3, Hayo Y. Shwen3, Arnab Pain4, Richard Culleton2
1Malaria Unit, Institute of Tropical Medicine (NEKKEN), Nagasaki University, Nagasaki, Japan; 2Department Protozoology, Institute of Tropical Medicine (NEKKEN), Nagasaki University, Nagasaki, Japan; 3Institute of Immunology and Infection Research, University of Edinburgh, Edinburgh, UK; 4Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Cambridge, United Kingdom; 5Computational Bioscience Research Center, Chemical Life Sciences and Engineering Division, King Abdullah University of Science and Technology, Thuwal, Kingdom of Saudi Arabia

BACKGROUND: Malaria parasites exhibit genetically controlled differences in their intrinsic virulence, or pathogenicity, to their hosts. These differences are most apparent between species, but are also manifest at the strain level. Discovering the genetic determinants of virulence differences between strains will enable the design of anti-virulence measures which could lead to a reduction in the severity of malaria infections, and protect against severe disease.

METHODS: Using the rodent malaria parasite Plasmodium yoelii, we identified parasite strains that differ in their virulence (i.e. the severity of disease they cause in mice). Genetic crosses were then performed with these strains, and the resulting progeny grown in mice so that the fast growing, virulent parasites outcompeted the slower growing, less virulent parasites. Whole genome sequencing at greater than 200 times coverage was then performed on both parental strains, and on the progeny pre- and post-selection, approximately 30,000 SNP markers quantified and genomic regions under growth rate selection were identified.

RESULTS: We show that a major difference in growth-rate phenotypes between two P. yoelii strains is controlled exclusively by a gene on chromosome 13, the most likely candidate being Pye1. We are currently performing replacement transfection of this allele in order to formally prove the involvement of this gene in the virulence phenotype.

CONCLUSIONS: Combining the rodent malaria system, classical genetic linkage analyses and next generation sequencing technologies offers a powerful approach to dissecting the genetic determinants of medically important phenotypes in malaria parasites.
P007: The effect of agriculture on the insecticide resistance in the malaria vector Anopheles arabiensis in Khartoum State/ Sudan

Sara Aboelmaali

BACKGROUND: Agricultural pesticides may play a profound role in selection of resistance in field populations of mosquito vectors. The objective of this study is to investigate possible links between agricultural pesticide use and development of resistance to insecticides by the major malaria vector Anopheles arabiensis in northern Sudan.

METHODOLOGY/RESULTS: Entomological surveys were conducted during two agricultural seasons in six urban and peri-urban sites in Khartoum state. Agro-sociological data were collected from 240 farmers subjected to semi-structured questionnaires based on knowledge attitude and practice (KAP) surveys. Susceptibility status of An. arabiensis (n=6000) was assessed in all sites and during each season using WHO bioassay tests to DDT, deltamethrin, permethrin, bendiocarb and bendiocarb. KAP analysis revealed that pesticide application was common practice among both urban and peri-urban farmers, with organophosphates and carbamates most commonly used. Selection for resistance appears potentially greater in peri-urban sites where farmers apply pesticide more frequently and are less likely to dispose of surpluses correctly. Though variable among insecticides and seasons, broad-spectrum mortality was slightly, but significantly higher in urban than peri-urban sites and most marked for bendiocarb, to which susceptibility was lowest. An. arabiensis from all sites showed evidence of resistance or suspected resistance, especially pyrethroids. However, low-moderate frequencies of the 1014F kdr allele in all sites, which was very strongly associated with DDT, permethrin and deltamethrin survivorship (OR=6.14-14.67) suggests that resistance could increase rapidly.

CONCLUSIONS: Ubiquitous multiple-resistance coupled with presence of a clear mechanism for DDT and pyrethroids (kdr 1014F) in populations of An. arabiensis from Khartoum-Sudan suggests careful insecticide management is essential to prolong efficacy. Our findings are consistent with agricultural insecticide use as a source of selection for resistance and argue for coordination between the integrated vector control program and the Ministry of Agriculture to permit successful implementation of rational resistance management strategies.

P009: Trends in the prevalence of Artemether lumefantrine resistance associated alleles within Pfmdr1 and Pfcr1 genes in Kenya

Angela O. Achien1,2, Walter Jura3, Edwin Kamau4

1Global Emerging Infections Surveillance (GEIS) Program, United States Army Medical Research Unit-Kenya (USAMRU-K)/ Kenya Medical Research Institute (KEMRI) - Walter Reed Project, Kisumu, Kenya; 2 Department of Zoology, Maseno University, Kisumu, Kenya

BACKGROUND: Artemether-lumefantrine (AL) is the most widely used Artemisinin-based combination therapy (ACT) in Africa, introduced in Kenya in 2006. AL selects for the pfcr1 76K, pfmdr1 86N, 184F, and 1246D alleles, whereas only Pfmdr1 184F is mutant-type. These alleles are linked to increased sensitivity to chloroquine and amodiaquine, but decreased sensitivity to lumefantrine, halofantrine, mefloquine, and artemisinins. Pfmdr1 1034C, 1042D and increased copy number are also associated with ACTs reduced sensitivity, observed outside of Africa. This study purposed to ascertain the change in prevalence of polymorphisms in pfcr1 and pfmdr1 genes in Kenya 2008-2013. In addition, correlation between AL in vitro activity and parasite genetic polymorphism was assessed.

METHODS: Single nucleotide polymorphisms in Pfcr1 76, Pfmdr1 86, 184, 1034, 1042 and 1246 were determined for 333 field isolates by real-time PCR, allelic discrimination assay and/or sequencing. Pfmdr1 copy number was determined using real-time PCR. Immediate ex vivo or in vitro susceptibility were determined using SYBR green I dye assay.

PRELIMINARY RESULTS: Pfmdr1 86Y decreased from 45% to 18% while 184F, 1034C, 1042D increased from 21% to 60% (P < 0.001), 0% to 5% and 0% to 4% during the study period respectively. Pfmdr1 1246D was 100% with no SNP and Pfmdr1 copy number was 1 for all the assayed samples. Pfcr1 76T decreased from 72% in 2008 to 15.6% in 2013 (P<0.001). Artemether median IC50s were (Interquartile range [IQR]) 8.7 (5.8-21.0) nM to 5.7 (3-10) nM and Lumefantrine, were 41.9 (24-63.0) nM in 2008 and 12.5 (8.6-49.2) nM. Artemether reduced in vitro response correlated positively with Pfmdr1 86N allele (R² = 0.70).

CONCLUSION: These in vitro findings indicate increasing tolerance to AL in Kenya. However we recommend further clinical monitoring of the efficacy of ACTs for a comprehensive assessment of artemisinin resistance.
P010: The role of signal transduction in mating of Anopheles gambiae provides a putative target for vector reproductive control in the mosquito.

**Daniel A. Achinko**, Paul Mirejii1,2, Daniel Masiga1, Flaminia Catteruccia1,2,3
1 International Centre of Insect Physiology and Ecology (icipe), Nairobi, Kenya; 2 Egeerton University, Njoro, Nakuru, Kenya; 3 Kenya Polytechnic University College, Nairobi, Kenya; 4 Universita' degli Studi di Perugia, Dipartimento di Medicina Sperimentale e Scienze Biochimiche, Terni, Italy; 5 Harvard School of Public Health, Boston, Massachusetts 02115

**BACKGROUND:** Reproduction as a vital process in living organisms leads to the formation of the mating plug in the mosquito Anopheles gambiae. This plug derives from male accessory glands (MAGs) proteins and transferred to the female during reproduction hence initiating post-mating effects (ovulation, oviposition and lifetime refractoriness to mating) in the female mosquito. The individual functions of mating plug proteins and their post-mating effects in the female during mating are still to be identified. This study presents proteomic analysis on the function of a female Trypsin-like serine protease (AGAP005195) whose interaction with specific plug proteins could putatively drive post-mating phenotypes in the female hence a putative target for vector control.

**OBJECTIVE:** Molecular characterization and expression analysis of the main Trypsin-Like Serine Protease (AGAP005195) secreted in the atria of the female Anopheles gambiae mosquito and involved in the process the mating plug.

**METHODS:** Anophelines gambiae mating plug sequences were obtained from Vectorbase and their Pfam domains predicted through the Geneious software. Male and female mosquitoes of the G3 strain were sexed as pupae and kept to emerge as virgins in 30 x 30 x 30cm cages. They were mated as 3 day old adults and females were later dissected for the presence of the plug in the atria and sperm in the spermatheca. Specific Trypsin-like serine proteases (AGAP005194 and AGAP005195) were stained with specific fluorescent antibodies, mounted in dapi stain and observed on the microscope for their localizations within the cell. RNA extraction from atrial and spermathecal tissues in the female were used for cDNA synthesis, qRT-PCR, RT-PCR analysis and sequencing of AGAP005195.

**RESULTS:** Pfam domain search predicted a Response regulator on AGAP005195. Immunostaining specific AGAP005195 to the plug and that it was only secreted after mating flowing from the atria to the spermatheca. Sequencing analysis identified three variants of the AGAP005195 transcript with two functional variants identified solely in the spermatheca. It showed that these variants resulted from alternative splicing and a non synonymous mutation at the G200S amino acid position deviates this protease from the suggested Trypsin related function. Expression variations of the AGAP005195 transcript in the atria of the experimental against the control after ATP injections shows that this transcript could be involved in signal transduction events in the female mosquito.

**CONCLUSION:** The specificity of AGAP005195 on the plug makes it specific to mating in the female and could be responsible for driving post-mating phenotypes in the female. Therefore RNAi studies on AGAP005195 transcript will better elucidate the functional pathway for this transcript and hence provide insight to further targeting it for vector reproductive control in the female Anopheles gambiae mosquito.

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**Akyala Ishaku Adamu, Adebola Olayinka, Patrick Nguku**
Nigeria Field Laboratory Epidemiology Training Program, Abuja, Nigeria.

**BACKGROUND:** Malaria rapid diagnostic test (RDT) is an antigen capture assay that enables rapid diagnosis of malaria without the need for electricity or highly skilled technicians. Though potentially useful, its adoption needs to be guided by local test sensitivity. Objective: To evaluate the diagnostic performance of a commercially available RDT (Malaria PF rapid device, Biotech Laboratories Limited, United Kingdom) among febrile children in Nasarawa state, Nigeria.

**METHODS:** This was a prospective observational study involving 400 children (aged 6months to 12years) who presented to the Paediatric Outpatient Department (POD) of Dalhatu Araf Specialist Hospital, Lafia with fever between March and October, 2009. Finger prick blood samples were collected from each of the patient (day 0) and immediately tested for falciparum malaria by both Giemsa microscopy and rapid diagnostic test (RDT). Patients with positive RDT and microscopy on day 0 were simultaneously retested on day 7 (after antimalarial therapy) by both diagnostic methods.

**RESULTS:** The prevalence of malaria among the study cohort was 40.8% by microscopy and 39.5% by RDT. The RDT had a sensitivity of 90.2% and specificity of 95.4%; with positive and negative predictive values of 93.0% and 93.4% respectively. Test accuracy was 95.3%, whereas reliability was 85.3%. Test sensitivity is reduced by low parasite density (100% at > 1600/µl Vs 69.2% at <800/µl). Of the 69 patients who were retested on day 7 after antimalarial treatment, 18 (26.1%) still had positive RDT test even though negative by microscopy and afebrile at the time of follow up.

**CONCLUSION:** The diagnostic performance of the RDT in this study is good. Hence, it is recommended as an alternative method for diagnosis of malaria, especially when microscopy is not feasible.

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P012: Antimalarial potential of kolaviron, a biflavonoid from Garcinia kola seeds, against Plasmodiumberghei infection in Swiss albino mice.

**Oluwatosin Adaramoyo1, Tolulope Akinpelu2, Ayokulehin Kosoko1, Patricia Okorie1, Aderemi Kehinde2, Catherine Falade4,5, Daniel Masiga1,2,3, Daniel Achinko1,2**
1 Departments of Biochemistry, 2Pharmacology and Therapeutics, 3Medical Microbiology, 4Institute for Advanced Medical Research and Training, College of Medicine, University of Ibadan, Ibadan, Nigeria.

**BACKGROUND:** Garcinia kola is commonly eaten for medicinal purpose in Nigeria. One of its active components is a biflavonoid called Kolaviron (KV) which has been reported to elicit antihelipotoxic, antioxidative, antioxidant and antiviral properties. We investigated the antimalarial potential of KV against Plasmodium berghei (P. berghei) infection in Swiss albino mice. Chloroquine (CQ) served as standard antimalaria.

**RESEARCH QUESTION:** Would intake of KV suppressed the growth of P. berghei and also alleviate malaria-induced anaemia in the mice?

**METHODS:** The study consists of seven groups of ten mice each. Groups I, II and III were normal mice that received corn oil, KV1 and CQ, respectively. Groups IV, V, VI and VII were infected mice that received corn oil, CQ, KV1 and KV2, respectively. CQ, KV1 and KV2 were given at 10-, 100- and 200-mg/kg daily, respectively for three consecutive days.

**RESULTS:** Administration of KV1 and KV2 significantly (p<0.05) suppressed P. berghei-infection in the mice by 85% and 90%, respectively, while CQ produced 87% suppression after the fifth day of treatment. Also, KV2 significantly (p<0.05) increased the mean survival time of the infected mice by 175%. The biflavonoid prevented a drastic reduction in PCV from day 4 of treatment, indicating its efficacy in ameliorating anaemia. Significant (p<0.05) oxidative stress assessed by the elevation of serum and hepatic malondialdehyde (MDA) were observed in untreated P. berghei-infected mice. Specifically, serum and hepatic MDA levels increased by 93% and 78%, respectively in the untreated infected mice. Antioxidant indices, viz; superoxide dismutase, catalase, glutathione-s-transferase, glutathione peroxidase and reduced glutathione decreased significantly (p<0.05) in the tissues of untreated P. berghei-infected mice. KV significantly (p<0.05) ameliorated the P. berghei-induced decrease in antioxidant status of the infected mice.

**CONCLUSIONS:** This study shows that kolaviron, especially at 200 mg/kg, has high antimalarial activities in P. berghei-infected mice, in addition to its known antioxidant properties.
BACKGROUND: Malaria is endemic in Ghana and it is a major cause of morbidity. The insecticide treated nets have proven to be one of the most efficient ways of preventing malaria in both adults and children. About 12.8 million long lasting insecticide nets (LLIN) were distributed in all ten regions of the country by the Ghana Health service and its collaborators between December 2010 and August 2012 through a universal mass distribution campaign. The campaign was implemented in all 10 regions plus the capital city of Accra; with volunteers also supporting the campaign through conducting behavior change communication, pre-registration of persons and their sleeping places, door to door distribution and hanging of the LLINs and a post distribution “keep up” campaign to ensure the use of the nets.

This evaluation was in three of the region, Brong Ahafo, Central and Western Regions to ascertain the effectiveness of the campaign in increasing ownership and use by the population and also to evaluate the value of the hung up activities to inform future campaigns.

METHODS: The evaluation employed a mixed-methods approach. Quantitative data on key net indicators was collected through a two-stage cluster household survey while qualitative data was collected through focus group discussions and in-depth interviews with key stakeholders involved in campaign implementation.

Results: Findings indicate that there are improvements in ownership and use of ITNs and this is attributable to the hung up strategy. Both implementers and volunteers believed that BCC was successful and impactful. The registration phase was crucial but not without challenges. Effective training and the importance of literacy of volunteers, effective supervision by implementers, understanding the validation of registration data, compensation for volunteers, time lapse between registration and distribution, complaints of invasion of recipients’ privacy were some issues that impacted volunteers’ work and the campaign.

CONCLUSION: The role of volunteers for the success of such campaigns is significant. Issues affecting their input and performance in such campaigns must be considered to improve success if such campaigns will be replicated.

P015: Prevalence and Severity of Anaemia among Children Under Five in Nigeria: Results from a National Malaria Indicator Survey

Samson B. Adebayo1, Dayo Arogundade2, Ezra Gayawan3

1Planning, Research & Statistics Directorate, National Agency for Food and Drug Administration and Control, Wuse Zone 7, Abuja, Nigeria; 2PLAN-Health Project, Management Sciences for Health, Abuja, Nigeria; 3Department of Mathematical Sciences, Redeemer’s University, Redemption City, Ogun, Nigeria

BACKGROUND: This paper describes the epidemiology and analyzes factors associated with iron deficiency anaemia in children under 5 in Nigeria in a population-based household survey of women of reproductive age 15–49 years. In addition to other objectives, this survey aimed at measuring the prevalence of malaria and anaemia among children age 6-59 months

METHODS: Data for this paper was based on 2010 Nigeria Malaria Indicator Survey, a household-based population survey. All women age 15-49 in the selected households were eligible for individual interviews. Sample was selected using a stratified, two-stage cluster design of urban and rural areas. A representative sample of approximately 6,000 households was selected, with a minimum target of 920 individual women’s interviews per zone. Within each state, the number of households was distributed proportionately among urban and rural areas. The survey incorporated anaemia and malaria testing using RDTs, and thick blood smear and thin blood film sample preparation on microscope slides. In addition, all children age 6-59 months were eligible to be tested for malaria and anaemia. Two dependent variables: binary (anaemic or normal) and ordinal (measuring severity of anaemia as: severe, moderate, mild or normal) were created from the haemoglobin level of the children.

RESULTS: Without controlling for possible factors that are suspected to be associated with anaemia, findings revealed a clear-cut North-South divide in prevalence of anaemia in Nigeria. Similarly, the net effect of spatial effect remained significant after controlling for possible factors that are assumed to be associated with anaemia level. These findings can guide policymakers in identifying states or districts that are associated with significant risk of anaemia among children under five. This can in turn be used in designing effective strategies and in decision making. These findings can also point in the direction of effective utilisation of scarce resources: a major challenge to those effecting interventions in developing countries.

Samson B. Adebayo1, Ezra Gayawan2, Dayo Arogundade3
1 Planning, Research & Statistics Directorate, National Agency for Food and Drug Administration and Control, Wuse Zone 7, Abuja, Nigeria; 2 Department of Mathematical Sciences, Redeemer’s University, Redemption City, Ogun, Nigeria; 3 PLAN-Health Project, Management Sciences for Health, Abuja, Nigeria

BACKGROUND: Everyone living in all parts of Nigeria is at risk of malaria infection. Plasmodium falciparum accounts for 90-95% of malaria infections in Nigeria. The geographical location of Nigeria makes the climate suitable for malaria transmission throughout the country. Transmission of malaria occurs throughout the year with the intensity higher in the southern parts of the country because of the longer rainy season that favours the breeding of mosquitoes. Therefore, this paper explores possible geographical variations in the prevalence of malaria in Nigeria among children under 5.

METHODS: Data for this paper was based on 2010 Nigeria Malaria Indicator Survey, a household-based population survey. All women age 15-49 in the selected households were eligible for individual interviews. During the interviews, they were asked questions about malaria prevention during pregnancy and the treatment of fever among their children. In addition, the survey included testing for anaemia and malaria among children age 6-59 months using finger (or heel) prick blood samples. Test results were available immediately and were provided to the children's parents or guardians. Thick blood smears and thin blood films were also made in the field and microscopy to determine the presence of malaria parasites and to identify the parasite species. Slide validation was carried out for quality assurance. We adopt Bayesian spatial analysis for modelling possible correlates of malaria prevalence.

RESULTS: Antibody response to GMZ2 was 3.4-fold (95% confidence interval: 1.6, 7.4) higher in Trichuris trichiura negative subjects compared to Trichuris trichiura positive subjects. Memory B-cell response was moderately increased in T. trichiura negative individuals, although the difference was not significant. Vaccine-specific antibody concentrations and memory B-cell response against the vaccine antigens and parasitological status were assessed. Vaccine-specific antibody concentrations and memory B-cell numbers were compared in worm infected and non-infected participants.

CONCLUSION: The result shows a high prevalence of P. falciparum and urinary schistosomiasis co-infections among children with considerable degree of anaemia in rural communities of Nigeria.

P018: Reduced antibody responses against Plasmodium falciparum vaccine candidate antigens in the presence of Trichuris trichiura.

Meral Esen1,2, Benjamin Mordmüller1,2, Pablo Martinez de Salazar1, Ayola, Akim Adegbe1,2, Selidji Todagbe Agnandji1,2, Frieder Schaumburg1, Aurore B. Hounkpatin1,2, Sina Brückner3, Michael Theisen1,2, Sabine Bélard1, Ulysse Ateba Ngoa1, Saadou Issifou1,2, Maria Yazdanbaksh1,3, Peter G. Kremsner1,2
1 Centre de Recherches Médicales de Lambaréné, BP: 118 Lambaréné, Gabon 2 Institute of Tropical Medicine, University of Tübingen, Tübingen, Germany 3 Leiden University Medical Center, Department of Parasitology, Leiden 2300, Netherlands 4 Department of Clinical Biochemistry and Immunology, Statens Serum Institut, Copenhagen, Denmark 5 Centre for Medical Parasitology at Department of International Health, Immunology and Microbiology, University of Copenhagen, Copenhagen, Denmark

BACKGROUND: Helminth infections are highly prevalent in the tropics and may have an effect on immune responses to vaccines due to their immunomodulatory effect. The prevalence of helminth infections in young children, the target group for malaria and most other vaccines, is high. Therefore we assessed the influence of helminth infection on vaccine-induced immune responses in a phase I clinical trial of the malaria vaccine candidate GM22.

METHODS: Twenty Gabonese preschool-age children were vaccinated with GM22, a blood stage malaria vaccine candidate. Humoral immune response against the vaccine antigens and parasitological status were assessed. Vaccine-specific antibody concentrations and memory B-cell numbers were compared in worm infected and non-infected participants.

RESULTS: Antibody response to GM22 was 3.4-fold (95% confidence interval: 1.6, 7.4) higher in Trichuris trichiura negative subjects compared to positive participants, whereas immunoglobulin subclass distribution was similar. Memory B-cell response was moderately increased in T. trichiura negative individuals, although the difference was not significant.

CONCLUSIONS: Future malaria vaccine development programs need to account for worm-mediated hyporesponsiveness of immune reactions.

P017: P.falciparum and Schistosomiasis co-infections among children in rural communities of Kwara State, Nigeria.

D Adefokun

BACKGROUND: Malaria and helminthes are parasitic diseases causing high morbidity and mortality in most tropical parts of the world, where climatic conditions and sanitation practices favor their prevalence. This study determine the prevalence and impact of malaria schistosomiasis co-infection in rural communities in northern parts of Nigeria.

METHODS: A total of 1,024 children age range 4-12 years from primary schools located in rural communities of Kwara state Nigeria were enrolled. Blood was collected for the detection of malaria parasite using thick film methods and also for PCV determination. Urine was collected for the detection of Schistosoma haematobium.

RESULT: 196 (19.1%) were positive for P. falciparum, 450 (44.0%) were positive for S. haematobium while 104(10.2) had P. falciparum and S. haematobium co-infections. Low PCV (17-28%) was observed in 108 children of which 51% and 79% were positive for malaria and schistosomiasis respectively. Normal PCV (>34%) was observed in 359 children of which 7.8% and 28.1% were positive for malaria and schistosomiasis respectively. Differences in prevalence between sexes were significant in both malaria and schistosomiasis (p<0.05).

CONCLUSION: The result shows a high prevalence of P. falciparum and urinary schistosomiasis co-infections among children with considerable degree of anaemia in rural communities of Nigeria.
P019: Effect of co-administration of artesunate on the disposition of amodiaquine in patients treated for uncomplicated falciparum malaria

O.N. Adejedi1, 2, O. Osonuga3, C.O. Falade4, 2, O.O. Bolaji2, O.G. Ademowo1, 2
1Department of Pharmacology & Therapeutics, University of Ibadan, Ibadan, Nigeria; 2Department of Pharmacology, Obafemi Awolowo University, Sagamu, Nigeria; 3Institute for Advanced Medical Research and Training, College of Medicine, University of Ibadan, Ibadan, Nigeria; 4Department of Pharmaceutical Chemistry, Obafemi Awolowo University, Ile-Ife, Nigeria

BACKGROUND: Artemisinin-based combination therapy has been adopted by several African countries as first line treatment for uncomplicated falciparum malaria. With this comes the need to balance the advantages of the combination against the possible effect of interaction between the component drugs. Therefore, we investigated the effect of co-administration of artesunate (AS) on the disposition of amodiaquine (AQ) in patients treated for uncomplicated falciparum malaria.

METHODS: This is a randomised, open-label trial in which twelve patients with P. falciparum infection were assigned into two treatment arms namely, AQ alone or in combination with artesunate (AQ/AS). AQ (600mg) or a fixed dose combination of AQ/AS (AQ 306.3 mg/AS 100 mg × 2 tablets) was administered once daily for 3 days. Blood samples were collected at pre dose and at 0.5, 1, 2, 4, 6, 12, 24, 48, 72, 144, 336 h post-treatment. Patients were followed up for 28 days to assess response to treatment. Plasma was obtained and assayed for AQ and DAQ levels using a high performance liquid chromatography technique. The pharmacokinetics parameters of AQ and DAQ were determined by non-compartmental analysis using the computer program WinNonlin Version 5.0.

RESULTS: There were higher peak plasma concentration, C max (22.7±0.01 vs. 20.43±0.12 ng/ml) and area under the concentration-time curve, AUC (59.63±0.05 vs. 57.52±0.24ng/ml), longer elimination half life, T ½ (2.8±0.02 vs. 2.69±0.01 h) for AQ in combination group (AQ/AS) when compared with AQ alone (P<0.05). AQ oral clearance, Cl/F (9729.5±7.61 vs. 9857.2±4.25 ml/h) was less rapid (P<0.05) in AQ/AS group relative to AQ monotherapy. Time to peak plasma concentration, T max was two hours in the two groups. The pharmacokinetic parameters of DAQ were however similar in both treatment groups. Parasites cleared in all patients in the two groups by day 2 on the average except in a patient in AQ group in whom parasites were seen on Day 14.

CONCLUSION: The study showed that artesunate affected the disposition of amodiaquine and not desethylamodiaquine when AQ/AS was orally administered in patients with malaria as the bioavailability of AQ was significantly increased. The total exposure to DAQ was reduced but not significantly in patients treated with Q/AS.

P020: First report of bendiocarb resistance in Anopheles gambiae s.l. population in Benin: a threat for malaria vector control

Rock Yves Alkpon

BACKGROUND: Face to the resistance of An. gambiae to pyrethroids, carbamates and organophosphates insecticides are regarded as alternatives or supplements to pyrethroids used in nets treatment. Resistance monitoring is therefore essential to investigate on the susceptibility of An. gambiae s.s. to these alternative products.

METHODS: Two – three day old female adult Anopheles mosquitoes reared from larval collections in five districts (Kouandé, Natitingou, Matéri, Pêhunco, Tanguêta) from the department of Atacora in Benin were exposed to WHO test papers impregnated with carbamates (0.1% bendiocarb, 0.1% propoxur) and organophosphates (0.25% pirimiphos-methyl, 1% fenitrothion). Then, PCR assays were run to determine the members of the An. gambiae complex, the molecular forms (M) and (S), as well as phenotypes for insensitive acetylcholinesterase (AChE1) due to ace-1 mutation.

RESULTS: Bioassays showed bendiocarb resistance in all tested populations of An. gambiae s.s. Propoxur resistance was observed in Matéri, Pêhunco and Tanguêta, while it was suspected in Kouandé and Natittingou. As for the organophosphates, susceptibility to pirimiphos-methyl was assessed on all populations. Fenitrothion resistance was detected in Kouandé, Pêhunco and Tanguêta, while it was suspected in Matéri and Nattingou. The S-form was predominant in tested samples (94.44%). M and S molecular forms were sympatric but no M/S hybrids were detected. The ace-1a mutation was found in both S and M molecular forms with frequency from 3.6 to 12%. Although the homozygous resistant genotype was the most prevalent genotype among survivors, the genotypes could not entirely explain the bioassay results.

CONCLUSION: Evidence of bendiocarb resistance in An. gambiae populations is a clear indication that calls for the implementation of insecticide resistance management strategies. The ace-1a mutation could not entirely explain the resistance to bendiocarb observed and highly suggests involvement of other resistance mechanisms such as metabolic detoxification.

P021: Prevalence and interaction of malaria and helminth co-infections among symptomatic and asymptomatic children in Southwest Nigeria

George Ademowo1, Olawunmi Rabiu2, Ayokulehin Kosoko2, Hannah Dada-Adegbola3, Ganiyu Alola1
1Institute for Advanced Medical Research and Training, College of Medicine, University of Ibadan, Ibadan, Nigeria; 2Department of Zoology, 3Department of Medical Microbiology; 4Department of Chemical Pathology, College of Medicine, University of Ibadan, Nigeria.

INTRODUCTION: Malaria and intestinal helminth infections are common tropical diseases in developing countries. Little is understood about their interaction when they coexist. Some workers reported that helminth infected individuals are susceptible to plasmodium infection while others did not. We therefore investigated the effect of co-infection of helminth and Plasmodium infections among children.

METHODS: Asymptomatic school children (304) with age range 3-10 years and febrile children (495) with age range 1- 10 years were recruited from selected primary schools and out-patient clinic of Adeyio Hospital, Ibadan, Nigeria. Blood samples were obtained from each subject and used for haematocrit determination and Giemsa stained smears that were used for malaria parasite screening by microscopy. Stool samples were also collected and used for helminth diagnosis done by Kato-Katz method. All subjects were clinically examined and personal details documented in a case record forms.

RESULTS: Among the school children, 142 (46.7%) were positive for malaria, 181 (59.5%) had helminth only (Ascaris lumbricoides, AL - 43.1%, Trichuris trichiura TT -2.3% and AL/TT - 14.1%), while 57 (18.8%) had co-infection of helminth and Plasmodium. Among the febrile children, 116 (23.4%) were positive for malaria, 45 (9.1%) for worms only (AL- 7.3%, TT- 0.2%, AL/TT- 1.4%, Taenia spp- 0.2%) while 16 (3.2%) had co-infection of malaria and helminth. Among asymptomatic children, Plasmodium infection was significantly (P<0.05) reduced in helminth positive relative to helminth negative. Whereas the opposite was the case among febrile children, as Plasmodium infection was increased in helminth positive relative to helminth negative. Anaemia was significantly higher in Plasmodium infection alone compared with those with helminth infection. A. lumbricoides was the most prevalent helminth.

CONCLUSION: The prevalence of helminth and Plasmodium coinfecion was markedly higher among asymptomatic than symptomatic children. Plasmodium was negatively and positively associated with helminth infection in asymptomatic and febrile children respectively. Work on the immunological interplay during the course of the infections is still in progress.
P022: Awareness, accessibility and use of malaria control interventions among at-risk groups in Lagos metropolis, Nigeria

Adeneye AK1, Osai PO2 and Awolola TS1
1Public Health Division, Nigerian Institute of Medical Research, Lagos, Nigeria; 2Department of Public Health, Kent State University, Kent

INTRODUCTION: With two years to the MDGs deadline, there is limited evidence of decreases in malaria-related mortality and morbidity in Nigeria. We therefore evaluated the awareness, accessibility and use of malaria control interventions among at-risk groups in Lagos metropolis, Nigeria.

METHODS: A descriptive, cross-sectional pilot study of consented 19 pregnant women and 61 mothers of under-five children was carried out using a household survey questionnaire and observation. Data were analysed using Epilinfo 6.04a software.

RESULTS: All (100.0%) respondents identified mosquito as malaria vector. Respondents’ preferred drugs for malaria treatment were: sulphadoxine-pyrimethamine (31.3%); ACTs (20.2%); artemisinin monotherapies (15.0%); chloroquine (13.8%); and anthelminics (12.5%). Only (30.0%) had used ACTs, 55.0% of these on self-medication; 97.5% and 70.0% knew and had LLINs. From room observation, only 53.0% (31.5% mothers of under-five vs. 11.3% pregnant women) actually hanged LLINs for use. Reasons for non-use of LLIN included: “prefer house spraying” (28.8%) and “causes heat” (7.5%). LLIN use was positively associated with education (p<0.05), ranging from 50.0% (no education) to 77.8% (post-secondary). 41.8% got their LLINs through house-to-house mass distribution. With an average of 3-time wash within an average of 21.7 months of use, LLIN washing practices showed that 30.5% used toilet soaps compared to detergents and hard soaps (66.7%), and 19.4% sun-dried it. 52.6% and 42.1% of pregnant women knew IPTp and had received at least one dose with an average of two doses respectively. Pregnancy stage and how long the pregnant women had visited antenatal clinics significantly influenced their awareness of IPTp and whether they had received any IPTp dose or not (p<0.05).

DISCUSSION: Results showed high awareness but low and poor use of malaria control interventions in populations studied. Public health education on the different malaria control interventions need to be intensified among women, emphasising the significance of using prescribed recommended ACTs, dangers of self-medication, LLIN benefits and its appropriate washing and drying mechanisms, and benefits of IPTp for improved pregnancy outcomes. This is important if the malaria-related MDG targets are to be realized in the metropolis and in Nigeria in general.

P023: Baseline malaria vector transmission dynamics in communities in Ahafo mining area in Ghana

A Adeneye

BACKGROUND: Malaria vector dynamics are relevant prior to commencement of mining activities. A baseline entomology survey was conducted in Asutifi and Tano communities in the Brong-Ahafo geo-political region of Ghana during preparatory stages for mining activities by Newmont Ghana Gold Limited.

METHODS: Centre for Disease Control light traps were set weekly in randomly generated compounds to collect mosquitoes. Information on individual mosquito prevention measures were assessed for each indexed person in a room that a light trap was set.

RESULTS: Of the houses surveyed, 90% were roofed with corrugated zinc sheets, 4% with mud or logs and 6% with thatch. Majority (66%) of houses had no eaves. Mosquito bed nets coverage in Asutifi (49%) and Tano (47%) shows low coverage and of these 32% (Asutifi) and 36% (Tano) were treated with insecticides (long lasting insecticide nets) at the time of the survey. Other measures of preventing mosquito bites; 20% (coil), 7% (insecticide spray), 2% (repellent cream), 1% (smoky fire), demonstrate very low individual mosquito preventive measures. Mosquito abundance was influenced by type of roofing material (grass or thatch) to a large extent (1.7 Odds Ratio) and marginally by the presence of eaves (0.75 Odds Ratio). EIRs in communities in Asutifi (27-395 /ib/p/m) and Tano (24-659 /ib/p/m) depict relatively high transmission of malaria in Ahafo.

CONCLUSIONS: The survey shows areas to intensify vector control activities. It also demonstrates that transmission in Asutifi and Tano is high even before the commencement of mining operations. The study has built a platform for future vector control studies and interventions.

P024: Preliminary evidence of genetic differentiation between populations of Anopheles coluzzii within the forest ecological zone in Nigeria

Adedapo O. Adeogun1,2, Kehinde O.K. Popoola1, Lizzette L. Koekemoer3, Choi Kwang-Shik4, Abiodun K. Okaligbe5, Samson T. Awolola1
1 Molecular Entomology and Vector Control Research Laboratory, Nigerian Institute of Medical Research, PMB 2013, Ibadan, Lagos, Nigeria; 2 Department of Zoology, University of Ibadan, Ibadan, Nigeria; 3 Vector Control Unit, National Institute for Communicable Disease, Johannesburg, South Africa and the WITS Malaria Research Institute, School of Pathology, Faculty of Health Sciences, University of the Witwatersrand.

BACKGROUND: Patterns of Anopheles gambiae s.s. population have received particular attention as these are considered suitable sites for experimental trials on transgenic-based malaria control strategies. We studied the population structure of Anopheles coluzzi from the forest ecological zone in Southwest Nigeria and determined genetic variation at 10 microsatellite loci located on chromosome 2.

METHODS: Larval samples were collected from six localities each in Lagos and Oyo state separated by geographical distance ranging from 150 - 200km. PCR species identification was conducted on 360 female Anopheles mosquitoes (180 from each region) selected at random and subsequent identification of the An. coluzzi species.

RESULTS: All samples identified in the Lagos populations were An. coluzzi while the samples from Oyo state contained 40% of the species. To determine the effect of selection, loci were located within and outside chromosome inversions. Analysis of genetic differentiation using Fst estimate revealed that four loci (AG2H26, AG2H637, AG2H772 and AG2H703) located within inversion 2Rb and 2La are responsible for the differentiation between the two populations.

CONCLUSION: This observation suggests that genes within inversions in chromosome II counter the homogenizing effect of gene flow among populations of Anopheles coluzzi in South western Nigeria. This will seriously undermine the success of GMM in South west Nigeria if candidate genes are located within these inversions.
P025: Cytokines in Malaria-HIV co-infected mothers and their neonates post delivery

Adeoti O. Michael 1, 2, Awobode Henrietta 2 Anumudu I. Chika 2
1Cellular Parasitology Programme, Department of Zoology, University of Ibadan, Nigeria; 2Face Out Malaria and AIDS Foundation, The Polytechnic Ibadan, Saki Campus; 3Parasitology Unit, Department of Zoology, University of Ibadan, Nigeria

BACKGROUND: Cellular immune responses play a vital role in maintaining pregnancy as well as the well-being of the foetus in utero. The degree of parasitemia may have a modulatory effect on the integrity of cellular immune responses and the fate of the pregnancy. The present study investigated the impact of co-infection of malaria and HIV on the number of CD4 lymphocytes compared with WHO standards and cytokine levels in co-infected pregnant mothers and their babies after birth.

METHODS: A total of 149 mothers and 30 babies born to HIV-infected mothers were engaged in a longitudinal study for 18 months in the endemic area of Saki and followed-up for six months post-delivery. Rapid diagnostic tests kits were used for HIV diagnosis in mothers; HIV screening was conducted for the babies using polymerase chain reaction at six months post delivery. CD4+ counts of the patients were enumerated by using FACS count techniques. Four cytokines were profiled: TNF-α, IL-2, IL-10 and INF-γ by Enzyme Linked Immunosorbent Assay. The data generated were analyzed using student t-test for equality of means while ANOVA was used to test for the independence among quantitative variables of the mothers and babies by their class of infection at α-level of P<0.05.

RESULTS: A higher percentage of Ikeja and Mushin residents used Baygon while GO 90 was only widely used in Mushin LGA. The use of conventional insecticides increased with respondents’ level of education in both LGAs and vice-versa with GO 90. At 4ml/cage insecticidal exposure to mice, 33.3% mortality rate was recorded with GO 90 and 16.7% with Baygon. Weight loss was observed among animals exposed to GO 90 and weight gain in animals exposed to Baygon except at 4ml/cage. The hematological parameters of treated mice were within normal ranges except the white blood cell count. Histological examination showed Inflammation of the lung’s interstitial cell, pulmonary congestion, oedema and vacuolization of the kupffer’ cells.

DISCUSSION AND CONCLUSION: The most commonly used insecticides in the two Local Government Areas were Baygon and GO 90. This study highlights the health hazards associated with man’s frequent use of insecticides against mosquitoes, especially with the locally formulated insecticide, GO 90.

P026: Comparative effects of insecticides against mosquitoes in two Local Government Areas of Lagos State, Nigeria

Grace O. Adeoye and Olawale S. Farinar
Department of Zoology (Parasitology & Bioinformatics Unit), University of Lagos, Akoka, Yaba, Lagos, Nigeria

BACKGROUND: Malaria is the most significant public health problem in Nigeria. The spread of malaria is dependent on the survival of the vector, Anopheles, and the parasite, Plasmodium. Different control measures employed against mosquitoes include the use of insecticides such as Baygon and GO 90. Baygon is a conventional insecticide while GO 90 is a locally formulated insecticide. This study aimed to assess the frequency of usage and health risks of the two insecticides in two local government areas of Lagos State.

METHODS: A cross-sectional study was carried out on 350 randomly selected individuals in two Local Government Areas of Lagos State, Mushin and Ikeja. Structured questionnaires were administered to the individuals on the preventive measures employed against malaria. The questionnaires were analyzed statistically using SPSS. Values of less than 0.05 were regarded as statistically significant. Haematological and pathological effects of the two insecticides were investigated using the albino mice Mus musculus.

RESULTS: A higher percentage of Ikeja and Mushin residents used Baygon while GO 90 was only widely used in Mushin LGA. The use of conventional insecticides increased with respondents’ level of education in both LGAs and vice-versa with GO 90. At 4ml/cage insecticidal exposure to mice, 33.3% mortality rate was recorded with GO 90 and 16.7% with Baygon. Weight loss was observed among animals exposed to GO 90 and weight gain in animals exposed to Baygon except at 4ml/cage. The hematological parameters of treated mice were within normal ranges except the white blood cell count. Histological examination showed Inflammation of the lung’s interstitial cell, pulmonary congestion, oedema and vacuolization of the kupffer’ cells.

DISCUSSION AND CONCLUSION: The most commonly used insecticides in the two Local Government Areas were Baygon and GO 90. This study highlights the health hazards associated with man’s frequent use of insecticides against mosquitoes, especially with the locally formulated insecticide, GO 90.

P027: In vivo evaluation of a herbal decoction and its component plants for antimalarial activity

Awodayo O Adepiti 1, Anthony A Elujoba 1, Oluseye O Bolaji 2
1 Department of Pharmacognosy, Obafemi Awolowo University, Ile-Ife, Nigeria 2 Department of Pharmaceutical Chemistry, Obafemi Awolowo University, Ile-Ife, Nigeria

BACKGROUND: The use of decoctions of different plant materials is common practice in antimalarial ethnomedicine in Africa. Scientific evaluation of such herbal combinations to verify the claims is important. The antimalarial activities of MAMA Decoction (MD), a multicomponent herbal medicinal product, and its component plants: leaves of Morinda lucida Benth [Rubiaceae], Azadirachta indica A. Juss [Meliaceae], Alstonia boonei De Wild [Apocynaceae and Mangifera indica L [Anacardiaceae], were investigated in Plasmodium berghei-infected mice.

METHODS: Each decoction was prepared by boiling the powdered leaf in water, concentrated in vacuo and freeze-dried. The acute toxicity of MD was determined using Lorke’s method. Decoctions of MD and its component plants were prepared, concentrated in vacuo and freeze-dried. The freeze-dried extracts of MD and its component plants were evaluated using the 4-day antimalarial test model at 15–240 mg/kg, while the positive control, amodiaquine, was tested at 1.25–10 mg/kg. Distilled water was used as the negative control.

RESULTS: The median lethal dose (LD50) of MD was found to be 3.8 g/kg. Dose-dependent chemosuppressive activities were observed. The results were expressed as (ED90, ED50 [mg/kg]): MD (43, 202); Alstonia boonei (79, 202); Azadirachta indica (140, 291); Morinda lucida (134, 408); Mangifera indica (208,480) and AQ (9, 9.2).

CONCLUSIONS: This study represents the first report on the in vivo antimalarial activities of the aqueous extracts of the leaves of Alstonia boonei and Mangifera indica as single plant preparations. MD appeared relatively safe and demonstrated high chemosuppressive activity thus providing scientific credence to its ethnomedical usage. Its component plants have contributory roles in the observed antimalarial activity.
P028: In vivo anti-plasmodial screening of *Nicotiana tabacum* and its effects on hepatic and renal function in swiss albino mice

Adewale O.O.; Oyeniyi, T.T.; Famodimu, M.T.; Adejoba, A.D.; Orubima, C.V.; Adeleke, M.A.
1Biochemistry Unit, Department of Chemical Sciences, 2Department of Biological Sciences, Osun State University, Osogbo, Nigeria

BACKGROUND: Malaria remains one of the leading public health diseases in developing countries and efforts to keep the infection at bay have suffered a lot of setbacks compounded by the increasing cases of resistance and cross resistance to frontline antimalarial drugs. Data emanating from recent ethnobotanical studies in developing countries revealed paradigm shift in the choice of malaria treatment with preference for herbal decoctions. However, there is paucity of information on the efficacies and toxicities of these herbal plants. We investigated the anti-plasmodial efficacy of ethanolic and aqueous extracts of *Nicotiana tabacum* on *Plasmodium berghei* infected swiss albino mice and its effects on liver and kidney function.

METHODS: The ethanolic (10mg/ml) and aqueous (2%) extracts were administered *ad libitum* three days post inoculation of the mice with chloroquine susceptible *Plasmodium berghei* while kidney and liver tissues of the mice were analyzed for biochemical enzymes (Alkaline phosphatase (ALP), Aspartate transaminase (AST), Alanine transaminase (ALT) and markers (urea and creatine) after five days of consecutive treatment.

RESULTS: The comparison of changes in parasite load of the infected mice before and after treatment showed that the parasitaemia level reduced significantly (p<0.05) in the mice treated with both ethanolic and aqueous extracts of *N. tabacum*, while there was a significant (p<0.05) increase in parasitaemia level in the untreated mice. The activities of (ALP), (AST), (ALT) and creatinine concentration varied marginally in the treated groups but the values were statistically comparable with control group (untreated) (P>0.05). However, the concentration of urea was statistically higher in treated groups than the control (p<0.05).

CONCLUSION: Our results therefore demonstrate the anti-plasmodial potential of *N. tabacum* and its relative safety for human consumption at the tested doses.

P029: Antimalarial and toxicological activities of *Clerodendron polycephalum*

Adewoyin F. B., Odaibo A. B. Adewunmi, C. A. Omosore, N. O and Iwalewa G. E.

*Clerodendron polycephalum* is a medicinal plant used traditionally to treat malaria. The leaves were collected and investigated for effectiveness against *Plasmodium berghei* in vivo using mice. Using appropriate standard methods, prophylaxis, curative and suppressive activities were carried out. The methanolic extranolic extract showed significant antiplasmodial activity compared to standard drug used. Toxicity studies of methanolic extract was also done using haematological parameters. There are slight changes in body weights compared to the control. The control animals generally showed increase in body weights while the test animals had slight decrease and then increase. The incidence of the later increase could be inferred as possible recovery from any toxic activity of the extract. The use of *C. polycephalum* could be encouraged as a good herbal material against malaria.

P030: Barriers to community case management of malaria in Ghana

Shadrach Adu-Poku

BACKGROUND: Health workers in Ghana can now diagnose and treat malaria in the field, using rapid diagnostic tests and artemisinin-based combination therapy in areas without microscopy and widespread resistance to previously effective drugs.

OBJECTIVE: This study evaluates communities’ perceptions of a new community case management of malaria programme in the district of Ahafo-ano south in Ghana, the effectiveness of lay health worker trainings, and the availability of rapid diagnostic tests and artemisinin-based combination therapy in the field.

METHODS: The study employed qualitative and quantitative methods including focus groups with villagers, and pre- and post-training questionnaires with lay health workers.

RESULTS: Communities approved of the community case management programme, but expressed concern about other general barriers to care, particularly transportation challenges. Most lay health workers acquired important skills, but a sizeable minority did not understand the rapid diagnostic test algorithm and were not able to correctly prescribe artemisinin-based combination therapy soon after the training. Further, few women lay health workers participated in the programme. Finally, the study identified stock-outs of rapid tests and anti-malaria medication products in over half of the programme sites two months after the start of the programme, thought due to a regional shortage.

CONCLUSION: This study identified barriers to implementation of the community case management of malaria programme in Mim in Ghana that include lay health worker training, low numbers of women participants, and generalized stock-outs. These barriers warrant investigation into possible solutions of relevance to community case management generally.

P031: Phytoceutical with antimalarial efficacy isolated from *Zizyphus spina-christi* root bark

Bulus Adu1*, Abdu Kaita Haruna2, Ben Ahmed Chindo2,2, Karniyus Shingu Gamaniel1
1Department of Pharmacology and Toxicology, National Institute for Pharmaceutical Research and Development, Abuja, Nigeria; 2Department of Pharmaceutical and Medicinal Chemistry, Ahmadu Bello University, Zaria, Nigeria; 2Department of Pharmacology and Toxicology, Faculty of Pharmacy, Kaduna State University, Kaduna, Nigeria.

BACKGROUND: Phytoceuticals are products of plant’s secondary metabolic pathways with proven activity on biologic systems. Such products have shown to be potent sources of antimalarial compounds or novel molecule template. They have also helped in controlling the increase threat of malaria to lives, especially in Africa and other tropical regions of the world. *Zizyphus spina-christi* Willd (Rhamnaceae) root bark is used in northern Nigeria for the treatment of fever. This study is aimed to search for antimalarial phytoceutical from the plant’s root bark.

METHODS: The plant’s powdered root bark was subjected to bioassay-guided fractionation and isolation procedure, and evaluated for prophylactic, suppressive and curative potency against *Plasmodium berghei* infected mice. The *P. berghei* in vivo model is used for studying the activity of potential antimalarial and has an advantage of taking into account any prodrug effect and likelihood of immune system in controlling infection. Identification of the phytoceutical and its structural analysis was based on thermo-analysis (DSC), spectroscopic (UV-visible, GC-MS, FTIR, 1H NMR, 13C NMR, DEPT, 1H NMR, and 1H – 1H COSY) data, and comparison with cited literature.

RESULTS: The search leads to the isolation of 3β-ol-lup-20(29)-en-28-oic acid, a pentacyclic triterpene as the main active antimalarial constituent. The agent exhibited antimalarial effect against established infection by 37 – 67% and suppressive models (41 – 66% inhibition) of the test. The agent also improved the survival time from an average of 9.6 days for untreated group, to 25.4 days for group that received 100 mg/kg, p.o. of the agent.

CONCLUSIONS: The study support detailed investigation of such traditionally used medicinal plants, for antimalarial drug discovery with a view of developing them into phytodrugs or used as drug leads for malaria treatment.
P032: Stimulation of early cytokines against malaria infection using *morinda lucida* stem bark extract

Funmilayo Afolayan1,2, Olayemi Adegbolagun1, Kellern Wafula1, Nicholas Nwikwabe1, Alex Wamachi3, Jennifer Orwa1 and Chiaka Anumudu1
1Department of Zoology, University of Ibadan, Ibadan, Nigeria; 2Department of Pharmaceutical Chemistry, University of Ibadan; 3Centre for Traditional Medicine and Drug Research, Kenya Medical Research Institute, (KEMRI), Nairobi, Kenya

BACKGROUND: Pro-inflammatory cytokines are essential mediators of protective immunity to erythrocytic malaria. The difference between lethal and non-lethal infections depends on the ability to mount early pro-inflammatory cytokine response. However, over production of pro-inflammatory cytokines predisposes to severe pathology. The profile of early cytokines released during malaria predicts the final outcome of infection.

STATEMENT OF RESEARCH PROBLEM: Investigations have shown that *Morinda lucida* has antimalarial activity. This study aimed to assess the effect of *M. lucida* on malaria immune response through stimulation of pro-inflammatory and anti-inflammatory and consequent effect on parasite density.

METHOD: Two groups of 20 in-bred balb/c mice were used for the experiment. Group I mice were given 100mg/kg/day Aqueous extract of *Morinda lucida* stem bark orally for 3-day to induce inflammatory response while group II received water. Pre-treatment blood samples were collected before drug administration while the post-treatment blood samples were obtained on the fourth day after which animals were challenged with *Plasmodium chabaudi chabaudi* AS. Smears were made and blood samples were collected on days 7, 9 and 11. Plasma samples were assessed for pro-inflammatory cytokines (IFN-γ, TNF-α, IL-12p70, IL-6) and anti-inflammatory cytokines (IL-10) using flow cytometry. Percentage parasitaemia was determined.

RESULT: The post-treatment plasma samples showed higher values of IFN-γ, TNF-α, IL-12, IL-6 and IL-10 when compared to pre-treatment samples in Group I while there was no significant change in the values of pre- and post – treatments values of Group II (control). Group I showed lower percentage parasitaemia on days 7, 9 and 11 of infection and higher survival days than the control group.

CONCLUSION: Aqueous extract of *Morinda lucida* stem bark induced cytokine production in naïve mice which consequently causes reduction in parasitaemia. This justifies its use as an antimalaria herb in folk medicine. Furthermore, its possible use as adjuvant to enhance the efficacy of probable malaria vaccine is predicted.

P033: Long-Lasting Microbial Larvicides for Malaria Vector Control: A Field Trial

Yaw Afrane1, Andrew Githelo2 and Guiyun Yan3
1 School of Health Sciences, Jaramogi Oginga Odinga University of Science and Technology, Bondo, Kenya; 2 Climate and Human Health Research Unit, Centre for Global Health Research, Kenya Medical Research Institute, Kisumu; 3 Program in Public Health, College of Health Sciences, University of California, Irvine, CA, USA

Larval control is a promising intervention for the control of outdoor transmission of malaria. Bio-larvicides are suited to malaria control as they can target both indoor and outdoor biting mosquitoes and do not have negative impact on non-target organisms. However, the currently available bio-larvicide formulations have a short effective duration, and consequently larval control incurs a high operation expense due to requirement for frequent re-treatment of larval habitats. Therefore, formulation of biological larvicides that has long-lasting effects is highly desired. A recently developed fourStar™ slow release Bacillus thuringiensis israelenis (BTi) and Bacillus sphaericus (Bs) was evaluated under semi-natural and natural conditions to test its effectiveness in reducing mosquito population in western Kenya. This formulation is designed to be effective against mosquito larvae for up to 6 months. In semi-natural habitats containing soil and rain water, second-instar larvae of Anopheles gambiae were introduced and FourStar™ briquettes dissolved in rain water with appropriate concentrations were added. The number of pupae produced from the larvae was recorded daily as the outcome. Formulation was also tested in natural productive habitats. Formulation was then tested for its efficiency to reduce mosquito population during the transmission season. Larval control was undertaken in field trials in three sites and with three other sites taken as control. We found that FourStar briquettes totally inhibited mosquito pupal production in the first 3 months, and then reduced pupal productivity by 87.2-98.0% 4-6 months after application. In natural habitats, FourStar briquettes reduced malaria vector pupal productivity by 100% in the first 2 months and then by 63.4-90.2% 3-5 months after application. In field randomly cluster trial, FourStar briquettes reduced indoor biting mosquitoes by 68.2-80.6% during the 3-month monitoring period, and reduced outdoor malaria vector abundance by 53.6-63.4%. This study suggest long-lasting microbial larviciding is a promising complementary malaria control tool.

P034: The cultivation of artemisia

Kokouvi Semenou Agbelekpo

*Artemisia annua* is a medicinal herb grown widely in China. Taken as tea, it has been used for thousands of years to treat malaria, and intestinal worms. It is known to be effective against many microbes but more significant effect is noticed in malaria parasites. Artesunate, a derivative of Artemisia has been recommended by WHO for the treatment of malaria. Due to its value in malaria treatments, especially falciparum (malignant) malaria, common in tropical Africa, interest in the growing of this plant has developed in Africa.

OBJECTIVE: To reduce morbidity from malaria so as to increase crop output in resource-limited through production and consumption of Artemisia.

METHODS: Support groups of people living with HIV/AIDS are trained on the production of Artemisia in the demonstration farm run by 2AVIE NGO at its headquarters in Vogan. The various steps of processing the tea are also supervised until when satisfactory results are obtained.

RESULTS: So far, out of 50 women living with HIV/AIDS, over 25 are taking it Artemisia tea regularly since 2007. The number of malaria attacks has drastically fallen among members and this is reflected in 25 average increases in the crop sales supply per member taking Artemisia. Attendance in meetings has registered an increase to over 50% as of now.

CONCLUSION: Harvesting yields can be significantly improved upon through integrated cultivation and consumption of Artemisia in resource limited regions.

FUTURE STEPS: As ARVs are being made freely available to the general public in Togo, we intend to monitor patients on Artemisia alongside ARVs as we hope that this will improve farm output further in resource limited regions.

BACKGROUND: As ARVs are being made freely available to the general public in Togo, we intend to monitor patients on Artemisia alongside ARVs as we hope that this will improve farm output further in resource limited regions.

CONCLUSION: Farming yields can be significantly improved upon through integrated cultivation and consumption of Artemisia in resource limited environment of people living with HIV/AIDS at little additional cost. We believe that is can be replicated elsewhere in Africa. Since it takes little farming space and input, it can be practiced both in the urban and rural settings.
**P035: Factors associated with risk of malaria infection among pregnant women in Lagos, Nigeria.**

Chieme O. Agomo¹ and Wellington A. Oyibo²
Malaria Research Laboratory, Nigerian Institute of Medical Research, Lagos, Nigeria, Department of Medical Microbiology and Parasitology, College of Medicine, University of Lagos, Idr-Araba, Lagos, Nigeria.

**BACKGROUND:** Pregnant women living in an area of stable malaria transmission like Lagos, Nigeria, have been identified as being at increased risk of the effects of malaria infection. In this area, most of the infections are asymptomatic which are overlooked and untreated to the detriment of the mother and foetus. This study aimed at identifying the factors associated with increased risk of malaria infection in pregnancy in Lagos, Southwest Nigeria.

**METHODS:** Demographic information and malaria prevention practices of the pregnant women were captured using structured questionnaire. Microscopy was used to establish malaria infection, species identification and parasite density.

**RESULTS:** The prevalence of malaria using peripheral blood from 1,084 pregnant women that participated in the study was 7.7%. *P. falciparum* was seen in 95.2% of the cases as either mixed infection with *P. malariae* (3.6%) or as mono-infection (91.6%). Malaria preventive practices associated with significant reduction (P<0.05) in malaria infection were the use of insecticide sprays (RR = 0.36, 95 C.I. 0.24-0.54) and the combined use of insecticide spray and insecticide-treated nets (ITN) (RR= 6.53, 95% C.I. 0.92-46.33). Sleeping under ITN alone (RR = 1.07, 95% C.I. 0.55-2.09) was not associated with significant reduction in malaria infection among the study participants with malaria parasitaemia. Young maternal age (<20years) (RR = 2.86, 95% C.I. 1.48 – 5.50) but not primigravidity (RR = 1.36, 95% C.I. 0.90 – 2.05) was associated with increased risk of malaria infection during pregnancy. After a multivariate logistic regression, and young maternal age (OR = 2.61, 95% C.I. 1.13 – 6.03) and use of insecticide spray (OR = 0.38, 95% C.I. 0.24 – 0.63) were associated with increase and reduction in malaria infection respectively.

**CONCLUSION:** Malaria prevalence is low among the pregnant women studied. Young maternal age and non-use of insecticidal spray were the main factors associated with increased risk of malaria infection during pregnancy in Lagos, Nigeria.

**P036: The molecular basis of the spread of pyrethroids and DDT resistance in field population of the major malaria vector Anopheles gambiae M form from BENIN, West Africa**

Fiacre R. Agossa¹, Christopher Jones¹, Martin Akogbeto¹,², Hilary Ranson¹
¹Centre de Recherche Entomologique de Cotonou, ²Université D’Abomey-Calavi, département de zoologie, Bénin, ³Liverpool School of Tropical Medicine, vector biology department, UK

**BACKGROUND:** To improve the design of new tools for vector control, it’s important to broaden our understanding of the modes of action and mechanisms of resistance to different insecticides. A time series of collections showed that the L1014F kdr allele has increased in frequency from 6% to 47% in 5 years and as a result there has been a drastic reduction in susceptibility to DDT and Pyrethroids in the north part of Benin (Malanville). In *Anopheles gambiae*, resistance to pyrethroid and DDT insecticides is strongly associated with the mutations L1014F and L1014S within the para voltage-gated sodium channel (VGSC).

**METHODS:** Here, we investigate the contribution of other potential resistance mechanisms. RT-qPCR was used to analyse CYP6M2, CYP6P3, GSTe2, GSTD3 and chymotrypsin (candidate genes associated lately with DDT resistance following microarray analysis) transcription profile from DDT and Deltamethrin resistant, *An. gambiae* M-form field collections from Houéyòhô and Malanville (vegetable growing areas) against the laboratory susceptible M-form strain from N’Gousso (Cameroun). We also sequenced the intron 1 of the VGSC region for the SNP detection.

**RESULTS:** Several detoxifying enzymes were up regulated, despite of the presence of kdr (0.81 in Malanville and 0.9 in Houéyòhô). GSTe2 and GSTD3 are significantly expressed in DDT resistant *An.gambiae* M form from northern Benin (Malanville) and CYP6P3 and GSTe2 are significantly expressed in DDT resistant *An.gambiae* M form from southern Benin (Cotonoou). However, the digestive enzyme (chymotrypsin) was down regulated. Furthermore, GSTe2 and CYP6M2 were significantly up regulated in natural (unexpressed *An.gambiae* M form population). The upstream intron 1 sequencing analysis revealed the presence of single nucleotide polymorphism (SNP) in wild resistant *An.gambiae* population. In addition we have reported for the first time the N1575Y mutation which also is associated with L1014F mutation in Malanville.

**CONCLUSION:** CYP6P3, and GSTe2 are expressed at higher levels in Houéyòhô than Malanville but this was only significant for GSTe2. GSTe2 looks like the strongest candidate gene for DDT resistance in Benin. 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.

**P037: Age-dependent carriage of alleles and haplotypes of Plasmodium falciparum sera5, eba-175, and csp in a region of intense malaria transmission in Uganda**

Connie Agyang, Joseph Erume, Joseph Olobo, and Thomas G. Egwang
Med Biotech Laboratories, PO Box 9364, Kampala

The development of malaria vaccines is constrained by genetic polymorphisms exhibited by *Plasmodium falciparum* antigens. We investigated the age-dependent distribution of alleles or haplotypes of three *P. falciparum* malaria vaccine candidates, circumsporozoite protein (csp), erythrocyte binding antigen 175 (eba-175) and serine repeat antigen 5 (sera5) in a region of intense malaria transmission in Uganda. A cross sectional study was carried out between August and November 2009. Blood samples were collected from 250 consenting individuals below 5 years, 5-10 years and above 10 years old. *P. falciparum* DNA was extracted from all samples. Alleles of sera5 and eba-175 were determined by polymerase chain reaction (PCR) and PCR products resolved by agarose gel electrophoresis stained in ethidium bromide. Haplotypes of CSP were identified by sequencing 63 PCR products using *P.falciparum* 7GB strain sequence as a reference. Both eba-175 FCR3 (48/178) and CAMP (16/178) alleles were observed, FCR3 (24/67) allele being predominant among the under 5 years old, the CAMP (12/67) allele was predominant among older individuals. Both sera5 alleles ORI (6/204) and ORI1 (103/204) were observed, ORI1 was more prevalent and significantly associated with age (P values < 0.0001), parasite density (P value <0.0001) and clinical outcomes (P value= 0.018). There was marked CSP diversity in the Thz2/Thz3 region. Out of 63 sequences, 16 conformed to the reference strain and one (1/16) was similar with a West African haplotype and the majority (14/16) of the haplotypes were unique to this study region. There was an age-dependent distribution of CSP haplotypes with prevalence < 5-year olds, (10/16) compared to adults (2/16). Interestingly, the CSP haplotype corresponding to 3D7 whose prototypical sequence is identical to the sequence of the leading malaria vaccine candidate RTS, S was not observed. Our data suggest that eba-175 FCR3 allele, sera5 ORI allele, and CSP haplotypes are targets of host immunity and under immune selection pressure in Apac District.
P038: Human antibodies induced against the vaccine candidate PfRh5 by natural infections of Plasmodium falciparum are limited in the population but neutralizing

Ambroise Ahoudi1, Saurabh D. Patel1, Amy K. Beil1, Tanadaka N. Dieye1, Souleymane Mbow2, Stephen C. Harrison4, Manoj T. Duraisingh5
1Harvard School of Public Health, 665 Huntington Ave Bldg 1 Rm 706, Boston, MA 02115; 2Children’s Hospital Boston, 300 Longwood Ave, Hunnillwound Ground, Dept. GI/Nutrition, Boston, MA 02115; 3Laboratory of Bacteriology and Virology, Le Dantec Hospital, and Laboratory of Parasitology, Faculty of Medicine and Pharmacy, Cheikh Anta Diop University, Dakar BP 7325, Senegal; 4Laboratory of Molecular Medicine, Children’s Hospital Boston, Harvard Medical School and Howard Hughes Medical Institute, Boston, MA 02115

BACKGROUND: Malaria, caused by the protozoan parasite Plasmodium, accounts for 1-3 million deaths a year mainly in children under 5 years old and pregnant women, with the preponderance of the disease burden in sub-Saharan Africa. The high parasitemia generated during the blood stage accounts for the clinical manifestations of the disease including cerebral malaria, and severe anemia. P. falciparum relies on multiple ligand-receptor interactions to invade the human erythrocyte, and both PfRH2b and PfRh5 have been implicated in the sialic-acid dependent pathway of erythrocyte invasion.

METHODS: To study the interaction of PfRh5 with the immune system, we have developed expression constructs the produce a stable, soluble form of PfRh5 without resorting to refolding, as well as the homologous portion of PfRh2, the P. falciparum RH domain with a sequence most similar to that of PfRh5. We have produced substantial quantities of full-length PfRh5 and the homologous portion of PfRh2 (PfRH2D1). These proteins have been analyzed biochemically, and we have characterized the humoral response by performing ELISAs on patient sera from high and low endemicity areas of Senegal. We conducted invasion inhibition assays using ex vivo Senegalese patient parasite isolates and affinity purified human IgG against PfRh2 and PfRh5.

RESULTS: In this study, we first show that antibodies from natural infections can recognize a recombinant form of the relatively conserved merozoite surface antigen, PfRh5. Furthermore, we show that affinity purified antibodies from endemic sera against PfRh2 and PfRh5 can inhibit erythrocyte invasion in vitro in both lab strains and clinical isolates of P. falciparum.

CONCLUSIONS: The search for possible antigens as components of a multi-subunit vaccine have been the focus of intensive research. Our demonstration of the presence of inhibitory antibodies to PfRh5 in vivo has important implications for the use of this protein in a multi-antigen vaccine strategy. Our data are the next step in the evaluation of PfRh5 as a vaccine candidate: they demonstrate that natural infection elicits antibodies that inhibit invasion.

P039: Spatio-temporal trends of malaria hotspots in Ilha Josina between 2003 and 2010

Pedro Aide1, Tiago Canelas1, Charlotte Pierrat2, Charufdin Sacoor1, John J. Aponte1,2,3 and Pedro Alonso1,2,3
1Maniça Health Research Center (CISM), Maniça, Mozambique; 2National Institute of Health, Ministry of Health, Maputo, Mozambique; 3Barcelona Centre for International Health Research (CRESIB), Hospital Clinic – Universitat de Barcelona, Spain

BACKGROUND: Identifying hotspots allows targeting preventive interventions where concentration of malaria cases occurs. The main objective of this study is to describe the temporal and spatial distribution patterns of clinical malaria in Ilha Josina from 2003 to 2010. We focused the seasonal variations of hotspots to assess whether their spatial patterns vary or not between wet and dry seasons.

METHODS: The study was performed in Ilha Josina Machel (25º08’S – 38º58’E), Maniça district (Maputo Province, Southern Mozambique). Malaria data used for the ascertainment of cases was collected through passive case detection in children under 15 years old attending the local health center. The spatio-temporal distribution of clinical malaria was assessed through a HotSpot analysis with ArcGis 10 (ESRI Inc., Redlands, CA, USA).

RESULTS: The overall spatial trend is the presence of a main major hotspot located in the Northern and North-western parts of Ilha Josina. However, there were variations throughout the study period in the importance of this hotspot. Until 2006 the concentration was moderate with occasionally high concentration of malaria cases appearing (in the Northwest in 2003–2005 and in the North in 2004–2006). At the end of 2006 we observed a peak in the number of households belonging to the hotspot area showing a peak in malaria transmission. The concentration trend has decreased since then. In 2009 we observed an exceptional increment of hotspots in the Northwest while the rest of territory showed high cold spots.

CONCLUSION: The hotspots of clinical malaria in Ilha Josina are located mainly in the North-Northwest over the study period. Variations in the hot spot follow seasonal trends as expected, but further exploration is needed to understand other underlying factors explaining this spatial pattern of malaria, like the role of the proximity to the health center.

P040: Mapping Malaria in South Africa: Informing Control and Targeting Elimination

Oluwagbemiga Aina

Malariorametric surveys generate data on malaria epidemiology and dynamics of transmission necessary for planning and monitoring of control activities. This study determined the prevalence of malaria and the knowledge, attitude and practice (KAP) towards malaria infection in Ibeshe, a coastal community. The study took place during the dry season in 10 villages of Ibeshe. All the participants were screened for malaria. A semi-structured questionnaire was used to capture socio-demographic data and KAP towards malaria. A total of 1489 participants with a mean age of 26.7 ±2.0 years took part in the study. Malaria prevalence was 14.7% (95% CI 13.0-16.6%) with geometric mean density of 285 parasites/µl. Over 97 % of participants were asymptomatic. Only 40(2.7%) of the participants were febrile while 227(18.1%) were anemic. Almost all the participants (95.8%) identified mosquito bite as a cause of malaria, although multiple agents were associated with the cause of malaria. The commonest symptoms associated with malaria were hot body (89.9%) and headache (84.9%). Window nets (77.0%) were preferred to LLIN (29.6%). Malaria is mesoendemic in Ibeshe during the dry season. The participants had good knowledge of symptoms of malaria, however there were a lot of misconceptions on the cause of malaria.

P041: Malaria prevalence in the rubber cultivated area of Niete, South region of Cameroon

Fodjo Airy

National Reference Unit for Vector Control

BACKGROUND: Malaria intervention strategies based on vector control and the use of drugs to eliminate circulating foci of Plasmodium falciparum, though useful, have been flawed by the upsurge in resistance to insecticides in the vectors and drugs in the parasites. New insecticides, drugs /drug combinations and vaccine molecules are needed, which must be primarily tried on the field. The success of such trials relies on their conduct in a stable population and a well-characterized field site. Thus, as part of a study to acquire baseline data for the preparation of Niete for future malaria drug/vaccine trials in Cameroon, three cross-sectional malaria prevalence surveys were conducted in January 2010 (dry season), September 2010 (wet season) and November 2011 (rainy-
dry transition season) in this locality. METHODS: Following physical and clinical examinations, finger prick blood samples were collected and used to prepare thin and thick blood smears for microscopy, while whole blood was collected to determine the packed cell volume (PCV) and for blood spots on Whatmann 3MM filter paper that were used to extract Plasmodium DNA for species identification by the Polymerase Chain Reaction (PCR) technique. RESULTS: Overall, 35.68% (n=488), 20.19% (426) and 66.03% (640) of the participants were found to have malaria infection in the dry, rainy and rainy–dry transition seasons respectively. The prevalence was inversely related to age. Unlike during the rainy and dry seasons where *P. falciparum* was the predominant parasite species with a prevalence of 94%, it represented only 64.6% during the rainy-dry transition season and generally occurred as a mixed infection with either *P. malariae* or *P. ovale*. Single infections with *P. ovale* were found only during the rainy-dry transition season. Malaria was highly associated with the development of anemia amongst children in Niete.

CONCLUSION: The findings clearly indicate that malaria occurs perennially in Niete and peak during the rainy-dry transition season when most cases of mixed *Plasmodium* species infections occur. These data complement previous entomological and socio-demographic information, which together provide a baseline to guide future malaria control efforts in Niete and other endemic areas with similar eco-epidemiological stature.

P042: Herbal Therapy for Malaria: Preclinical Evaluation of 20 Ethnomedical Aqueous Plant Extracts as Antimalarial Tea

**Edith O. Alajevoa, Dorcas A. Fadare, Omonike O. Ogbole, Olusegun G. Ademowo, Samuel O. Etautie, Tamuno F. OOkujagu, Muhammed I. Choudhary.**

**Background:** Ethnomedicine is a useful tool and has provided the basis for the drug discovery of and development of malaria chemotherapeutic agents. This coupled with a mimetic of the traditional preparation and use of traditional medicines, informed the present study of 20 medicinal plant aqueous extracts identified from two Nigerian ethnomedicines for suitability as antimalarial teas.

**Methods:** Powdered plant materials (200 g) were extracted by maceration in boiling water and were subjected to *in vitro* antiplasmodial assay using the multi-resistant K1 strain of *Plasmodium falciparum* in the parasite lactate dehydrogenase assay (pLDH) for determination of fifty percent inhibitory concentration (IC$_{50}$). Artemisinin and chloroquine diphosphate were included as controls. Extracts were also subjected to the brine shrimp lethality assay (BSL) and lethal doses at 50% (LD$_{50}$) were evaluated. Suppression of parasitemia in mice of the three most active aqueous extracts was studied in *vivo* using *Plasmodium berghei* with the four-day suppressive test. Acute toxicity in mice was done to lethal dose (LD$_{50}$) for the three active extracts. Extracts were subjected to student *t*-test of significance at 95% confidence limits.

**Results:** In the pLDH assay, *Lippia multiflora* leaf had the highest activity with an IC$_{50}$ of 3.49 µg/mL. *Phyllanthus muellerianus* leaf and *Trichilia monadelpha* leaf had IC$_{50}$ values of 4.12 µg/mL and 4.87 µg/mL, respectively. *Azadirachta indica* stem bark, *Khaya ivorensis* stem bark, *Annona senegalensis* root and *Ocimum gratissimum* leaf had IC$_{50}$ of 13.26-19.51 µg/mL. The BSL assay showed that *T. monadelpha* was the most toxic with an LD$_{50}$ of 5.06 µg/mL. *Morinda lucida* leaf and stem bark extracts (100.3 µg/mL each), *Azadirachta indica* stem bark (249.3 µg/mL) and *A. senegalensis* (5936.3 µg/mL). The other extracts were found not-toxic with LD$_{50}$ values greater 10%. Etoposide included as standard had an LD$_{50}$ of 7.4 µg/mL. *Lippia multiflora* displayed the highest suppressive activity in mice with a suppression of 90.0% at 500 mg/kg body weight of mice. *Trichilia monadelpha* and *P. muellerianus* had suppression of 88.12% and 64.19%, respectively.

**Conclusion:** The preclinical study has revealed that Nigerian ethnomedical plants have antimalarial properties and non-toxic as aqueous extracts. *Lippia multiflora* was the most active antimalarial aqueous extract. It was also found to be non-toxic. *Lippia multiflora* aqueous extract is the most suitable for use antimalarial herbal tea.

P043: Anti-inflammatory Activity of bark extract of *Hallea rubrostipulata* (Schum.)

J.-F. Leroy, a common Ethnomedicinal Antimalarial Plant Used in Uganda

Abayomi M. Ajayi, Julius K. Tanayen, J. O.C. Ezeonwumelu

**Background:** The use of medicinal plant has been the mainstay of traditional medicine for rural dwellers for thousands of years. Medicines based on traditional knowledge of wild plants serve as some of the most common treatments for acute malaria in Uganda. There are several medicinal plants that are used traditionally for malaria treatment and which have been shown to possess anti-inflammatory and analgesic activities. Cryptolepis sanguinolenta, Alstonia boonei, Vernonia amygdalina, Azadiracta indica, among a few has been reported to possess anti-inflammatory activities. The ethnomedicinal use of Hallea rubrostipulata stem bark as a remedy against malaria necessitated this study on its anti-inflammatory activity.

**Methods:** The aqueous bark extract was investigated for the presence of alkaloids, tannins, saponins, terpenoids and flavonoids. The Lorke's method was used in determining the acute toxic effects of the aqueous extract. The anti-inflammatory activity was examined using the carrageenan–induced oedema and formalin–induced paw licking in rats. The aqueous bark extract was investigated for the presence of alkaloids, tannins, saponins and terpenoids. Acute toxicity study of the aqueous extract showed that the extract was safe up to 2000 mg/kg with no obvious signs of toxicity following single oral administration. The extract at doses (200, 400 and 800 mg/kg) significantly (p<0.05) inhibited the carrageenan–induced oedema at the 3rd and 5th hour after carrageenan injection. The formalin test for aqueous extract of *H rubrostipulata* bark demonstrated a significant (p<0.005) percentage of inhibition in the inflammatory phase.

**Conclusion:** Traditional medicine uses crude extracts, the crude aqueous extracts results revealed the presence of phytochemical constituents which can work synergistically to dampen the inflammatory reaction. The anti-inflammatory activity of the aqueous extract of *H rubrostipulata* bark might partly explain the efficacy of the bark decoctions in the traditional management of acute malaria attack.
P044: Sustainability of Intervention for home management of malaria: The Nigerian Experience

Ikeoluwa O. Ajayi1,2, Ayodele Samuel Jegede1,2, Catherine O. Falade1,4

1Epidemiology and Biostatistics Research, Institute of Medical Research and Training, College of Medicine, University of Ibadan, Nigeria; 2Epidemiology and Medical Statistics Department, College of Medicine, University of Ibadan, Nigeria; 3Department of Sociology, Faculty of The Social Sciences, University of Ibadan, Nigeria; 4Department of Pharmacology & Therapeutics, College of Medicine, University of Medicine, University of Ibadan, Ibadan, Nigeria

BACKGROUND: An important challenge to community-based intervention is sustainability. This study evaluated sustainability of an intervention to improve Home Management of Malaria (HMM) in Ona-Ara Local Government Area of Oyo State, Nigeria two years after end of intervention.

METHODS: A total of 13 FGDs was conducted among trained Community Medicine Distributors (CMDs), mothers of children aged 0-5 years and community members; 14 Key Informant Interviews were held with community leaders, Primary Health Care (PHC) Coordinator and Rollback Malaria Manager. Observation was carried out on 13 CMDs to check AL stock and registers. Thematic approach was used to analysed the data.

RESULTS: Utilization of CMDs was said to be high when the project started but dwindled after the researchers left the community. Some of the CMDs have not had drug to distribute in the two years preceding this study. Thus, majority of the caregivers sought care at other alternative care providers or used herbs. While some CMDs have abandoned the assignment, a few continued to provide care to febrile children as their own contribution to the good of the community. The functioning CMDs prescribed paracetamol, sulfadoxine-pyrimethamine, amoxycillin and chloroquine when out of AL stock or refer to PHC center. Source of AL was still the nearest government health facilities but supply was irregular and hindered by incessant transfer of trained health workers. All the CMDs mentioned they did not receive any support from the community as promised and this was corroborated by community members/leaders and health facility workers. None of the CMDs observed had AL in stock or record of patronage in the last one year. They mentioned that health centres have not had AL stocked in recent times.

CONCLUSION: Mechanisms to draw unflinching commitments from the government and community to sustain community-based intervention, the major sustainability challenge identified in this study, should be explored.

P045: Nutritional status influenced pro-inflammatory cytokines response in Plasmodium falciparum malaria patients in Nigeria

Ajbaye O1, Osuntoki AA1, Orok AB1, Iwalokun BA1, Faneye OA1, and Akinibosun OA1

1Biochemistry & Nutrition Division, Nigerian Institute of Medical Research, Yaba, Lagos, Nigeria; 2Virology Unit, Microbiology Department, University of Ibadan, Ibadan, Nigeria; 3Biochemistry Department, College of Medicine, University of Lagos, Nigeria.

BACKGROUND: In malaria endemic regions, malnutrition has also been reported to be a public health problem and an important factor modulating the risk of malaria. Pro-inflammatory cytokines are known mediators of malnutrition with higher risks in sub-Saharan African countries. Given the fact that the pattern of host innate immunity mediated by pro-inflammatory cytokines is critical in determining malaria outcomes, understanding the impact of malnutrition on innate immune response in Plasmodium falciparum (PF) infected patients is very important for malaria control. In Nigeria, no studies have examined the relationship between nutritional status, pro-inflammatory cytokines and malaria. We specifically chose to examine the interaction between Tumour Necrosis Factor (TNF) α, Interleukin 18 and Interleukin 12 (IL-18 & IL-12), malaria parasite density and nutritional status of Plasmodium falciparum malaria patients in Nigeria.

OBJECTIVE: To determine nutritional status and evaluate the influence of malnutrition on immune response among PF infected patients in Lagos, Nigeria.

METHODS: Volunteers (307) with history of fever within 72hrs were screened microscopically for PF in a cross-sectional study at IJEDE General Hospital, Lagos, Nigeria. The nutritional status of participants was determined using the International Reference Population defined by the U.S National Center for Health Statistics (NCHS). Blood film was used to identify malaria parasite. TNF-α, Interleukin 18 & Interleukin 12 were determined by ELISA. Statistical analysis was done using SPSS Version 17. Study protocol was approved by NIMR IRB.

RESULTS: A total of 64 patients comprising of 47% males and 53% females with a median age of 10 years were recruited. Significant proportion (42.2%) of the participants were stunted (height-for-age, z-score < -2). Malaria rate was 20.85% and undernutrition, 62.5%. TNF-α was associated with age and higher in < 5 yrs (P = 0.001). Mean levels of TNF-α, Interleukin 18 & Interleukin 12 were significantly lower in underweight patients (P<0.05). There was no significant relationship between fever and MPD (P > 0.05) in the age groups.

CONCLUSION: This preliminary investigation suggests that nutritional status modulates malaria outcomes and pattern of progression in all ages.

P046: Plasmodium falciparum malaria burden in rural setting of Dienga in southeastern Gabon

Olusola Ajbaye

INTRODUCTION: Malaria remains a public health problem. Several studies have shown a lower prevalence in endemic countries. The epidemiological studies are quite needed to develop new strategies for disease control and even its eradication.

OBJECTIVE: The present study was conducted to assess the true prevalence of P. falciparum infection and therefore to improve malaria surveillance.

MATERIALS AND METHODS: From March to May, P. falciparum infection was sought by microscope and PCR STEVOR, in 375 subjects (≥3 years old) living in the village of Dienga, south eastern Gabon.

RESULTS: Overall, 25.06% (95% CI = [20.68 to 29.31]) of P. falciparum patents infected individuals (microscopy positive=ME+) and 18.13% (CI 95% = [6.47 to 12.67]) of submicroscopic infections (PCR+) were observed. The true prevalence of P. falciparum malaria was therefore 43% (95% CI [38.1 to 47.9]). The prevalence of gametocyte carriers was 3.20% (95% CI = [20.68 to 29.31]). The median value of parasitemia was 1100 parasites/μl (minimum 39 parasites/μl and maximum of 40000 parasites/μl). The prevalence of P. falciparum in 3-12 years old children was 33.12%, this prevalence was 26.8% in 1995. The prevalence of P. falciparum in 13-85 years old was 19%, this prevalence was 7.2% in 2003.

CONCLUSION AND PERSPECTIVES: These data show that the prevalence of P. falciparum malaria has increased in Dienga. This could be due to the decline of efforts (prevention, treatment) to fight against this disease locally.
P047: Malaria treatment in Nigeria: the burden of payment and coping in South East Nigeria

Miriam Ajuba1,2, Chinenye Okwuoza2,3, Alex Adjagba1, Benjamin SC Uzochukwu1,4, Obinna E Onwujekwe1,5
1Health Policy Research Group, Department of Pharmacology and Therapeutics, University of Nigeria, Enugu, Enugu State, Nigeria; 2PATH Malaria Initiative (MVI), PATH, Ferney-Voltaire, 01210 France; 3Department of Community Medicine, University of Nigeria Teaching Hospital, Enugu, Enugu State, Nigeria; 4Department of Health Administration and Management, Faculty of Health Sciences, University of Nigeria, Enugu, Enugu State, Nigeria

INTRODUCTION: Malaria infection is known for its enormous cost burden to individuals. It affects mostly the poor and as such referred to as the disease of poverty. Worldwide about half a billion illness episodes and 1-2 million deaths occur from malaria. This paper tries to determine how households pay and cope for malaria treatment in Nigeria.

METHODS: The study was carried out in two communities with high malaria incidence rates both in Oji-River Local Government Area of Enugu State, Southeast Nigeria. It was a facility based descriptive cross sectional study. Exit interviews were employed for 200 patients leaving the health facilities after consultation and treatment for malaria. Treatment costs were calculated and distributed across different socio-economic groups to ascertain the burden of payment and coping methods.

RESULTS: Findings show a mean cost of N946.90 was spent as direct medical and N119.77 as direct non-medical cost. A higher N2,461.8 amount was spent as indirect-medical cost. This was based on 154.06 Naira = 1USD (CBN, Nigeria exchange rate) at 2011. The most employed method of coping with payment was savings (79.5%). The least poor spent N1,160 (1454.84) on direct medical cost while the most poor spent N 944(1017.17) of their total income. None of the respondents used the health insurance mechanism (p<0.05).

CONCLUSION: The high burden of payment and coping with the costs of malaria illness largely occurred at the level of the household on the most poor families. Burden of payment and coping can be reduced by education of public on the affordable health insurance system e.g. the Community Based Health Insurance mechanisms.

P049: Antiplasmodial activity of methanolic extract of anogeissus leiocarpus and its effect on liver function

Olusegan Akanbi

Malaria is a deleterious disease that affects man, especially in the tropics. Anogeissus leiocarpus is one of the medicinal plants commonly used in Nigeria as alternative medicine to treat malaria infection, but its efficacy and effect on the liver function have not been studied. Therefore, this work studied the antiplasmodial activity Anogeissus leiocarpus its effect on the liver function of mice infected with P. berghei. Mice weighing between 18-25g were infected with P.berghai and distributed into five groups. The first group was not infected with the parasite (normal control). The second group was infected but not treated with antimalarial drug (negative control). The third group was infected and treated with 5.0mg/kg of artesunate (positive control). The fourth and fifth groups were infected and treated with 100 and 200mg/kg of methanolic extract of Anogeissus leiocarpus. The was significant reduction in the parasitaemia in the positive control and the groups treated with Anogeissus leiocarpus when compared with the negative control. The rate of parasite reduction was higher in the group treated with 200mg/kg than other groups studied. The serum and liver alanine aminotransferase (ALT) level was significantly higher in the group treated with 200mg/kg than the normal and positive control (P<0.05). The mean serum and liver aspartate aminotransferase (AST) levels were significantly higher (P<0.05) in the negative control than other groups studied. The total bilirubin level was significantly higher in the negative control and the group treated with 200mg/kg than the group treated with 100mg/kg. The serum and liver protein and albumin levels were significantly lower in the negative control when compared with other groups studied. This study showed that methanolic extract of Anogeissus leiocarpus has high antiplasmodial activity at 200mg/kg and that it is capable of reducing the serum and liver ALT, AST, bilirubin, protein and albumin levels.

P048: The geographical study of prevalence of malaria infection in pregnant women in Akoko South West of Ondo state in Nigeria

Akanbi F Rebecca, Ikwyatum Godwin
Department of Geography, University of Ibadan, Ibadan, Nigeria

BACKGROUND: Malaria is an endemic disease prevalent in the tropic and subtropical regions of the world. Younger women and primigravidae are more susceptible to the disease than older women and multigravidae. Most of the studies on prevalence of malaria has been scientific, but for malaria to be eradicated in our society there is a need to study the impact of environmental factors on its prevalence, therefore this work studied the impact of the environmental factors in the prevalent of malaria in Akoko South west of Ondo state, Nigeria.

METHODOLOGY: The study used both primary and secondary data. Data such as interviews, raw data collection, questionnaires and verbal interview of health workers were collected. Statistical methods were employed to analyze the data.

RESULT: Hospital records revealed that only 14.6% of pregnant women were malaria positive. On average while of the 204 pregnant women respondents to questionnaire, 66.7% were infected with malaria. 67.0% of pregnant women who lived in single rooms were malaria positive, 72.2% of those who lived in a room and parlour had malaria and 37.5% of those without toilet facility were malaria positive. 71.4% of the 77 respondents with no drainage system had the malaria infection, 77.3% of 182 respondents who used open air refuse dump were malaria infected. Of the 57 respondents having stagnant water close to their houses, 73.7% had malaria, while 89.9% of the 116 respondent who had bushes around their houses had malaria infection. 76.1% of those who did not use mosquito nets had malaria episode.

CONCLUSION: This study shows that environmental factors have a great influence in the prevalence of malaria infection in pregnancy.

P048: The geographical study of prevalence of malaria infection in pregnant women in Akoko South West of Ondo state in Nigeria
P050: Assessment of the relationship between treatment given and decision making in home management of malaria among children in South West Nigeria

Adekunle Akerele

In the home management of malaria (HMM) strategy, the person who makes treatment decision is important to the type of treatment outcome of an episode of malaria. To assess the factors that enhance caregivers ability to make treatment decisions this study was a part of a larger study evaluating the effectiveness of arteether-lumefantrine(AL) in HMM in a rural community in southwestern Nigeria where ‘mother trainers’ have been trained to distribute AL to caregivers of children suffering from malaria. A descriptive cross sectional study was carried out in thirty villages among 552 caregivers whose children had fever two weeks prior to a survey using an interviewer administered questionnaire. Caregivers were asked who advised them on drug to give when child was sick, time it took for action to be taken, distance of place where orthodox treatment was obtained and where treatment was received. Multinomial logistic regression model was fitted to determine factors that enhance decision making in HMM.

Mean age of caregivers was 32±9.6 years of which 529 (95.8%) are females. The mean time action was taken after child was noticed to have fever was 2 hours ± 015.11. A total of 43 (39.8%) of the 166 caregivers who started treatment greater than 2 hours after illness was observed sought advice from mothers/grandmothers, 33 (7.4%) of the 35 caregivers who sought advice from fathers started treatment before 2 hours after the illness was observed while 288 (64.9%) of the 351 caregivers who sought advice from CBD started treatment before 2 hours after the illness was observed (p=0.011). A total of 91 (32.4%) of 166 caregivers who obtained advise from mothers or grandmothers walked less than 10 minutes to where orthodox medicine was received (p=0.008) while 21 (50.0%) of caregivers who obtained advice from mothers and grandmothers obtained the orthodox medicine from mother trainers (p=0.037). Caregivers who obtained drugs from mother trainers were about 3 times more likely to seek advice from mother/grandmother (RRR = 2.89, 95% CI: 1.15 – 7.22, p=0.037). Caregivers who obtained advice from mothers/grandmothers obtained the orthodox medicine from mother trainers (p=0.03). P25sRNA was not detected among the microcopy-negative samples from Senegal whereas it was found in 3.6% and 15.4% of those from Abidjan and Gabon. Gametocyte carriage increased with age in Senegal (15.4% in children below five years versus 43.9% in 5-14 years old children, p<0.01) whereas a trends toward a decrease rate in adults was found in Ivory Coast (32.3% versus 21.1% in younger children).

CONCLUSION: In areas with low malaria prevalence such as Dakar, pure SMG is uncommon compared to other cities with higher infection rate. Moreover, the relationship with age differed between the study areas suggesting a different type of exposition and of parasite transmission.

P052: Characterization of malaria transmission during military crisis in urban area of Bouake, Central Cote d’Ivoire

ADJA Akre Maurice1,2, YOBO Mabot Celine1, ASSI Serge Brice1
1Institut Pierre Richet, Institut National de Santé Publique, Bouaké, Côte d’Ivoire; 2Laboratoire de Zoologie et Biologie Animale, Université F. Houphouët Boigny, Abidjan, Côte d’Ivoire

BACKGROUND: During the past 10 years, Cote d’Ivoire has experienced a serious military and political crisis that disrupted the environment of towns and villages. From the change of the initial environment emerge new landscapes favourable to tropical diseases vectors and pests. To investigate the real situation of malaria in a war zone, a survey was conducted from April 2008 to March 2009 in the urban area of Kennedy in Bouake, central Cote d’Ivoire.

METHODS: Entomological data were collected from the human landing catches and parasitological data were obtained from blood smear and thick film in children aged 0 to 15 years, following a clinical examination.

RESULTS: The Culicidae fauna collected is characterized by three main malaria vectors, An. gambiae, An. funestus, An nili. However, An. gambiae and An. funestus transmitted Plasmodium with means sporozoite rates of 2.8% and 12.5%, respectively. The aggressive rates of both vectors were 29 and 0.7 b/m/n respectively. The endemicity of the study area estimated by the entomological inoculation rate (EIR) was 296 infected bites /man/year (b/m/ y) for An. gambiae and 33 b/m/n for An. funestus. Mean prevalence of Plasmodium falciparum was estimated to 83% and Plasmodium falciparum is responsible for 100% of infections.

CONCLUSIONS: During the political contributed to modify transmission ecology of Plasmodium in urban Bouake as malaria vectors found previously in rural areas were collected in this high urbanized area of Kennedy in Bouake.

P051: Pfalciparum submicroscopic gametocyte carriage in urban areas of Central and West Africa

MK Bouyou Akotet1,2, W Yay1, B Faye3, DP Mawili Mboumba4,5,6, A Lembet Mikolo1, ML Tshibola1, F Kassi1, O Gaye4, M Kombila3,4,7
1Department of Parasitology, Faculty of Medicine, Libreville, Gabon; 2Department of Parasitology, Faculty of Medicine, Douala, Cameroon; 3Malaria Clinical and Operational Research Unit, Melen Regional Hospital, Gabon; 4Malaria Research and Control Center, NIPH, Abidjan, Côte d’Ivoire; 5Laboratoire de Zoologie et Biologie Animale, Université F. Houphouët Boigny, Abidjan, Côte d’Ivoire

BACKGROUND: A rebound of malaria prevalence is observed in some countries, suggesting a continuous high level of transmission. This transmission depends on the presence of mature gametocytes which circulate at low densities in human peripheral blood.

OBJECTIVE: To analyze and compare the frequency of submicroscopic gametocytaemia (SMG) in urban areas of three countries with different malaria prevalence.

METHODS: Blood samples from febrile patients living in Libreville, Abidjan and Dakar in 2011 (global malaria prevalence of 31%, 24% and 7% respectively) were collected into paper filter. After nucleic acids extraction, Pf25 mRNA Q7-NASBA was performed for gametocyte detection. Results were compared between countries according to the age and the presence of asexual parasites.

RESULTS: None of the analyzed samples had microscopic gametocytaemia. SMG carriage was the lowest in patients with positive blood smears from Abidjan (25.5%) compared to Senegalese (44.9) and Gabonese (52.4%) individuals (p<0.03). Pf25smRNA was not detected among the microcopy-negative samples from Senegal whereas it was found in 3.6% and 15.4% of those from Abidjan and Gabon. Gametocyte carriage increased with age in Senegal (15.4% in children below five years versus 43.9% in 5-14 years old children, p<0.01) whereas a trends toward a decrease rate in adults was found in Ivory Coast (32.3% versus 21.1% in younger children).

CONCLUSION: In areas with low malaria prevalence such as Dakar, pure SMG is uncommon compared to other cities with higher infection rate. Moreover, the relationship with age differed between the study areas suggesting a different type of exposition and of parasite transmission.
P053: A Five Year Review of the Trends for In-patient Cases of Malaria at the Children’s Ward of Volta River Authority (VRA) Hospital, Akosombo, Ghana

Ralph K. Akyea
Korle-Bu Teaching Hospital, Accra, Ghana

BACKGROUND: Malaria continues to claim one to two million lives a year, mainly those of children in sub-Saharan Africa. Reduction in mortality depends, in part, on improving the quality of hospital care, the training of healthcare workers and improvements in public health. This study examined the trends of severe malaria treatment outcomes and quality of care for inpatients admitted to the Children’s Ward of the VRA Hospital, Akosombo over a five year period.

METHODS: A retrospective study of 856 admitted (in-patient) malaria cases to the Children’s Ward of the VRA Hospital in Akosombo between January, 2006 and December, 2010 was conducted to review the trends.

RESULTS: There was an increase in the admitted cases of malaria, directly related to the increase in National Health Insurance Scheme (NHIS) clientele over the same period, with a corresponding increase in the number of patients who improved over the period. For 79.9% of the patients admitted, the duration of stay was for 1-5 days, with 0.5% of the patients staying beyond 15 days. Fever, vomiting and convulsions were the most common clinical presentations with severe anaemia, cerebral malaria and generalized convulsions being the most common complications patients presented with. Over the 5 year period, 95% of the admitted patients improved with a rise from 78 in 2006 to 215 in 2010, 1.4% of the patients were referred to other health facilities for further management and 3.6% of the patients died.

CONCLUSION: There was an overall yearly increase in the number of malaria cases admitted to the Children’s Ward of the VRA Hospital, Akosombo from 2006 to 2010 which corresponded to the yearly increase in the number of NHIS clientele for the hospital.

P054: Successful large scale production of the insect parasitic nematode, Romanomermis iyengari, for sustainable malaria vectors control in Benin, West Africa

Thiery B C Alavo1, Ayaba Z Abaglii, Rafael Pérez-Pacheco2, Edward G Platzer2
1Laboratoire d’Entomologie appliquée / Centre Edward Platzer, Université d’Abomey-Calavi, BP 215; 2CIDIR Oaxaca, Instituto Politécnico Nacional, Xoxocotlan, Oaxaca, C.P.71230, Mexico; 3Department of Entomology, University of California, Riverside, CA 92521-0415, USA

BACKGROUND: Control of Anopheles mosquitoes in malaria endemic areas of Sub-Saharan Africa has become increasingly difficult because of widespread insecticide resistance. There is an urgent need for malaria control programs to adopt more integrated mosquito management approaches that include sustainable, nonchemical solutions. The insect parasitic nematode, Romanomermis iyengari, has shown potential as mosquito biocontrol agent, in Benin. Present work is aimed to develop a large scale production procedure using affordable local materials for this nematode in tropical Africa.

METHODS: Culex quinquefasciatus was used as the host for the nematodes. Mosquitoes were fed on blood meal of chicken (Gallus gallus domesticus). Four days after blood meal, eggs rafts were transferred into plastic trays each containing 2 L of water. The larvae were fed with fish food. Second instar larvae were infected with preparasitic nematodes (J2), (three J2 / larva). Six days after infection, post-parasite juveniles emerged, were collected, and washed several times. A volume of 7 ml of post-parasitic nematodes are then transferred into a plastic container containing 500 ml of washed and sterilized coarse sand with sterilized water. Once all nematodes moved into the sand, water was drained completely, the container closed, and stored for 8 weeks.

RESULTS: Every six days, we collected approximately 400 mL of post parasitic nematodes. Seven mL of these post-parasites yielded approximately 350 000 infectious nematodes. Within 2 months, about 300 million infectious nematodes were produced and stored in the Centre. This amount is enough to suppress Anopheles larvae in about 150 000 m2 of breeding sites.

CONCLUSIONS: Large scale production of R. iyengari is possible at low costs in Sub-Saharan Africa. Production and inundative release of this nematode around malaria endemic areas can help reduce reliance on insecticides.

P055: High Prevalence of 1264G non-sense mutation of CD36 in the Southern Non-Fulani Populations of Cameroon

Innocent M. Ali1,2, Patrice N. Mimbche1,3, Marie-Solange Evehe, Palmer M. Netongo4, Alkideh M. Nji5, Isabel Akaragwe6, Ireneé Domkam1, Diakité Mahamadou4, Tracey J. Lamb3, Wilfred F. Mbacham7
1Laboratory for Public Health Research Biotechnologies, The Biotechnology Centre, University of Yaoundé 1, Yaoundé, Cameroon; 2Department of Biochemistry, University of Dschang, Dschang, Cameroon; 3Department of Paediatric Infectious Diseases, Emory University of Medicine, Atlanta, USA; 4Malaria Research Training Centre, Bamako, Mali

BACKGROUND: We determined the distribution of a non-sense codon in exon 10 of the gene encoding CD36 in two geo-ethnic populations endemic for malaria in Cameroon in which variability in the ability to clear malaria parasites with resistance conferring mutations exist.

METHODS: The WHO 2003 protocol for assessing clinical and parasitological outcome was used to classify patients within a randomized double blind control trial of fansidar (SP)-amodiaquine (AQ) treatment of Plasmodium falciparum malaria in Cameroon. Human DNA from pre-treatment samples was subjected to primer extension pre-amplification and genotyped using Sequenom technology to evaluate selected single nucleotide polymorphisms in the CD36 gene. Allele frequencies were calculated for the SNPs and allelic distribution compared between the study population in the Northern and Southern regions. Chi Square Odd ratio was used to assess association with clearance of AQ resistant parasites and differences in distribution of SNPs. The homozygous dominant genotype was considered as reference in a co-dominance model. A P-value (OR) <0.0008 was considered significant.

RESULTS: No association was found between studied SNPs in the CD36 and ability to clear AQ resistant parasites. However, analysis of the 1264G mutation indicated a moderate correlation with delayed rate of clearance of AQ resistant parasites (OR 2.99, 95% CI: 1.21-7.04, P-value <0.05). The frequency of 1264G SNP was significantly different between participants resident in the northern and southern study ecologies (P-value: 5.78 x10^-5 OR: 0.27, 95%CI 0.13-0.54). No geographical differences were observed with analysis of other CD36 SNPs. CD36 deficiency was absent in the northern population.

DISCUSSION: Absence of CD36 deficiency in the Northern population suggesting that deficiency might enhance efficient anti-malaria immune responses in this region. High frequencies of the 1264G SNP in Africa have been reported only among the Yoruba ethnicity south of Nigeria and presently in southern Cameroon. The reason for this foci evolution is yet unclear.
P056: Study of the effectiveness of bacillus thuringiensis israelensis for the control of anopheles gambiae sl resistant to pyrethroid, Benin, West Africa

Roland ALIA2, Armel T. DJENONTIN1,3, Martin C. AKGBETO1,2
1Centre de Recherche Entomologique de Cotonou (CREC), Bénin; 2Faculté des Sciences et Techniques / Université d'Abomey-Calavi (FAST / UAC), Bénin

BACKGROUND: The chemical control based on the widespread use of insecticides, has led to vectors resistance to insecticides. The use of biological insecticides has appeared as a great alternative to circumvent this phenomenon. In public health, Bacillus thuringiensis and Bacillus sphaericus are most commonly used. In this study, we report the results of the effectiveness of Bacillus thuringiensis for the control of Anopheles gambiae sl resistant to pyrethroid in Benin.

METHODS: Three different doses (1g/m², 1.5g/m² and 2g/m²) of the commercial formulation Vectobac GR 3,33% AI (200 ITU/mg) of Bti were initially tested in the laboratory on instars II of the wild population of Anopheles gambiae by measuring the inhibition rate of adults. The optimal dose of the product was then, applied under natural conditions to determine the persistence of the product through the measurement of the density reduction of larval.

RESULTS: The linear regression model used, by considering the dose 1g/m² as a reference, showed that the dose 2g/m² inhibited 1/76,71 = 0,01 – relative risk = 76,71 [69,41 ; 84,77] – times, the emergence of adults more than doses 1g/m² et 1,5 g/m². Under natural conditions, the larval density reduction observed after the application of the product on the advanced larval stage, (L₄) was higher (99,8%) than that observed (73,5%) with the young larval stage (L₁). The mixed linear regression model showed that the persistence of product was approximately of 3 days on advanced larval stage.

CONCLUSION: The commercial formulation of Bti used in this study is not so effective to be considered at indicated doses for a large scale use against mosquitoes in Benin.

P057: Population genetics of Plasmodium falciparum in the Arabian Peninsula and prospect of malaria elimination

Salama Al-Hamidhi1, Mohammed A.K. Mahdy2, Saad M. Bin Dajem3 Adel Ali H. Al-Sheikh4, Zainab Al-Hashami4, Hissa Al-Farsi5, Abdulsalam M. Al-mekhlafi1, Mohamed Ahmed Idriss1, Albano Beja-Pereira6 and Hamza A. Babiker7
1 Department of Biochemistry, Faculty of Medicine and Health Sciences, Sultan Qaboos University, Oman; 2Department of Microbiology and Immunology, Faculty of Medicine and Health Sciences, Sultan Qaboos University, Oman; 3Department of Parasitology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia; 4Biology Department, College of Science, King Khalid University, Abha, Saudi Arabia; 5National Centre for Training and Research, MOH, Jazan, Saudi Arabia; 6Department of Parasitology, Faculty of Medicine, Sana’a University, Sana’a, Yemen; 7Research Centre in Biodiversity and Genetic Resources (CIBIO), University of Porto, Rua Padre Armando Quintas 7, Vairão 4485-661, Portugal

BACKGROUND: Malaria control efforts in the Arabian Peninsula have been boosted by political commitment and increased funding of governments of the Gulf States. Consequently, the burden of the disease is being reduced dramatically and transmission has been interrupted in a number of countries throughout the region. However, malaria remains endemic in limited sites, in Yemen and southwest of Saudi Arabia. In addition to local transmission, imported malaria sustains an extra source of parasites. The present study examined genetic diversity of Plasmodium falciparum parasites in Yemen, and Saudi Arabia to elucidate parasite structure, and how the current control efforts is reflected on parasite diversity and its structure.

METHODS: Ten microsatellites were genotyped in 108 P. falciparum isolates collected in three sites in Yemen (Taiz, Dhamar and Hodeidah) and 203 samples from Saudi Arabia (Jazan). All samples were collected from confirmed P. falciparum cases in 2008. All isolates were types for 10 putative neutral microsatellites located on 6 different chromosomes. Extend of diversity, multi-locus haplotype and inter-population differentiation were examined using.

RESULTS: All of the examined microsatellites were found to be highly polymorphic in all sites in Yemen and Jazan (Saudi Arabia). Allelic diversity at each locus, summarized as unbiased heterozygosity (He) from the distribution of allele frequencies, revealed higher levels of genetic diversity among parasites in Hodeida (HE = 0.615 ) and Taiz from (HE = 0.66) than from Dhamar (HE = 0.481). In Jazan (Saudi Arabia), the level of genetic diversity (HE = 0.76) was higher than among parasites in Yemen. Most microsatellites were distributed widely across different populations, and ‘private’ alleles (detected only in one population) were at very low frequencies. Pairwise comparisons of populations showed that Yemen populations, Taiz and Hodeidah display low population differentiation values Fst (0.074). The three population can probably be considered as one population. However, parasites in Taiz was among the Yemen parasites that are most closer to Jazan (Saudi Arabia), followed by Hodeidah. Dhamar is clearly a distinct population

CONCLUSION: Although current control efforts have reduced risk of malaria in Saudi Arabia, the extent of parasite diversity and genetic structure was similar to that seen in Yemen where malaria transmission is high. The current control efforts should consider strategies to curb flow of imported malaria into the region.

P058: Active Pharmacovigilance of Artemisinin-based Combination Therapies in Benin

Aurel C. ALLAB1, David Kannmadozo, Achille Massougbdji

Artemisinin-based Combination Therapies are widely prescribed in endemic countries to treat uncomplicated malaria. The widespread prescription of these new drugs requires the implementation of a monitoring system for their safety in a real-life prescription. The present study aims to contribute to evaluate the safety profile of Artemisinin-based Combination Therapies including Artemether-Lumefantrine, Artesunate-Amodiaquine and Dihydroartemisinine-Piperaquine in the health district of Cotonou in Benin and to use the results for strengthening the national pharmacovigilance system of Benin. It is a prospective, observational study of active pharmacovigilance held from June to August 2011 in Cotonou. Among the three Artemisinin-based Combination Therapies studied, Artemether-Lumefantrine is the most prescribed. The frequency of patients in whom the diagnosis was biologically confirmed before applying Artemisinin-based Combination Therapy was 57.5%. The frequency of patients with at least one Adverse Event was 14%. Main Adverse Events are represented by digestive (3.5%), neurological (2.5%) and general (2%) symptoms. Treatment failures are the only serious Adverse Events reported by patients. All treatment failures are experienced by patients under Artemether-Lumefantrine, with an overall incidence of 3.82% with 3.18% for Early Treatment Failures. The implementation of pharmacovigilance is a challenging for Benin. But the implementation of this study and preliminary results obtained demonstrate its feasibility. The occurrence of a considerable number of treatment failures under Artemether-Lumefantrine requires periodic assessment of the effectiveness of Artemisinin-based Combination Therapy in Benin.
P059: Testing the uptake of low-cost rapid diagnostic tests for malaria in Tanzanian private sector drug dispensing outlets.

Sean P Allen1, Kathleen Maloney1, Bonnie Krenz1, Sigbert Mkude1, Clinton Health Access Initiative, Dar es Salaam, Tanzania; 2National Malaria Control Program, Ministry of Health and Social Welfare, Dar es Salaam, Tanzania

BACKGROUND: The private sector serves approximately 40% of people seeking treatment for febrile illnesses in Tanzania. Although malaria is the most commonly perceived cause of febrile illness, malaria prevalence is declining 10% nationwide in 2012 down from 18% in 2007. There is a need for additional research in order to determine if malaria rapid diagnostic tests (mRDTs) can and should be scaled up in private accredited drugdispensing outlets (ADDOs). In particular, this study was designed to determine whether trained dispensers in these outlets can safely and correctly administer mRDTs, and whether the introduction of diagnostic testing improves antimalarial targeting.

METHODS: After the completion of a baseline assessment in March 2013, 328 ADDO dispensers were trained to appropriately store and administer mRDTs in two districts in Morogoro Region. The outlets represented by the dispensers were licensed to purchase mRDTs from specially contracted local distributors and use them to test febrile customers. The selected distributors in one district were given access to mRDTs at the price of $0.46 per test and in the other district they were given access to subsidized mRDTs at the price of $0.15 per test. A midline evaluation will be conducted seven months after the baseline and an endline conducted at 12 months in both intervention districts, as well as a control district.

PRELIMINARY RESULTS: Results suggest a favourable environment for increasing uptake of mRDTs. Willingness to pay data (WTP) collected as part of the baseline showed that 92% of respondents were willing to pay increasing uptake of mRDTs. Willingness to pay data (WTP) collected as part of the baseline showed that 92% of respondents were willing to pay for an mRDT, with a mean WTP ($0.92) above the recommended retail price of mRDTs in the non-subsidized intervention district ($0.68). Of the ADDO dispensers invited to training, 97% were certified to perform mRDTs. The initial monitoring visit showed that the majority of ADDOs were able to safely stock and administer mRDTs. Overall, these results suggest that ADDOs can serve as viable mediums for increasing uptake of mRDTs in the private sector in rural Tanzania.

RESULTS: We included 52 responses from 25 counties; 87% working at an investigational site and 75% reporting about and interventional study. For AEs, questioning in 31% of interventional studies was a combination of general (e.g. open questions about health) and structured (e.g. reference to specific health-related items), 26% used structured only and 18% general only. No observational studies used general questioning alone. A minority incorporated pictorial tools. Rationales for the questioning approach included: standardisation of assessment or data capture, specificity or comprehensiveness of data sought, avoiding suggesting a response, feasibility, and seeking to understand participants’ perceptions. Most respondents considered the approach they reported as optimal, though several later reconsidered this. Four AE grading, and 3 causality assessment approaches were reported. Combining general and structured questions about non-study drug use were considered useful for revealing and identifying specific medicines, while pictures were said to enhance reports, particularly in areas of low literacy.

CONCLUSIONS: It is critical to evaluate the safety of the antimalarial drugs that are being deployed in large, diverse populations. Many studies would be suitable for contributing to a larger body of evidence for answering questions on harm, however our survey showed that various methods are used to obtain relevant data. We plan to facilitate the antimalarial drug clinical research community working towards consensus about the selection and/or design of optimal methods.

P060: Evaluating harm associated with antimalarial drugs: a survey of methods used by clinical researchers to elicit, assess and record participant-reported adverse events and related data

Elizabeth Allen1, Clare Chandler2, Nyaradzo Mandimika1, Cheryl Pace3, Ushma Mehta1, Karen Barnes1

1 Division of Clinical Pharmacology, Department of Medicine, University of Cape Town, Cape Town, South Africa; 2 Department of Global Health & Development, London School of Hygiene & Tropical Medicine, London, United Kingdom; 3 Department of Clinical Sciences, Liverpool School of Tropical Medicine, Liverpool, United Kingdom

BACKGROUND: Participant reports of medical histories, adverse events (AE) and non-study drugs are integral to evaluating harm in clinical research, but how data are obtained can influence study results and meta-analyses. We conducted an online survey of antimalarial clinical researchers between August 2012 and January 2013 to understand how such data were elicited, how AEs were assessed for severity and relationship with study drug(s), and how study drug adherence was evaluated.

METHODS: The survey was advertised through emails, via collaborators and at conferences. Questions aimed to capture the detail, rationale and application of methods used to obtain relevant data within various study designs and populations. Closed responses were analysed using proportions, open responses through identifying repeating ideas and underlying concepts.

RESULTS: We included 52 responses from 25 counties; 87% working at an investigational site and 75% reporting about and interventional study. For AEs, questioning in 31% of interventional studies was a combination of general (e.g. open questions about health) and structured (e.g. reference to specific health-related items), 26% used structured only and 18% general only. No observational studies used general questioning alone. A minority incorporated pictorial tools. Rationales for the questioning approach included: standardisation of assessment or data capture, specificity or comprehensiveness of data sought, avoiding suggesting a response, feasibility, and seeking to understand participants’ perceptions. Most respondents considered the approach they reported as optimal, though several later reconsidered this. Four AE grading, and 3 causality assessment approaches were reported. Combining general and structured questions about non-study drug use were considered useful for revealing and identifying specific medicines, while pictures were said to enhance reports, particularly in areas of low literacy.

CONCLUSIONS: It is critical to evaluate the safety of the antimalarial drugs that are being deployed in large, diverse populations. Many studies would be suitable for contributing to a larger body of evidence for answering questions on harm, however our survey showed that various methods are used to obtain relevant data. We plan to facilitate the antimalarial drug clinical research community working towards consensus about the selection and/or design of optimal methods.

P061: Identification of parasite genetic factors implicated in cerebral malaria

Talleh ALMELLI1,2, Nicaise Tiuikue NDAM1,3, Philippe DELoron1,3, Rachida TAIBI1,4,5,6, Philippe DELoron1,3, Rachida TAIBI1,4,5,6

1IRD, Institut de recherche pour le développement, UMR216, Mère et enfant face aux infections tropicales, Paris, France; 2Centre d'étude et de recherche sur le paludisme associé à la grossesse et à l'enfance (CERRAGE), Faculté des Sciences de la Santé, Université d'Abomey-Calavi, Benin

BACKGROUND: Cerebral malaria (CM) is a severe complication of Plasmodium falciparum malaria infection in children living in endemic areas. The major factor of disease complication is the cytoadherence of red blood cells infected by the mature parasites (iRBCs) in vascular beds, thus reducing the microvascular blood flow leading to organs dysfunction and coma. This phenomenon is partly due to parasite proteins expressed on the surface of iRBCs and mediate the cytoadherence on endothelial cells. Our study aims to identify the parasite genetic factors implicated in cerebral malaria. We hypothesized that the transcription profiles of P. falciparum genes vary between parasites causing cerebral malaria and those implicated in mild infections.

METHODS: We performed a whole-genome microarray analysis of P. falciparum 3D7 genes. We used Cameroonian isolates from patients who had asymptomatic parasitemia (AP), uncomplicated malaria (UM) and severe complicated disease like cerebral malaria (CM). As controls, we used 3D7 strain (wild type) and Pf 3D7LIB-line which displays a severe malaria phenotype.

RESULTS: Microarrays analysis showed 100 up regulated in parasites from children with CM comparing to children with AP and 149 up regulated genes in 3D7LIB comparing to 3D7. The results were confirmed by RT-qPCR using the Cameroonian isolates employed for the hybridization and isolates from cerebral and mild malaria infections collected in Benin. The most outstanding up regulated genes in both isolates from CM and 3D7LIB encode for UPS A1 and UPSA3 var genes containing DBL domains previously shown to be implicated in cytoadherence to host cell receptors. A set of DBL domains belonging to the regulated genes as [PF11_0521and PFD0020c] were selected for further characterization. PF11_0521 displays DBLα-1.7 and CDR-g2, while PFD0020c displays DBLα-1.2 and CDR-a1.1. These architectural domains characterize Domain Cassettes DC13 and DC8, respectively, and were shown to be more transcribed in parasites from children with severe malaria.

CONCLUSION: We identified a list of genes presenting distinct expression profiles in CM isolates comparing to mild ones. We are looking forward to investigate the roles of the proteins encoded by the selected genes in inducing immune response and the potential role of antibodies directed against them in the protection from cerebral malaria.
P062: Malaria Vector Susceptibility/Resistance Status to Indoor Residual Spraying (IRS) Insecticides in Four States of Northern Nigeria

Amajoh C.N., Samdi, L.M., Inyama, P.U., Mwanasat, G.S. and Mafuyai, H.B.; Community Vision Initiative, Abuja, Nigeria; PMI AIRS Project, Abt Associates, Lafia, Nigeria; Department of Zoology, University of Jos, Nigeria; Nigerian Institute of Medical Research, Maiduguri Outstation, Borno State.

BACKGROUND: Malaria is a leading public health problem in Nigeria. Although The Federal Ministry of Health plans to reduce malaria morbidity and mortality by 50% at an affordable cost by scaling up IRS, not much is known about Insecticide susceptibility in Northern Nigeria. This study aimed to lay the foundation for malaria vector insecticide/susceptibility resistance management in four northern States of Nigeria implementing IRS.

METHODS: Insecticide susceptibility tests were carried out using the standard WHO protocol insecticide susceptibility test kits and impregnated papers. Two-to-three day old non-blood-fed adult female Anopheles mosquitoes were exposed. Batches of 20-25 mosquitoes were exposed to test papers impregnated with 0.1%, Bendiocarb 0.05% Deltamethrin, 5% Malathion and 4% DDT. Controls included batches of Anopheles exposed to untreated papers. PCR analysis was conducted on the mosquitoes tested.

RESULTS: PCR analysis showed An. arabiensis and An. gambiae s.s are both the most broadly distributed across all study sites in the Sudan and Guinea savannah. A significant difference was observed between species composition in all study sites (P<0.5). In Jigawa State, the proportion of An. gambiae s.s was 7% and An. arabiensis 91%. In Kano State, An. gambiae was 5% while An. arabiensis was 95%. In Gombe State An. gambiae was 3%, An. arabiensis 84% and An. funestus 12%. Resistance and susceptibility were observed in the study areas. In Plateau State, Anopheles gambiae was susceptible to Permethrin 0.75%, DDT 4%, 98% and 92% respectively. In Jigawa State, resistance was observed to 0.05% Deltamethrin and 4% DDT at 79.36% and 43.09% respectively. In Kano resistance to lambda-cyhalothrin 0.05% was observed for 45.09% and Deltamethrin 97%. In Gombe state 100% susceptibility to Propoxur 0.1%, Malathion 5% Cyfluthrin (0.1%) and Alphacypermethrin (0.75%) was observed.

CONCLUSION: An. arabiensis was predominant in the Sudan savannah study sites and An. gambiae s.s prevalent was predominant in Plateau state the Guinea savannah. Though pockets of resistance were observed in Kano and Jigawa States, pyrethroid resistance in Nigeria is still limited. IRS complements Long Lasting Insecticidal Net distribution.

P063: Coverage of patients and individually malaria information

Joseph Amoah

BACKGROUND: Although described in several reports, imported malaria in Ghana has not been surveyed nationwide with overall coverage of patients and individually rechecked background information. Plasmodium falciparum infections have been reported despite regularly taken appropriate chemoprophylaxis, yet the reliability of such questionnaire-based retrospective data has been questioned. This was the starting-point for conducting a prospective nationwide survey of imported malaria where compliance data was double-checked.

METHODS: Data was collected on all cases of imported malaria confirmed and recorded by the reference laboratory of Finland (population 5.4 million) from 2003 to 2011, and these were compared with those reported to the National Infectious Disease Register (NIDR). Background information was gathered by detailed questionnaires sent to the clinicians upon diagnosis; missing data were enquired by telephone of clinician or patient. Special attention was paid to compliance with chemoprophylaxis: self-reported use of anti-malarials was rechecked for all cases of P. falciparum.

RESULTS: A total of 265 malaria cases (average annual incidence rate 0.5/100,000 population) had been recorded by the reference laboratory, all of them also reported to NIDR: 54% were born in malaria-endemic countries; 86% were currently living in non-endemic regions. Malaria was mainly (81%) contracted in medina in Ghana. Plasmodium falciparum proved to be the most common species (72%). Immigrants constituted the largest group of travellers (44%). Pre-travel advice was received by 20% of those born in endemic regions and 81% of those from non-endemic regions. Of those with P. falciparum, 4% reported regular use of appropriate chemoprophylaxis (mefloquine or atovaquone/proguanil or doxycycline for regions with chloroquine-resistant and atovaquone/proguanil or doxycycline for regions with mefloquine-resistant P. falciparum); after individual rechecking, however, it was found that none of them had been fully compliant.

CONCLUSIONS: Information on compliance with chemoprophylactic regimen cannot be relied on, and it should be rechecked if malaria is suspected. The results of the present study suggest that mefloquine, atovaquone/proguanil and doxycycline are effective as chemoprophylaxis against P. falciparum malaria, when taken conscientiously.

P064: Relationship between the stage of parturition and the infection with Plasmodium falciparum in Anopheles gambiae

Rodrigue ANAOGOUN 1,2, Martin AKOGBETO 1,2

1Centre de Recherche Entomologique de Cotonou (CREC); 2Faculte des Sciences et Techniques de l’Université d’Abomey Calavi, Benin

BACKGROUND: Longevity of vectors and their infectivity for Plasmodium falciparum are two indicators used to evaluate the effectiveness of vector control programs. While it is clear that older mosquitoes are supposed to be the most infected for P. falciparum, no clear demonstration was done. This study aims to demonstrate the relationship between age grading of An. gambiae and its infection showing that multiparous mosquitoes are more infected by P. falciparum than the uniparous.

MATERIAL AND METHODS: To achieve this goal, human landing catches were performed overnight in two localities in Bénin (Adjarra and Ifangni). These two localities, where anophelines density is high, are located in the southeast of Benin. The physiological age of mosquitoes was determined microscopically by counting follicular dilatations using the technique of oil injection. The infectivity of mosquitoes for circumsporozoitid (Cs) of P. falciparum was determined by ELISA- CSP.

RESULTS: In Adjarra as in Ifangni, the infected anophelines are those having laid at least twice. In Adjarra, the infectivity rate is 35.75% in the biparous mosquitoes and 66.67% in the triparous mosquitoes. The same observation has been done in Ifangni where, with 21 biparous and 4 triparous mosquitoes, the infectivity rates are respectively 33.33% and 100%.

CONCLUSION: Mosquitoes epidemiologically dangerous are those spawned at least twice. Thus, the older mosquitoes are most infected with Plasmodium.
P065: Antenatal care visit attendance and intermittent preventive treatment (IPT) in Mutengene, Mount Cameroon Area

Judith K Anchang-Kimbi 1, Eric A Achidi2, Blaise Nkengou3, Joseph-Marie Mendimi4, Eva Sverremark-Ekstrom4, Marita Troye-Blomberg1
1Department of Zoology and Animal Physiology, University of Buea, Cameroon, 2Department of Microbiology and Parasitology University of Buea, Cameroon, 3Department of Anatomy and Pathology, University of Yaounde Teaching Hospital, Cameroon, 4Department of Immunology, Wenner-Gren Institute, Stockholm University, Sweden.

INTRODUCTION: The antenatal care (ANC) clinic provides a good opportunity for delivering interventions to control malaria in pregnancy in endemic regions. A previous study carried out in Mutengene, Cameroon reported that about 90% of women attending ANC clinic received IPT-SP at least once during pregnancy but only 52 % of these women had the recommended two or more doses. Thus this study evaluated the determinants of ANC clinic attendance and IPT-SP uptake among parturient women in the study area.

METHODOLOGY: Consented parturient women at the Mutengene Medical centre were consecutively enrolled into the study in 2007. A structured questionnaire was administered to document socio-demographic data including age, marital status, educational level, ANC clinic attendance, gestational age (GA), fever history and reported use/dosage of IPT-SP. Complete ANC attendance was considered to be at least four ANC visits during pregnancy.

RESULTS: A total of 287 women were interviewed of whom 277 (96.5%) attended ANC at least once (median visits (IQR): 5(3-6)); range from 0-12). Only 69.3% (199) had a complete ANC attendance (≥ 4 ANC visits). Among ANC attendees, 2.2%, 59.7%, and 38.1% made their first visit in the first, second and third trimester respectively. Early clinic attendance was associated (P < 0.001) with fever history where women with fever (OR= 0.51; 95%CI: 0.3-0.89) were more likely to attend four or more ANC visits. Younger age (≤ 20years) (OR=1.98; 95%CI: 1.16-3.38) and being unmarried (OR= 2.19; 95%CI: 1.25-3.83) were significant factors associated with fewer clinic visits (< 4visits). Women who received one SP dose (OR=3.7; 95%CI: 2.0-6.8) were more likely not to have attended four or more visits. Equally, a higher proportion (P<22.18; P < 0.001) of women with first visit during the third trimester (68.9%) received only one dose during pregnancy compared with those who had two or more doses (31.1%).

CONCLUSION: In the study area, mother’s age and marital status influenced ANC attendance while one SP dose was associated with late first ANC visit and fewer clinic visits. Education of pregnant women to encourage early ANC clinic attendance to scale-up uptake of two or more SP doses is recommended.

P066: Risk of rebound malaria following treatment of asymptomatic Plasmodium falciparum malaria in children residing in Western Kenya

Ben Andagalu

BACKGROUND: Asymptomatic Plasmodium falciparum carriers constitute a reservoir of parasites and thus a real public-health risk. The treatment of asymptomatic carriers with ACT is an innovative tool for breaking the cycle of infection in some transmission settings. However, little is known about the impact that the treatment of these asymptomatic carriers has on the subsequent risk of developing multiple episodes of malaria, given that that partial immunity to malaria is considered to develop following repeated exposure to the malaria parasite.

METHODS: A longitudinal cohort study of the epidemiology of pediatric malaria was conducted in Kombewa Division, Western Kenya between 2003 and 2004, in which 270 healthy children were randomized to receive either Artemether-Lumefantrine (AL) or placebo at the beginning of the study, and then followed up for one year. Active surveillance consisted of weekly visits by field workers and monthly visits at the study clinic, during which samples for malaria blood films were collected. Passive surveillance consisted of unscheduled visits, when participants had specific complaints, malaria films were done if needed and the results made available to the attending clinician.

RESULTS: Out of the 270 subjects enrolled, a total of 187 subjects [69%] completed the 12 month follow-up. A total of 328 and 344 multiple malaria episodes were recorded in the placebo and AL arms respectively over the observation period. The mean number of episodes per participant was 4 in both arms, with the number of episodes per participant ranging from 0 – 8 in the placebo arm and 0 – 6 in the AL arm. The monthly event rates were generally similar in the two arms, with an overall rate of 15.43 episodes/100 person-weeks in the placebo arm and 13.70 episodes/100 person-weeks in the AL arm. The event rate ratios in the AL arm to the placebo arm also showed no evidence of increased risk in either treatment the overall adjusted rate ratio (AL/placebo) was 0.90 (95% CI of 0.76 to 1.06, p-value=0.205).

CONCLUSION: AL administered in pediatric asymptomatic carriers have little influence on the risk of multiple clinical malaria episodes and therefore would not result in rebound malaria morbidity.

P067: Antiplasmodial Activity, β-hematin Inhibition and Cytotoxicity of 3-Hydroxyypyridin-4-one-Chloroquine Hybrids

Warren A. Andayia, Timothy J. Egana, Jiri Gut, Philip J. Rosenthal, Kelly Chibalea,b,*
1Department of Chemistry, University of Cape Town, Rondebosch, 7701, South Africa. 2Institute of Infectious Disease and Molecular Medicine, University of Cape Town, Rondebosch, 7701, South Africa. 3Department of Medicine, University of California, San Francisco, CA 94143, USA

BACKGROUND: Resistance of malaria parasites to previously widely used drugs such as chloroquine (CQ), and of mosquitoes to pesticides remain big challenges in the sustainable control of the disease. There is therefore need for the discovery of new effective medicines which are safe and efficacious against resistant P. falciparum the main causative agent for malaria. Molecular hybridization has been used in the discovery of new antimalarials. The approach can take advantage of desirable properties of one drug; for example, the transport mechanism of one compound to enhance access of the other drug to the active site. However one drug may suffer from liabilities of the other drug. The aim of this study was to synthesize HPO-CQ hybrid analogues that have activity against CQ resistant parasites elucidate the mechanism of action of these compounds with respect to inhibition of β-hematin formation and evaluate their cytotoxicity. The hybridization was justified by the synergistic antiplasmodial effect of combining N-alkyl-3-hydroxyypyridin-4-ones (precursors) with chloroquine.

RESULTS: In vitro antiplasmodial activity of the precursors was negated by blocking the chelator moiety and none of the precursors inhibited β-hematin formation. Most hybrids were more potent inhibitors of β-hematin formation than CQ, and a correlation between antiplasmodial activity and inhibition of β-hematin formation was observed. The hybrids exhibited lower in vitro cytotoxicity than CQ. This study confirms that the chelation or the free chelator moiety in these hybrid molecules could be responsible for enhancing the potency of the hybrids against resistant strains. However, data from the N-alkyl-3-hydroxyypyridin-4-ones indicate iron chelation by itself is not sufficient to cause significant antiplasmodial activity.

CONCLUSION: The antiplasmodial activity of the synthesized hybrid molecules depends largely on β-hematin inhibition. The antiplasmodial activity of the precursor enhanced in hybrid molecules could be due to accumulation in the digestive vacuole mediated by CQ moiety or via polyamine transporters. Hybridization has potential to enhance the efficacy of CQ against resistant malaria as well as mitigate against its toxicity.
P068: Statute of sensitivity to the insecticides of Anopheles funestus in a district of the Central Highlands of Madagascar

**INTRODUCTION:** Anopheles funestus, one of the major vectors of the malaria and the most endophilic of anophelian species, has been incriminated as responsible for the lethal epidemics (Bemangovitra) towards the end of the years 80 in Madagascar. The indoor residual spraying of insecticide and the use of the long lasting insecticidal impregnated net are currently the two main strategies of anti-vectorial work, according to the standard protocol of the Ministry of Health. This paper describes the sensitivity tests conducted in 2012 at the village of Alatsinainikely in the district of Murinarivo.

**METHODOLOGY:** It is about a descriptive study of vector sensitivity and all tests have been done according to the standard protocol of the WHO on seven insecticides: deltamethrin 0.05%, lambdacyhalothrin, permethrin 0.75%, propoxur 0.1% DDT 4%, fenitrothion 1% and bendiocarb 0.1%.

**RESULTS:** Anopheles funestus has been found sensitive to the three pyrethroids, to the propoxur, to the DDT and to the fenitrothion. Mortality rates were from 100% for each insecticide except for deltamethrin (99%). It was resistant to the bendiocarb with an observed mortality of 44%. 

**CONCLUSION:** Seen the apparition of the resistance of anopheles to bendiocarb in this locality, this preliminary result is essential for the choice of insecticide to use subsequently. The studies of mechanism of resistance and molecular specification of the tested species will be the subject of ulterior research.

P069: High prevalence and fixation of pfdhfr triple mutant Plasmodium falciparum in Maevatanana (Madagascar)

Voahangy Andrianaranjaka, Léonora Ravolanjarasoa, Henrielle Emasignay, Arthur Andriamiananena, Jemima Ravelonarivo, Zanah Rahasilievolo, Milliajona Randrianarivojosiosio

1Institut Pasteur de Madagascar, Antananarivo 101, Madagascar
2Ministère de la Santé Publique, Madagascar
3Faculté de Médecine, Université de Mahajanga, Madagascar

**BACKGROUND:** Antimalarial drugs play a key role for achieving malaria elimination in Madagascar. Since 2006, sulfadoxine-pyrimethamine (SP) has been recommended for intermittent preventive treatment in pregnant women in Madagascar. Thus, as part of the national network for antimalarial drug resistance surveillance activity, Plasmodium falciparum dihydrofolate reductase (pfdhfr) as drug resistance genetic marker is typed for P. falciparum isolates collected from Maevatanana.

**METHODS:** P. falciparum isolates (n=169) were collected in Maevatanana in 2009 and 2012. Samples were kept at -20°C until use. PCR followed by sequencing was used to determine the alleles of pfdhfr.

**RESULTS:** Our results demonstrated high prevalence of pfdhfr triple mutant (51, 59, 108 codon) P. falciparum in Maevatanana. It was 86.2% in 2009 (IC95%: 74.8 – 93.1%) and 82.7% in 2012 (IC95%: 73.8 – 89.2%).

**CONCLUSIONS:** There is a proven increase and a fixation of the triple mutant prevalence in Maevatanana during the last 5 years. Triple mutant prevalence in Maevatanana was 47% in 2007. The situation in 2012 is alarming. Fortunately, SP treatment still remains efficacious. Molecular markers of SP resistance including P. falciparum dihydrofolate reductase (pfdhps) must be closely monitored in Madagascar. Given that seasonal workers come (for gold mining and agricultural reason) and leave regularly in Maevatanana, the risk of mutant and drug resistant parasites from Maevatanana – linked to human population mobility – is discussed in our presentation. Understanding the emergence and the spread of mutant malaria parasite resistant to drug following malaria treatment policy change is crucial. Population-based studies should be important to evaluate the effects of drug selection on P. falciparum.

P070: High prevalence of asymptomatic malaria in urban settings in Douala, Cameroon

Léopold Gustave Lehman, Jeanne Dina Nfon Prisoo, Calvin Tonga, Hervé Nyabeyeu, Natanael Banda, Arlette Linda Ngapmen, Yamadi, Lafortune Kangam, Antoine Mouangue, Adolphe Dikoume

1University of Douala, Douala, Cameroon; 2Cameroon Business Coalition against Malaria, Tuberculosis and HIV/AIDS (CCA/SIDA), Douala, Cameroon; 3University of Yaoundé I, Yaoundé, Cameroon

**BACKGROUND:** Malaria remains a major health problem in Cameroon with 38% of consultations and 24% of deaths. The negative economical impact of malaria has encouraged a new approach targeting companies with counseling and distribution of prevention kits for workers and their families.

**METHODS:** A cross sectional study was undertaken from October 2012 to June 2013 in the town of Douala to collect preliminary data to assess the impact of the Exxon Mobil foundation control program in enterprises and communities through indoor spraying and distribution of Long Lasting Insecticidal Nets (LLINs). 2600 inhabitants of six neighborhoods, 829 workers of three enterprises were interviewed and screened with a mass diagnosis method based on malaria rapid blood test using pre-stained slides for fluorescence microscopy (CyScope®, Partec GmbH, Germany). Alongside, 785 children were screened in five schools. All positive cases were treated on the spot.

**RESULTS:** The prevalence of malaria in the 4212 participants was 37.23%, most of the infected persons (79.81%) being asymptomatic. The prevalence of malaria infection in enterprises, communities and schools was 24.49%, 38.81% and 45.47% (Chi-2=83.1, p<0.0001) respectively. Children aged less than five years recorded the highest prevalence (41.09%, Chi-2=28.9, p<0.0001). Only 38.18% of the 3641 respondents possessed a LLIN of which 31.73% were damaged. The average coverage of LLINs is alarming. Fortunately, SP treatment still remains efficacious. Molecular markers of SP resistance including P. falciparum dihydrofolate reductase (pfdhps) must be closely monitored in Madagascar. Given that seasonal workers come (for gold mining and agricultural reason) and leave regularly in Maevatanana, the risk of mutant and drug resistant parasites from Maevatanana – linked to human population mobility – is discussed in our presentation. Understanding the emergence and the spread of mutant malaria parasite resistant to drug following malaria treatment policy change is crucial. Population-based studies should be important to evaluate the effects of drug selection on P. falciparum.
P071: Health workers experiences and perceptions of the impact of clinical trials on health care services in rural Coastal Kenya

Angwenyi Vibian1, Nancy Mwangome1, Patricia Njuguna2, Sassy Molyneux1,3,4 and Caroline Jones1,5

Malaria treatment

P072: Using Innovative approaches to elicit barriers to rational use of injections in Malaria treatment

N Anjia


Gifty D. Antwi1, Imelda Bates2, Harry Tagbor1

BACKGROUND: This presentation describes the processes that led to the implementation of an enhanced antenatal care service package for malaria and anaemia in pregnancy. The purpose of implementing the package is to assess whether encouraging pregnant women to participate in the diagnosis of malaria and anaemia will improve their adherence to recommendations given at the antenatal clinics and thus improve maternal and infant outcomes. The enhanced package comprises the use of a pictorial guide for malaria and anaemia in pregnancy, rapid diagnostic testing for malaria and the haemoglobin colour scale for haemoglobin concentration estimation in addition to current antenatal practices. The pictorial guide and a desk guide to aid implementation were developed in consultation with non-health professionals, antenatal care staff and pregnant women. Antenatal care staff of 7 randomly-selected antenatal clinics were then trained and pre-testing of the package done. Development of the guides through pre-testing lasted 9 months. Implementation began in all 7 clinics in September 2012 and is currently on-going. Following implementation, monitoring visits were conducted twice monthly for first three months and then monthly till date.

RESULTS: We are currently assessing the impact of this enhanced antenatal care package using a cluster randomized controlled trial. The details of the feasibility of implementing this package, the results of the intervention and the acceptability of integrating the package into routine antenatal care will be presented at a later conference.

CONCLUSION: Antenatal care staff have been helped to integrate the package into current antenatal care practices without any disruption to service provision.
P074: Frequent but poorly documented presenting symptoms of Malaria, and less commonly observed adverse effects of anti-malarial drugs.

Chikere A. Anusiem and Eijke Arodiwe
College of Medicine, University of Nigeria Enugu Campus, Nigeria.

BACKGROUND: The typical symptoms as well as the common adverse effects of the commonly used anti-malarial drugs are apparently well known among doctors working in malaria endemic communities. However, a search through contemporary literature shows that some fairly common symptoms of malaria in Enugu Nigeria seem to be unknown or ignored in literature. Sometimes these subtle symptoms are the only presenting symptoms of malaria in an adult. There are also some noteworthy adverse effects of some anti-malarials including lesions in the external genitalia that some patients prefer to conceal and some that are less discussed with respect to West African patients. In this presentation, we highlight some of these subtle symptoms and adverse effects for the purpose of providing information for clinicians that are new to malaria endemic West Africa.

METHODS: The various symptoms of malaria documented in our clinics over the past five years were written down and the interesting commonly encountered ones were included for this poster presentation. The uncommon adverse effects of anti-malarial drugs observed in our clinical practice were also written down and those that had been convincingly pinned down to a specific anti-malarial were selected for inclusion in this presentation. Clinical photographs of genital cutaneous drug reactions were kept in a file for the benefit of clinicians who would request to see them.

RESULTS: Nightmares, and other non-classical symptoms frequently volunteered by malaria patients and adverse effects of currently used anti-malarial drugs are listed in our poster.

CONCLUSION: Whereas fever, chills, headache and vomiting are classical symptoms of malaria, adults resident for several years in malaria endemic areas (unlike non-immune persons) could have significant malaria parasitemia without exhibiting the classical symptoms. Awareness of other symptoms of malaria as highlighted in this presentation should increase the clinician’s index of suspicion and lead to early diagnosis and prompt treatment.

P075: Efficacy of two ACTs for the treatment of malaria in pregnancy in India: A randomised controlled trial

Anupkumar Anvikar1, Irene Kuepfer2, Jane Bruce2, Bina Srivastava1, DR Mishra1, Rajesh Mohanty4, PK Tyagi5, Jayne Webster2, SK Mishra1, Brian Greenwood1, Daniel Chandramohan1 and Neena Valecha1

1National Institute of Malaria Research, New Delhi, India; 2London School of Hygiene and Tropical Medicine, London, United Kingdom; 3Ispat General Hospital, Rourkela, India; 4Tata Main Hospital, Jamshedpur, India; 5National Institute of Malaria Research Field Unit, Rourkela, India

BACKGROUND: In India, national policy for treatment of malaria in pregnancy in second and third trimesters is artesunate+sulfadoxine-pyrimethamine (AS-SP). However, data on safety and efficacy of Artemisinin based combination therapy in pregnancy is scarce. We assessed the safety and efficacy of AS-SP and artesunate+mefloquine (AS-MQ) for treatment of falciparum malaria in pregnancy in India.

METHODS: This open-label, randomised clinical trial was initiated in October 2010 at three sites (Ranchi and Jamshedpur, Jharkhand state and Rourkela, Odisha state). Informed consent was obtained from women attending antenatal care (ANC) clinics with a gestational age between 12 and 36 weeks to screen for malaria at each ANC visit. Pregnant women having P. falciparum mono-infection of any parasite density with or without fever were randomised to either AS-MQ or AS-SP arm of the trial. Blood slides and filter paper samples for PCR were collected on day 0,1,2,3,14,28,42 and 63 post treatment and followed up until day 42 postpartum.

FINDINGS: Between October 2010 and June 2013, two hundred and fourteen women were found to have P. falciparum mono-infection among 6684 pregnant women. They were randomised to receive either AS-MQ or AS-SP. There were no therapeutic failures on day 28, 42 or 63 in both arms of the study. There were twenty one serious adverse events during the study. None of the severe adverse events were related to the study drugs.

CONCLUSION: Both artesunate+SP and artesunate+mefloquine are safe and effective for treatment of uncomplicated malaria in pregnancy in India.

P076: Reduction in prevalence of malaria among pregnant women and investigation on the uptake of intermittent preventive treatment in Anambra State, Nigeria

Dennis Aribodor, Stella Obianumba and Onyebuchi Ozor
Department of Parasitology and Entomology, Faculty of Biosciences, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria

BACKGROUND: Part of the efforts to control endemic malaria in Nigeria, targeted at pregnant women. Malaria during pregnancy has been a big burden in Nigeria. Monitoring progress in malaria control among pregnant women involves periodic assessment of prevalence and uptake of intervention tools. This study aimed to assess malaria prevalence and uptake of intermittent preventive treatment among pregnant women in largely rural community, Ozubulu, and relatively urban community, Awka, both in Anambra State, Nigeria.

METHODS: Microscopy of peripheral blood samples collected from 243 pregnant women during routine antenatal visits was used to determine malaria parasite. Self-administered structured questionnaire was used to determine the uptake of intermittent preventive treatment among the pregnant women. With the aid of a software SPSS version 20, data were analyzed using percentages, chi-square and logistic regression and compared with existing statistics.

RESULTS: Prevalence of malaria among pregnant women was 53.9% (131/243) in 2012, a reduction of 10% from previous studies. The compliance rate of intermittent preventive treatment was 29.2% (71/243).

CONCLUSION: Though the prevalence of malaria among pregnant women was high at 53.9%, it nevertheless was a reduction of 10% over the case within the last five years. This may not be unconnected with progress in delivery of intervention tools especially intermittent preventive treatment.
P077: Assessment of the efficacy of artesunate–amodiaquine recommended by the National Malaria Control Program in Madagascar

Ratsimbosaso Arsene

BACKGROUND: In order to assess the efficacy Madagascar, of 3-day regimens of artesunate-amodiaquine (ASAQ), the antimalarial therapies recommended by the National Malaria Control Programme (NMCP) after 7 years of adoption as first-line treatment, the efficacy of this combination were evaluated in children presenting with uncomplicated malaria.

METHODS: Children between six months and 5 years with uncomplicated P. falciparum malaria were enrolled in May 2012 to September 2012, in Mahatanga, in Southern Est, an endemic area of Madagascar. Primary endpoints were the day-28 treatment failure rate evaluated after 28 days follow-up, either unadjusted or adjusted by genotyping. Risks of clinical and parasitological treatment failure after adjustment by genotyping were estimated using Kaplan-Meier survival analysis. Secondary outcomes included fever clearance, parasite clearance, change in haemoglobin levels between Day 0 and the last day of follow-up, and the incidence of adverse events.

RESULTS: We enrolled 65 patients with uncomplicated malaria from 292 outpatients screened at the health centers. Fifty six had completed treatment and follow-up to day 28. Treatment regimen resulted in clinical cure rates above 96.4% by day-28 (adjusted by genotyping). Parasite and fever clearance was more rapid with artesunate plus amodiaquine than chloroquine, but the extent of haematological recovery on day-28 did not differ significantly between the days 0 and the last day of follow-up, and the incidence of adverse events.

CONCLUSION: These findings (i) constitute a follow-up on the efficacy of antimalarial drugs recommended by the NMCP, (ii) show that artesunate-amodiaquine drug remains effective in Madagascar, and (iii) support the current policy of ASAQ as the first-line treatment in uncomplicated falciparum malaria.

P078: Sensitivity to the insecticides of Anopheles funestus in a Central Highlands of Madagascar (example: Miarininarivo district).

Andrianaivo T.1, Randrianarivelo O.1, Rakotomanga T.1, Raharimanga R.2, Ranaivo L.1, Ramarosandratanana B.1, Ratsimbosaso A.1,2.1 National Malaria Control Program, 2 Faculty of Medicine Antananarivo

BACKGROUND: Anopheles funestus, one of the major vectors of the malaria and the most endophlic of anopheline species, has been incriminated as responsible for the lethal epidemics (Bemangovitra) towards the end of the years 80 in Madagascar. The indoor residual spraying of insecticide and the use of the long lasting insecticidal impregnated net are currently the two main strategies of vector control in work, according to the epidemiological strata of the country. Aim of this study is to tests to test sensitivity to the insecticides of Anophele funestus

P079: Diagnostic performance of CareStart™ malaria HRP2/pLDH (Pf/pan) combo test versus standard microscopy on falciparum in malaria endemic, Southern Est of Madagascar

Rakotomanga T.1, Randranianomanana M.1, Rakotondrandriana A.1, Ralino R.1, Razanandrazana B.1, Ralaibasonina Y.1, Zolinaianarisoa T.1, Rakotoavo L.1, Ramarosandratanana B.1, Randrianirimanana V.2, Rakotomanga JDM.1, Razanarisoa A.1,2, Ratsimbosaso A.1,2.1 National Malaria Control Program, 2 Faculty of Medicine Antananarivo

BACKGROUND: Microscopy is considered the gold standard for malaria diagnosis but has limitation. Rapid diagnostic test (RDT) is becoming an alternative way of establishing quickly the diagnosis malaria infection, by detecting specific malaria antigens in suspected patients’ blood, although their application at the field level is currently feasible. This study was aimed to evaluate the performance of CareStart™ Malaria Pf/Pan RDT kit for the diagnosis of malaria infections in suspected patients. Blood examination by microscopy was taken as gold standard to evaluate performance of CareStart™ kit’s sensitivity, specificity and predictive value.

METHODS: The study was conducted from health care center in malaria endemic of Southeastern of Madagascar in February to June 2012. Blood samples were collected from febrile patients referred for malaria diagnosis by clinicians.

RESULTS: Overall 104 of 257 (40.47%) malaria cases were detected by microscopy compared to 109 of 257 (42.41%). CareStart™ kit’s sensitivity and specificity for the diagnosis of malaria were 98.11% (CI 95 %: 96.4% - 99.8%) and 96.73% (CI 95 %: 94.6% – 98.9%), respectively, compared to standard microscopy. The CareStart™ positive predictive values were 95.41 (CI 95 %: 92.9% – 98%), and the negative predictive values were 98.67% (CI 95 %: 97.3% – 100%).

CONCLUSION: The RDT could be used in place of light microscopy in the most peripheral health facilities which microscopy is not available.
P080: Assessing the impact of the strategies being implemented at the community level

Ratasmibasa A1,2, Franchard T1, Rakotondraria A2, Daouda S3, Rakotarivoeny C1, Ravoy H1, Ranavo L1, Ratovonjahary M1, Menard D1, Rapelanoro R2,1, Millet P1, Malvy D1,2, Rakotomanga JDM2
1 National Control Malaria Program, 2 Faculty of Medicine Antananarivo, 3 Centre Medical d’Ampasimanjeva, 4 Université Bordeaux Segalen - France

BACKGROUND: The National Malaria Control Program decided to phase out the systematic fever treatment with antimalarial drug based on the systematic use of rapid diagnostic tests (RDTs) at Community level. We conducted a community-level study aimed to measure indicators related to malaria morbidity and identify the risk factors for fever during the year.

METHODS: we conducted study in Ampasimanjeva, Manakara District in Southern Est of Madagascar, in high area transmission between the months of October 2009 to April 2011, a longitudinal study of the study was based on a series of active case detection (ACD) with asymptomatic children every four months (October 2009, February 2010, June 2010, October 2010 and February 2011). A fever passive longitudinal follow-up (PD) on admission to the health facility was conducted with community workers from February 2009 to March 2011.

RESULTS: A total of 1932 children were identified. In ACD, the prevalence rate of malaria in the area was 9%. The multivariate analysis confirms the variation of prevalence rate depending on the villages, age, month, and body temperature. Based on health facility attendance, the incidence rate of malaria was 2.5 cases per 100 person-months, and fever incidence rate was 31.25 cases/100 person-months. The etiologies of non-malaria fevers were mostly respiratory infections of viral or bacterial. Between July to October, the risk of malaria was low.

CONCLUSION: These results are needful for orders of RDTs and ACTs. These results show a variation in risk of acquiring malaria in the current year, as well as the need to include children more than 5 years of age in fever’s case management programs at Community level.

P081: Plasmodium parasites interaction with reactive oxygen species during uncomplicated and complicated malaria infection in Ghanaian children

Richard Asmahi1, Litowell Asare1, Isaac Sarsah1, Charles Brown1, Selorme Adupko1, M Cham1, Ben Gyan1, Micheal Ofor1, David Adjei, Edwin Wiru1, Patrick Ayeh-Kumi1
1 Department of Medical Laboratory Sciences, University of Ghana School of Allied Health Sciences, Accra; 2 Noguchi Memorial Institute for Medical Research, College of Health Sciences, University of Ghana 3 Comboni Hospital, Sogakope, Ghana

BACKGROUND: Malaria is an infectious disease affecting over 200 million people of which 1-2 million die yearly mainly children. The increasing and widespread resistance of malaria parasites to current anti-malarial drugs is a major problem in prevention and treatment of the disease. During malaria infection, oxidative stress in the human host is increased due to reactive oxygen species (ROS) inducing parasite killing and tissue damage. Superoxide dismutase (SOD) represents an important enzymatic defense against oxidative stress. In this work we investigated the extent of resistance in the malaria vector An. gambiae towards developing a resistance management strategy (RMS), we have investigated the development of a RMS which will require comprehensive information on malaria vector species composition and susceptibility to insecticides used for their control.

METHODS: WHO bioassays were performed on adult Anopheles mosquitoes reared in the laboratory from larvae collected from different parts of the districts using the frequently used insecticides, notably pyrethroids (deltamethrin, permethrin and lambda-cyhalothrin), an organochlorine (DDT) and carbamate (Bendiocarbin). Molecular techniques were used for species identification, knockdown resistance (kdr) and ace-1R mutations in individual mosquitoes. These data were subjected to tests of genotype:phenotype association. The same molecular techniques were applied to mosquitoes captured using Human Landing Catches from randomly selected houses in the districts. These data were used to estimate resistance allele frequencies across the area.

RESULTS: Anopheles gambiae s.s was the predominant vector species. Bioassays showed phenotypic resistance (defined as <80% mortality) to the main insecticides. The L1014F kdr allele, often associated with resistance to pyrethroids and DDT, Ace-1R resistance alleles, associated with carbamate resistance and a new mutation termed N1575Y (an asparagine-to-tyrosine) change in the voltage gated sodium channel recently identified in the An. gambiae s.s. were detected at varying frequencies (76.2%, 28.9% and 54.7% respectively). Preliminary analysis of genotype:phenotype association tests showed a significant association (Pearson’s χ²=0.001) between the Ace-1R mutation and carbamate phenotypic resistance.

CONCLUSIONS: These data serve as baseline to inform the NMCP in the development of a RMS which will require comprehensive information on malaria vector species composition and susceptibility to insecticides used for their control.
Malaria still remains major public health problem in Ethiopia. Malaria Indicator survey (MIS) had been conducted in 2007 aimed at measuring key malaria interventions coverage and prevalence of malaria morbidity as well as parasitemia and under five children anemia. MIS-2011 has been conducted from October to December 2011 to measure the progress of malaria prevention and control efforts undertaken since 2007 and see whether the goals set forth in the FMOH National Strategic Plan for Malaria Prevention and Control 2005 - 2010 were achieved. The survey was a national level study that used a two-stage random cluster sample of 10,444 households in 440 census enumeration areas. A total of 47,248 people participated in the survey. Data were collected using Roll Back Malaria M&E Reference Group household and women’s questionnaires, which were adapted to the local context and assisted by PDA. Data collected were transferred in MS access data base and analysed using STATA and SAS statistical soft-wares. The results indicated that 55.2% of households have at least one mosquito net (of any type), and 54.8% of households have at least one long-lasting insecticidal net (LLIN). Of children U5, 38.2% slept under a net the night before the survey, and 64.5% of children US slept under a net in a household that owned at least one net. These figures were 35.3% and 63.8% respectively for pregnant women. IRS had been conducted in 46.6% of households in the last 12 months preceding the survey. It was reported that 19.7% of children US had suffered from a fever in the two weeks preceding the survey. Of these children, 51.3% sought medical attention within 24 hours of onset of fever; 32.6% took an antimalarial drug and 8.5% may reduce the cost associated to the resistance level to carbamate and organophosphate insecticides and the resistance level to carbamate and organophosphate insecticides and organophosphates (chlorpyrifos-methyl, p<0.002; fenitrothion, p<0.001) when compared to AcerKis strain. However, no differences were recorded between AcerKdrKis and KisKdr resistance level against permethrin (Pyrethroid, p=0.7) and DDT (Organochlorine, p=0.24). For adult bioassays, AcerKdrKis was less resistant to fenitrothion (p<0.001) and propoxur (p=0.03) than AcerKis but no differences were recorded for chlorpyrifos-methyl (p=1, and bendiocar (p=0.14). Furthermore, no differences were recorded between AcerKdrKis and KisKdr for permethrin (p=1), deltamethrin (p=0.4) and DDT (p=1). Concerning acetylcholinesterase enzyme, AcerKdrKis strain showed higher ACE1 activity than AcerKis (p<0.001).

CONCLUSION: The presence of both kdr and ace-1R alleles may increase the resistance level to carbamate and organophosphate insecticides and may reduce the cost associated to ace-1R. Our results provide strong evidence that concomitant kdr and ace-1R resistant alleles in individuals represent an important threat for malaria vector control in West Africa.
P086: Schistosoma and microfilaria infection are associated with an increase of antibodies to Plasmodium falciparum sexual stage antigens

Ulysses Ateba-Ngoa 1,2,3, Teun Bousema 4,5, Will Roelfsen 6, Bertrand Lell 1,2, Ayola Akim Adegnika 1,2, Peter G. Kremsner 4,5, Maria Yazdanbakhsh 1,2
1 Centre de Recherches Médicales de Lambaréné, BP : 118, Lambaréné, Gabon; 2 Institut für Trompenmedizin, Universität Tübingen, Wilhelmstrasse 27 D-72074 Tübingen, Germany; 3 Department of Parasitology, Leiden University Medical Center, Albinusdreef 2 2333 ZA Leiden, The Netherlands; 4 Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; 5 Department of Immunology and Infection, London School of Hygiene and Tropical Medicine, London, United Kingdom;

Helminth infections appear to be a potential confounder in immunological studies on Plasmodium falciparum. However, despite an increasing body of evidence documenting the effects of helminth on malaria indices it is still unclear whether a concurrent helminth infection can augment the susceptibility of the human host to gametocyte which could consequently increase malaria transmission. To address this we conducted a prospective study in Lambaréne (Gabon), an area where helminth and malaria are highly endemic. We hypothesized that helminth infected subjects will have a higher rate of gametocyte carriage and a higher antibody response to gametocyte antigens. Schistosoma haematobium and microfilaria infection were determined by urine filtration and by the Leucoconcentration technique, respectively. Gametocyte carriage was determined by microscopy and was used as a marker of current exposure to Plasmodium falciparum (Pf) sexual form. Antibody response to Pf gametocyte antigens Pf230 and Pf48/45 was assessed by ELISA. All malaria and helminth infected subjects were treated at inclusion and their infectious status and antibody response was assessed again 6 weeks following treatment. Overall a total of 287 subjects were included in our study. Among them 229 (81%) had either Schistosoma or microfilaria infection while 52 (19%) carried Pf sexual forms. Gametocyte carriage was not different between helminth infected and uninfected subjects (19% vs 20%, p=0.46) regardless the species. The proportion of responders to Pf230 and Pf48/45 was significantly higher in Schistosoma (76% vs 57%; p=0.004) and microfilaria infected subjects (80% vs 68%; p=0.038) compared to uninfected subjects. The proportion of responders to Pf48/45 significantly decreased following treatment of S. haematobium (74% vs 63%; p=0.001) and/or malaria (78% vs 60%; p=0.001; at the pre and post treatment time points, respectively). In contrary, no differences were found for the antibody response against Pf230. These results imply either an increased cumulative exposure of helminth infected subjects to Pf gametocytes or a better ability to produce antibody able to block gamete fertilization in the mosquito gut. In either case the implication for malaria control program can be important.

P087: House design modifications for mosquito-free homes: An innovative, effective and environmentally sound alternative to chemical use

H Atieli

Being a young research scientist from malaria endemic country, Kenya and a lecturer at Maseno University and equally affiliated to Kenya Medical Research Institute for the last 12years, this opportunity will benefit both my university and the country at large from the knowledge, expertise and experience that I would acquire from this seminars and conference. The opportunity to attend this conference last time in Nairobi changed my scientific life. Using the ideas from the previous meeting in Nairobi, I was able to develop an innovative malaria intervention strategy that won a grant from Canadian grand challenge. I would like to share results from this innovative idea (submitted abstract) with other researchers so as to get critique and comments on the best way to utilize this approach and some inputs that can make me scale up this vector control strategy. Likewise I would like to get opportunity to meet like-minded collaborators to work together in the modification and implementation of this innovative malaria control strategy. Apart from sharing my experience, I believe I will utilize researches displayed by world class scientist in this conference to strengthen my malaria research skills for the benefit of the country at large and also share with young scientist training at the university. The pool of trained young scientist from the university under my mentorship will have a paradigm shift in the best interest of malaria control and intervention approaches in our country. I would like to kindly request for sponsorship to facilitate my attendance to this very important event in my research career because of the financial constraints/challenge I have as a young scientist from developing country.

P088: HbC and HbS modify distinct Plasmodium falciparum binding interactions.

Oumar Attaher 1, Almahamoudou Mahamar 1, Moussa Kanoute 1, Kadidia Cisse 1, Bakary Diarra 1, Patrick Duffy 2, Alassane Dicko 2, Michal Fried 1
1 Malaria Research & Training Center, Faculty of Medicine, Pharmacy and Health Sciences, University of Dakar, Saly, Senegal; 2 Division of Clinical Immunology and Infectious Diseases, National Institutes of Health, Twinbrook 1, 5640 Fishers Lane, Rockville, Maryland 20852 USA.

BACKGROUND: Plasmodium falciparum is the deadliest of the human malaria parasites, and kills up to a million African children each year due to severe syndromes. Hemoglobinopathies reduce severe malaria risk, and existing data suggest that their protective effect may be related to an effect on parasite adhesion. Because Hbs protects from all severe syndromes while Hbc may preferentially protect against cerebral malaria, we hypothesized that host factors like Hbs and Hbc may differentially modify falciparum parasite binding to specific receptors.

METHODS: In assays using clinical isolates collected from children participating in longitudinal cohorts in Ouelessebogou, Mali, we identified novel endothelial molecules that support infected erythrocyte binding including extracellular matrix molecules and members of the integrin family.

RESULTS: Infected erythrocytes collected from children with sickle cell trait were less likely to bind to the receptor CD36 but not to other receptors, while infected erythrocyte collected from children with hemoglobin AC were less likely to bind to other endothelial receptors (E-selectin, P-selectin, ICAM1, Integrin α5b1 and ICAM2) but not to CD36.

CONCLUSIONS: In summary, our results support our hypothesis that different host factors differentially modify infected erythrocyte binding to endothelial receptors.

6th MIM Conference 2013
P089: Risk factors for malaria transmission in Engela District, of the Ohangwena region of Northern Namibia

J. Auala1, H. Sturrock2, I. Kleinischmidt3, I. Du Preez4, R. Bock4, R. Gosling4, S. Katokole4 and D.R. Mumbengegwi5

1Multidisciplinary Research Centre, Science, Technology & Innovation Division, University of Namibia, Windhoek, Namibia; 2Malaria Elimination Initiative, Global Health Group, University of California, San Francisco, USA; 3Faculty of Epidemiology and Population Health, Dept. of Infectious Disease Epidemiology London School of Hygiene and Tropical Medicine, London, United Kingdom; 4Faculty of Science, Dept. of Biological Sciences, University of Namibia, Windhoek, Namibia; 5National Malaria control program, Ministry of Health and Social Services, Windhoek, Namibia.

BACKGROUND: Malaria transmission in Namibia has declined dramatically from 477,786 in 2000 to 1546 cases in the 2012/13 malaria season. Namibia has adopted a policy of malaria elimination by 2020 (zero local transmission). This presents new challenges as interventions have been shown to have reduced malaria cases and deaths in malaria endemic areas. However, it is difficult to accurately quantify the reduction in the malaria burden in endemic areas because the common methods of assessing transmission intensity are imprecise, unreliable and costly. Therefore, there is need to evaluate better alternatives for estimating malaria disease transmission.

METHODS: Antibody levels to Plasmodium falciparum merozoite surface protein-1α (MSP-1α) was used to evaluate trends in malaria transmission after vector control using combined ITNs plus IRS and ITNs alone in Rachuonyo and Nyando districts respectively in western Kenya. Serum from 5,839 participants collected during cross-sectional surveys conducted before and after vector control were tested for anti-MSP-1α, immunoglobulin G (IgG) antibodies by enzyme-linked immunosorbent assay (ELISA).

RESULTS: The prevalence of antibodies to P. falciparum MSP-1α was significantly reduced in the ITNs+IRS district from 60.5% (95% CI: 57.7 – 62.8) to 48.7% (95% CI: 44.9 – 49.5) after the intervention, (χ2 = 11.762, df = 1, P = 0.001). In contrast, there was only marginal reduction in sero-prevalence in the control ITNs+No-IRS district, from 48.3% (95% CI: 44.5 – 51.0) to 47.2% (95% CI: 46.1 - 50.7) after the intervention, (χ2 = 3.307, df = 1, P = 0.069). There was a reduction in the age-specific sero-conversion rates from λ = 0.1272 to λ = 0.0571 in ITNs+IRS district and from λ = 0.1070 to λ = 0.0607 in the ITNs+No-IRS district at the two time points. Parasite prevalence reduced from 8.6% (95% CI: 7.2 - 10.1) to 6.9% (95% CI: 5.8 - 8.2) in the ITNs+IRS district following the intervention. In contrast, it increased significantly in ITNs+No-IRS district from 10.4 % (95% CI: 8.5 - 12.5) at baseline to 20.4% (95% CI: 18.5 - 22.3) at the second survey.

CONCLUSION: This study validates the use of antibody responses to MSP-1α to monitor and evaluate the effectiveness of malaria control interventions in malaria endemic areas.

P090: Evaluation of malaria transmission intensity using antibody responses to Plasmodium falciparum merozoite surface protein-1α after vector control in western Kenya

Shehu Shagari Awande1,2, Michael Gicheru3, Kephis Otieno4, Peter Otieno4, Philip Onyona5, Mary Hame1, John Gimng1 and Simon Kariuki2

1Kenyatta University, Department of Zoological Sciences; 2Centre for Global Health Research, Kenya Medical Research Institute/Centres for Disease Control and Prevention, Kisumu, Kenya; 3Centers for Disease Control and Prevention, Division of Parasitic Diseases, Atlanta, GA 30341, USA

BACKGROUND: The scale up of malaria control strategies such as insecticide-treated bednets (ITNs) and indoor residual spraying (IRS) have reduced malaria cases and deaths in malaria endemic areas. However, it is difficult to accurately quantify the reduction in the malaria burden in endemic areas because the common methods of assessing transmission intensity are imprecise, unreliable and costly. Therefore, there is need to evaluate better alternatives for estimating malaria disease transmission.

METHODS: Antibody levels to Plasmodium falciparum merozoite surface protein-1α (MSP-1α) was used to evaluate trends in malaria transmission after vector control using combined ITNs plus IRS and ITNs alone in Rachuonyo and Nyando districts respectively in western Kenya. Serum from 5,839 participants collected during cross-sectional surveys conducted before and after vector control were tested for anti-MSP-1α, immunoglobulin G (IgG) antibodies by enzyme-linked immunosorbent assay (ELISA).

RESULTS: The prevalence of antibodies to P. falciparum MSP-1α was significantly reduced in the ITNs+IRS district from 60.5% (95% CI: 57.7 – 62.8) to 48.7% (95% CI: 44.9 – 49.5) after the intervention, (χ2 = 11.762, df = 1, P = 0.001). In contrast, there was only marginal reduction in sero-prevalence in the control ITNs+No-IRS district, from 48.3% (95% CI: 44.5 – 51.0) to 47.2% (95% CI: 46.1 - 50.7) after the intervention, (χ2 = 3.307, df = 1, P = 0.069). There was a reduction in the age-specific sero-conversion rates from λ = 0.1272 to λ = 0.0571 in ITNs+IRS district and from λ = 0.1070 to λ = 0.0607 in the ITNs+No-IRS district at the two time points. Parasite prevalence reduced from 8.6% (95% CI: 7.2 - 10.1) to 6.9% (95% CI: 5.8 - 8.2) in the ITNs+IRS district following the intervention. In contrast, it increased significantly in ITNs+No-IRS district from 10.4 % (95% CI: 8.5 - 12.5) at baseline to 20.4% (95% CI: 18.5 - 22.3) at the second survey.

CONCLUSION: This study validates the use of antibody responses to MSP-1α to monitor and evaluate the effectiveness of malaria control interventions in malaria endemic areas.

P091: Evaluation of the antimalarial activity of the aqueous leaf extract of Gossypium barbadense (Malvaceae) in mice

Olufunso Awodele
Department of Pharmacology, College of Medicine, PMB 12003, Ibadan, Osun State, Nigeria

BACKGROUND: There is upsurge resistance to Artemisinin based combination therapy in the management of malaria thus the wide use of medicinal plants for malaria infections. Some medicinal plants have been shown to have antimalarial activity when used as combination therapy. Gossypium barbadense has been used by herbal medicine practitioners in combination with other herbs, and as a monotherapy in the treatment of malarial infection.

RESEARCH PROBLEMS: There has been no scientific evaluation of the antimalarial potentials of Gossypium barbadense. The study was, therefore, aimed at evaluating the antimalarial effect of the aqueous leaf extract of G. barbadense using mice infected with P. berghei.

RESEARCH METHOD: The suppressive effect was evaluated by administering 25 mice divided into five groups with 250, 500, and 1,000 mg/kg of aqueous leaf extract of G. barbadense, 5 mg/kg of chloroquine, and 10 mL/kg of distilled water, respectively, starting from the day of inoculation with P. berghei for four days. The curative effect was evaluated by administering 25 mice divided into five groups as above with treatment starting 72 h post inoculation with P berghei.

RESULTS: The results indicate that the aqueous leaf extract of G. barbadense, when used alone as monotherapy, has a non-significant (P ≥ 0.05) but slight suppressive antimalarial activity (23 %) when compared with that of chloroquine (100 %). The curative model also revealed that aqueous leaf extract of G. barbadense showed no significant antimalarial activity.

CONCLUSION: G. barbadense as monotherapy for malaria has no significant therapeutic effect. Therefore, it is recommended that it should be combined with other herbal medicine to manage malaria infection.
P092: Parasite neutralization by vaccine-induced antibodies against the blood-stage malaria antigen, *P. falciparum* Reticulocytoc-binding Protein Homologue 5 (PfRH5)

Dennis K. Awual1, Andrew R. Williams1, Julie M. Furze1, Alexander D. Douglas1 & Simon J. Draper1

1Jenner Institute, University of Oxford, Old Road Campus Research Building, Roosevelt Drive, Oxford, OX3 7DQ, UK; 2Current address: Department of Immunology, Noguchi Memorial Institute for Medical Research, College of Health Sciences, University of Ghana, Legon, Accra-Ghana

**BACKGROUND:** No vaccine has yet proven effective against the blood-stages of malaria infection in humans. Recent studies indicate that PfRH5, a *P. falciparum*-specific protein expressed in merozoites, is efficiently targeted by vaccine-induced, broadly-neutralizing antibodies. Additionally, PfRH5 is highly conserved across parasite lines and does not appear to be a major target of naturally-acquired immunity to *P. falciparum*. Despite the promise shown in pre-clinical studies by vaccines encoding full-length PfRH5, little is known regarding functional mechanisms of antibody-mediated neutralization. We therefore aimed to study the effectiveness of polyclonal and novel monoclonal antibodies against PfRH5 in assays of growth inhibition.

**METHODS:** Rabbit and mouse antibodies were raised to PfRH5 (full-length 3D7 sequence) using recombinant human adenovirus (AdRH5) and poxvirus (MVA) vectored-vaccines. Antibodies were purified using protein G columns and were capable of binding recombinant PfRH5 by ELISA and parasites by IFA; monovalent Fab IgG fragments were generated by treatment with papain/ ficin. *P. falciparum* clone 3D7 cultures were maintained and synchronized by the Percoll-sorbitol method. *In vitro* assays were performed under initial conditions of 2% haematocrit uninfected RBCs and ~0.4% schizonts; parasite levels were quantified after a single cycle of growth by measurement of pLDH.

**RESULTS AND DISCUSSION:** Bivalent polyclonal and monoclonal anti-PfRH5 IgG demonstrated a more effective inhibition of parasite growth compared to their respective monovalent Fab equivalents (*P* < 0.05; repeated measures two-way ANOVA). However, this reduced inhibition by monovalent Fab antibody forms was dependent on IgG concentration (*P* < 0.001; *P* < 0.05 for lower and higher concentrations respectively). Antibody-mediated growth inhibition via PfRH5 antigen crosslinking and/or direct steric inhibition of receptor binding may thus play some role in the mechanism of parasite neutralization. In further studies, although epitope-specific monoclonal anti-PfRH5 antibody combinations also achieved parasite neutralization, there was no evidence of a synergistic interaction on growth of *P. falciparum* (*P* > 0.05; Mann-Whitney tests).

**CONCLUSION:** Results demonstrated in this study provide further indication of the potential of PfRH5 as a suitable vaccine target. However, further analyses are required to determine whether simple antibody blockade of important sites on PfRH5 is sufficient for parasite neutralization, or whether cross linking of the antigen is important.

P094: Proportion of atypical memory B cells from a malaria holoendemic region of Kenya does not vary with age but correlates with antibodies to *Plasmodium falciparum* merozoite surface protein-3 and glutamate-rich protein.

Ayleko C.1, Wilmore J.1,2, Ondio B.1,2, Ofulla A.V.O.1, Jura W.G.Z.O.1, John C.C.1,3, Rochford R.4

1Maseno University, Maseno, Kenya; 2SUNY Upstate Medical University, Syracuse, NY, USA; 3Center for Global Health Research, Kenya Medical Research Institute, Kisian, Kenya; 4University of Minnesota Medical School, Minneapolis, MN, USA

**BACKGROUND:** Antibodies play a central role in protection against malaria infection and disease, evidence yet naturally acquired antibodies to malaria antigens are slow to acquire and apparently short-lived absence of persistent parasite exposure. Recent studies have associated acute and chronic *Plasmodium falciparum* infection with expanded populations of atypical memory B cells. However, the functions of atypical memory B cells in this context have not been elucidated.

**OBJECTIVE:** To assess the distribution of B cell subsets in a population exposed to holoendemic malaria and determine their relationship with specific antibody responses to *P. falciparum* antigens.

**METHODS:** B cell phenotypes and antibodies to pre-erythrocytic and blood-stage antigens were assessed in sixty randomly selected volunteers from a malaria holoendemic region of western Kenya. B cell phenotyping was done by flow cytometry while antibodies were measured by cytometric bead assay.

**RESULTS:** Age correlated negatively with the percentages of transitional B cells (*r* = -0.47, *P* = 0.002) and marginal zone B cells (*r* = -0.38, *P* = 0.014) and positively with IgM+ B cells (*r* = 0.34, *P* = 0.03). In contrast, there was no correlation between age and percentages of atypical naive B cells, resting classical memory B cells and atypical memory B cells by age (*P* > 0.05). A significant correlation was documented between atypical memory B cell frequencies and levels of antibodies to the *P. falciparum* antigens merozoite surface protein-3 (*r* = 0.06, *P* = 0.02) and glutamate rich protein GLURP region-2 (*r* = 0.04, *P* < 0.0001).

**CONCLUSIONS:** The frequency of atypical memory B cells is associated with levels of antibodies to MSP-3 and GLURP-R2. Further studies are required to demonstrate whether atypical MBC are responsible for generation of antibodies to these antigens, and if so, how this may affect antibody half-life.
P095: Validation of field-based real-time PCR testing for diagnosis of *Plasmodium falciparum* infection

**George Ayodo**, Eliud Onyango1, Priscah Cheruiyot1, M. N. Manjo2, Ibrahim Daudi1, Chandy C. John1

1Kenya Medical Research Institute, Kenya; 2University of Minnesota, USA; 3Bigtec Labs, Bangalore, India

**BACKGROUND:** For malaria elimination campaigns, testing methods more sensitive than microscopy are required to detect low-level parasitemia. Testing should also be specific and easy to conduct. We present results of testing using a portable real-time PCR machine (microPCR, bigtec Labs, Bangalore, India) for detection of *Plasmodium falciparum* infection in a field clinic setting.

**METHODS:** 400 individuals <15 years who visited Gobei Health Centre, Kenya with a temperature ≥37.5°C were tested for on site by microscopy and microPCR and later in our research by real-time PCR (qPCR).

**RESULTS:** Of the 400 individuals, 310 had interpretable microPCR results and were tested by qPCR. Of these 310 individuals, 197 (63.3%) were positive by both microscopy and microPCR, 56 (18.1%) were positive by microPCR alone, none were positive by microscopy alone, and 57 (18.4%) were negative by both testing methods. 196 (63.2%) were positive by both microscopy and qPCR, 47 (15.2%) were positive by qPCR alone and 66 (21.2%) were negative by both testing methods. Using microscopy as the gold standard, sensitivity and specificity were 100% and 50.4%, respectively, for microPCR and 99.4% and 58.4%, respectively, for qPCR. In the 231 persons positive for *P. falciparum* by microPCR and qPCR, parasite density correlated strongly (Spearman’s rho=0.66, P < 0.0001). However, 23 individuals were positive by microPCR but negative by qPCR (median parasite copy number [25th, 75th percentile] 770 [200, 4800]), and 13 individuals were positive by qPCR but negative by microPCR (median parasite copy number [25th, 75th percentile] 29.7 [17.8 – 232.6]).

**CONCLUSIONS:** The microPCR machine, a real-time PCR machine designed for field use, has the potential to accurately detect sub-microbial parasitemia and level of parasitemia in a clinic setting. Further refinement of sample processing and DNA extraction should decrease the frequency of uninterpretable results.

P096: Malaria in HIV patients attending the Mutengene Treatment Centre, Southwest Cameroon

**Bate Ayukenchengamba1**, Emmaculate Lum1, Helen Kimbi1, Elias Onyoh2 and Pascal Bessong3

1Department of Zoology and Animal Physiology, Faculty of Science, University of Buea, P.O. Box 63 Buea, Buea, SWR, Cameroon; 2Mbingo Baptist Hospital, Bogo Division, NWR, Cameroon; 3Department of Microbiology, University of Nenda, South Africa

**BACKGROUND AND AIMS:** HIV-positive children and pregnant women are more at risk of malaria because HIV and pregnancy worsen the malaria situation. Such patients need antiretrovirals (ARV) to reduce morbidity and mortality rates due to HIV/AIDS. This study was aimed at analysing the impact of HIV and pregnancy on malaria during pregnancy in a rural area in Cameroon.

**METHODS:** HIV patients were recruited. A structured questionnaire was used to record information on sex, age, economic status, education level, marital status, duration and time of 1st ARV collection and malaria preventative methods used. Blood samples were collected and blood films were Giemsa-stained for determination of malaria parasite prevalence and density. Haemoglobin concentration was determined using a haemoglobinometer and CD4 T cell count was determined using a Guava PCA instrument.

**RESULTS:** A total of 434 HIV patients (234 children aged 1 – 15 years and 200 pregnant women) were enrolled in the study. The overall malaria parasite prevalence was 24.4%. (24.8% in children and 24.0% in pregnant women). Malaria parasite prevalence was significantly higher (χ²=5.414, P =0.020) in children who were on ARV when compared to their counterparts that were not. The values were similar in pregnant women. Malaria parasite prevalence was significantly higher (χ²= 8.185, P=0.004) in females (31.9%) than males (16.4%). Prevalence was also significantly higher (χ²=6.671, P= 0.036) in the youngest age group of pregnant women, patients implementing at least 3 malaria preventive measures (χ²=11.376; P= 0.023) and children (36.4%) in clinical stage 1 (χ²=9.729, P = 0.021). The overall prevalence of anaemia in the general population was 53.5% and the value was significantly higher in children (χ²=16.500, P= 0.001) and pregnant women (χ²=11.873, P= 0.003) with CD4 T cells of 200-499 and <200 respectively. Factors such as CD4 T cell social class and duration on ARV did not affect malaria parasite and anaemia prevalence.

**CONCLUSION:** HIV patients need to be educated on the importance of adhering to ARV treatment in order to improve on their health conditions thus avoiding malaria related morbidity and mortality.

P097: Defining Correlates of Protection from Placental Malaria Using a Predictive Multi-Assay Approach

**Anna Babakhanyan1,2, Naveen Bobbili1, John Chen1, Philomina Gwamensia1, Rose G.F. Leke4 and Diane W. Taylor1**

1Department of Tropical Medicine, Medical Microbiology and Pharmacology, John A. Burns School of Medicine, University of Hawaii at Manoa, Hawaii, USA; 2Biotechnology Centre, University of Yaounde I, Yaounde, Cameroon

**BACKGROUND:** Malaria during pregnancy poses risk of serious health complications for approximately 85 million mothers and developing fetuses worldwide. *Plasmodium falciparum*-infected erythrocytes accumulate at the maternal-fetal interface of the placenta using the VAR2CSA adhesion molecule, creating a condition known as placental malaria. Antibodies against VAR2CSA improve pregnancy outcome and a vaccine based on VAR2CSA is feasible. However, VAR2CSA antibody function or functions that mediate protection from placental malaria are unknown. Knowing correlates of protection will expedite development and field-testing of a malaria vaccine for pregnant women. Therefore, the goal of the study was to identify correlates of protection from placental malaria.

**METHODS:** Plasma samples collected at delivery from Cameroonian women with (n=115) and without (n=345) placental malaria were screened in multiple assays to measure antibodies to recombinant full length VAR2CSA, its 6 DBL domains and 15 strain variants, the surface of infected erythrocytes, as well as IgG isotype, IgG avidity, FcγR-mediated opsonic phagocytosis, and antibody-mediated inhibition of binding.

**RESULTS:** Each assay was considered individually using receiver operating characteristic curve method to determine sensitivity and specificity. No correlation with protection was found using a single assay. We are in the process of analyzing all the assays in a multivariable logistic model, adjusting for important covariates and eliminating assays that do not significantly contribute to the overall model. Final model in a form of equation based on the selected assays will provide a way investigators can predict the probability a woman will have placental malaria at delivery.

**CONCLUSION:** Results will define a correlate(s) of protection from placental malaria, which will provide a way to evaluate vaccine efficacy in field trials. In addition, clinicians will have a method for early identification of women at risk of placental malaria and therefore early intervention.
P098: Activities of artesunate, amodiaquine and artesunate plus amodiaquine during repeat administration for treatment of *Plasmodium berghei* infection in mice: parasite clearance, behavioural response and organ histomorphological and biochemical alterations

Ahmed A Adedeji1, Sabur O Badmosi2, Mufiati T Akinwumi1, Abolane A Oduoliki1, Ahmed K Omotosh2, Adebola A Rasheed1, Adekunle S Adebola1, Mariam O Balogun1, Gbemiola C Adekunle1, Oluwatosin O Abiona1, Adekunle A Adesesin1, Nicholas A Adesoye1, Samuel O Ogungbola1, Ayobami A Adegumi1, Oyeronde T Oyeleke1, Aminat O Adegbite1, Abosede O Popoola2, Eniola O Ashaye1, Phillips Oguntolu1, Kubura M Okeowo1

1Communicable Diseases Research Unit, Department of Pharmacology, Olabisi Onabanjo University, Ogun State, Nigeria; 2Department of Pharmacology and Toxicology, School of Health Sciences, Kampala International University, PO Box 71, Bushenyi, Uganda; 3Department of Biochemistry, School of Health Sciences, Kampala International University, PO Box 71, Bushenyi, Uganda.

**BACKGROUND:** Although chemotherapy still remained a mainstay strategy for malaria control, safe use of antimalarial drugs obtained from drug stores in communities self-treating malaria is a growing concern. The effects of amodiaquine (AQ), artesunate (AS) and their combinations (AQAS) when orally administered repeatedly were investigated to determine the parasitological clearance, feeding behaviour and histomorphological and biochemical changes of kidney and liver of *Plasmodium berghei* infected mice.

**METHODS:** Sixty mice were grouped into, viz: infected placebo treated (IP-t), infected artesunate-treated (IAS-t), infected amodiaquine-treated (IAQt), infected artesunate/amodiaquine-treated (IAQAS)t, uninfected artesunate/amodiaquine treated (uASAQt) and uninfected control groups (uc); and treated at first exposure to *P. berghei*. The feeding behaviour and parasitaemia levels were determined, and blood/organ sample collected in 50% of the animals. Following parasitaemia clearance in the remaining mice and recovery period, a re-infection, treatment and follow-up procedures were done.

**RESULTS:** The feeding behavioural pattern assessment indicates that the IPT consumed more feeds and water. The body weight and Packed Cell Volume (PCV) were significantly reduced during second treatment in all the groups. The parasite clearance was similar in AQ, AS and AQAS treated mice in the first exposure but a significant rise by day 3 in parasitaemia was observed in the AS treatment group at second exposure relative to AQ and AQAS treatments (p= 0.0001). The histomorphology section of the kidney was normal in all animals, but the liver histomorphology of AQ treated groups showed numerous pigments of laden kupffer cells with focal hepatocyte necrosis compared with other groups. The Alkaline Phosphatase (ALP) concentration during the first and repeated treatment were similar (p-value >0.05) but the Alanine Transaminase (ALT) was significantly reduced at repeated treatment (p-value <0.05) in the AQ/AS infected, AQ/AS uninfected and IPT groups relative to uninfected control group.

**CONCLUSION:** The findings in this study suggest that the repeated use of AQ or AQAS antimalarials may be detrimental to body organs and the general well-being of individuals with consecutive use of the drugs for malaria treatments. AS play important protective role against parasite induced physiological alterations but its monotherapy appear to lose potential for parasitologic clearance.

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P099: Temporal variation in exposure to *Anopheles gambiae* salivary and risk of malaria transmission

Kingsley Badu, Ben Gyan, Maxwell Appawu, Guiyun Yan, Kwadwo Koram

Noguchi Memorial institute for medical Research, College of Health Science, University of Ghana, Accra, Ghana

Human biting rates and mosquito infection rates vary across space and time. Heterogeneity in the distribution of mosquito populations generate variability in the risk of human infection. Assessment of exposure to malaria vectors is important to our understanding of disease transmission risk, and facilitates planning of control efforts. *Anopheles gambiae* salivary peptide (gSG6-P1) has been designed to enhance its specificity and immunogenicity to detect human exposure to malaria vectors. We evaluated total IgG responses to gSG6-P1 and two malaria antigens (CSP, MSP-1) in an age stratified cohort (< 5, 5-9, >9) from Asutuare, South-western Ghana, an area of relatively low but perennial transmission. 200 randomly selected sera were analyzed from archived samples belonging to a cohort that were followed at 3 contact times (n = 600) as follows; February, toward the end of the dry season, May at the peak of the major rainy season and August a dry period before the minor rainy season, representing snap shots of the perennial transmission in the year. Seropositivity above threshold of negative group to the 3 antigens was detected in the cohort at all contact points across age groups. Although, seroprevalence showed temporal trends similar to rainfall and mosquito exposure patterns, specific responses to MSP 1 and CSP, Chi square analysis did not yield significant differences among the different time points. MSP 1, 30.3%, 30.6%, 36.7% (chi2= 1.91, p = 0.38), CSP; 21.7%, 19.4% and 23.3% (Chi2 1.53, p =0.46) in Feb, May and August in respective order. In contrast to the above, analysis of seroprevalence and median antibody levels to gSG6-P1 showed significant differences, detecting temporal variations in vector exposure among the cohorts at different time points. Where gSG6 - P1, from Feb, May and August respectively were; 46.2%, 49.7% and 35.7%, (chi2= 7.41, p = 0.02). Repeated measures ANOVA as well as post hoc Tukey multiple comparison test showed significant difference in antibody levels in mosquito exposure between the peak rainfall and dry period preceding minor rainy season. It is concluded that gSG6-P1 is robust and sensitive to detect temporal changes in human exposure.
Background: Artemisinin isolated from *Artemisia annua* is the most potent antimalarial against chloroquine-resistant *Plasmodium falciparum* malaria. *A. vulgaris*, an invasive weed, is the only *Artemisia* species in Sri Lanka. A prototype study investigated antimalarial activities of *A. vulgaris* ethanolic leaf extract (AVELE) in a *P. berghei* ANKA murine malaria model that elicit pathogenesis similar to falciparum malaria.

Methods: Air dried leaves of *A. vulgaris* were soaked in an organic solvent mixture of diethyl ether, methanol and petroleum ether at a 1:1:1 ratio. Resulting dark green solution was filtered and evaporated at reduced pressure using a rotavapour to obtain a crude extract paste at 5% ethanol to obtain the required dosages. The 4-day suppressive and curative assays determined anti-parasitic activity of four doses of AVELE (250, 500, 750 and 1000 mg/kg), Coartem® and 5% ethanol (negative control) in male ICR mice (N=6/group) infected with *P. berghei*. Anti-disease activity of *A. vulgaris* was evaluated in terms of effect on thrombocytopenia, end-stage disease, anti-pyretic activity, and antinociception, using standard methodology. Toxicity of the extract was determined by chronic administration of the high dose of AVELE, while phytoconstituents of AVELE was evaluated using preliminary phytochemical analysis and TLC.

Results: The 500, 750mg and 1000 mg/kg doses of AVELE significantly (P≤0.01) inhibited parasitaemia by 79.3%, 79.6% and 87.3% respectively, in the 4-day suppressive assay, but not in the curative assay. AVELE also significantly reversed the profound thrombocytopenia (P≤0.01), altered the end-stage disease (P≤0.05), increased survival, and manifested significant anti-pyretic and antinociceptive (P≤0.05) activities using the 1000 mg/kg dose. Chronic administration of the high dose of AVELE ruled out overt signs of toxicity and stress, plus hepatotoxicity, nephrotoxicity and haematotoxicity. Phytoconstituent analyses of AVELE suggested the presence of alkaloids, coumarines, and a group that contains higher alcohols, phenols, steroids and essential oils.

Conclusion: Oral administration of a crude ethanolic leaf extract of *A. vulgaris*, possesses potent and safe anti-malarial properties, in terms of both anti-parasite and anti-disease (anti-pyretic activity, peripheral and central antinociception, increased survival, averted end-stage disease and reversed thrombocytopenia) activities.

P101: Adherence to artemisinin-based combination therapy: a review of the evidence

Kristin Banek, Mirza Lalani, Sarah G. Staedke, Daniel Chandramohan

Department of Clinical Research, London School of Hygiene and Tropical Medicine, London, UK; 1 Department of Disease Control, London School of Hygiene and Tropical Medicine, London, UK

Background: Increasing access to and targeting of artemisinin-based combination therapy (ACTs) is a key component of malaria control programmes. To maximize efficacy of ACTs and ensure adequate treatment outcomes, patient and caregiver adherence to treatment guidelines is essential. This review summarizes the current evidence base on ACT adherence, including definitions, measurement methods, and factors associated with adherence.

Methods: A systematic search of the published literature was undertaken in November 2012 and updated in April 2013. Bibliographies of manuscripts were also searched and additional references identified. Studies were included if they involved at least one ACT and reported an adherence measurement for any population.

Results: The search yielded 1,412 records, 37 of which were found to measure adherence to ACTs. Methods to measure adherence focused on self-report and pill counts with varying definitions for adherence. Most studies only reported whether medication regimens were completed, but did not assess how the treatment was administered. Adherence data were available for four different ACT formulations: artemether-lumefantrine (AL) (range 39-100%), amodiaquine plus artesunate (AQ+AS) (range 48-94%), artesunate plus sulfadoxine-pyrimethamine (AS+SP) (range 39-75%) and artesunate plus mefloquine (AS+MQ) (range 77-95%). Association between demographic factors, such as age, gender, education and socio-economic status and adherence to ACT regimens was not consistent. Two studies found that children under 5 were less likely to be adherent. Three studies reported that caregiver education was significantly associated with adherence; caregivers with higher education levels were more likely to be adherent. Some evidence of positive association between adherence and patient drug preferences, health worker instructions, patient/caregiver knowledge and drug packaging were also observed.

Conclusions: Our review highlights the weak evidence base on ACT adherence. Results suggest that ACT adherence levels varied substantially between study populations, but comparison between studies was challenging due to differences in study design, definitions, and methods used to measure adherence. Standardising methodology for evaluating adherence of different formulations across diverse contexts would improve the evidence base on ACT adherence and effectiveness. Additionally, further studies of the individual factors associated with adherence are needed in order to make informed policy choices and to improve the delivery of effective treatment.

P102: Polyamine analogues, targeting epigenetic regulatory mechanisms, have anti-proliferative activity against *Plasmodium falciparum*

Bernice Barnard1, Bianca Verlinden1, Patrick Woster2, Braam Louw3 and Lyn-Marie Birkholz4

1Department of Biochemistry, Faculty of Natural and Agricultural Sciences, University of Pretoria, 0002, South Africa; 2Department of Pharmaceutical and Biomedical Sciences, Medical University of South Carolina, USA

Background: Due to increasing resistance of malaria parasites to current antimalarials, compounds are constantly tested for improved antiplasmodial activity. Histone post-translational modifications (PTMs) contributes to epigenetic regulation in *Plasmodium falciparum*. An epigenetic regulatory enzyme, Lysine-specific demethylase 1 (LSD1) regulates gene expression through the modulation of chromatin structure and the removal of methyl groups from lysine residues (Cui et al., 2008). A library of polyamine analogues were tested in cancer cells and found to specifically inhibit LSD1 (Huang et al., 2007). These were subsequently shown to have antiplasmodial activity with IC50 values in the low nanomolar range (Verlinden et al., 2011). A library of polyamine analogues were tested in cancer cells and found to specifically inhibit LSD1 (Huang et al., 2007). These were subsequently shown to have antiplasmodial activity with IC50 values in the low nanomolar range (Verlinden et al., 2011).

Methods and results: The leading compound was fluorinated at four different positions to advance metabolic stability, compound potency and was tested for enhanced antiplasmodial activity. The compounds showed >3000 fold selectivity towards the *P. falciparum* parasite compared to mammalian cells (HepG2) and a leading compound inhibited parasite proliferation irreversibly after 12 h treatment.

Conclusions: The fluorinated polyamine analogues have potent antimalarial activity and the future aim of this study is to determine if the two leading fluorinated polyamine analogues result in epigenetic regulatory changes within the malaria parasites by specifically targeting LSD1 activity.
**P103: Defining adequate lumefantrine exposure in patients with uncomplicated malaria treated with artemether-lumefantrine**

Karen I Barnes on behalf of the Worldwide Antimalarial Resistance Network [WWARN] Lumefantrine PK/PD Study Group

**BACKGROUND:** Achieving adequate antimalarial drug exposure is pivotal for curing malaria. Drug exposure (pharmacokinetic, PK) and response (pharmacodynamic, PD) data from individual studies are generally insufficient to accurately define the minimum drug exposure required to achieve a cure. The ‘therapeutic’ day 7 lumefantrine concentrations published to date range from 170 ng/mL to 500 ng/mL, suggesting that this threshold needs to be better defined.

**METHODS:** To address this question, the WWARN Pharmacology module has lead the largest pooled analysis of individual patient antimalarial PK-PD data to date. With the cooperation of researchers globally, PK-PD data from 26 studies in 3,926 patients was contributed, comprising 92.6% of all patients in lumefantrine PK-PD studies identified. The relationship between Day 7 lumefantrine concentrations and treatment response was examined with the aim of defining target Day 7 lumefantrine concentrations across all key target populations.

**RESULTS:** Day 7 lumefantrine concentrations and treatment response were available for 2,715 patients from 23 studies. PCR confirmed recrudescence occurred in only 80 patients (2.9%), while 332 patients acquired new Plasmodium falciparum infections during follow-up. The main drivers of recrudescence were Day 7 lumefantrine concentration [HR 0.53 (95% CI 0.43-0.67), p<0.001] and baseline parasitaemia [HR 2.20 (95%CI 1.46-3.29), p<0.001]. Adjusted for mg/kg dose, day 7 lumefantrine concentrations in children < 5years of age were 33% lower than concentrations in older patients. Day 7 lumefantrine concentrations were 81% higher with supervised than unsupervised treatment. The highest risk of recrudescence was observed in areas of emerging artemisinin resistance, very low transmission intensity and in malnourished children under 5 in areas with moderate/high transmission intensity. To achieve 95% efficacy in these high risk groups, Day 7 lumefantrine concentrations up to ≥1000 ng/mL (depending on baseline parasitaemia) would be required. For all other populations studied, Day 7 concentrations above 200ng/ml appear sufficient.

**CONCLUSIONS:** Current AL dosing recommendations are adequate for most patients. However, alternative dosing strategies may be needed in malnourished young children. In areas with artemisinin resistance and the very low transmission areas we studied, it is unlikely that effective AL concentrations could be achieved, so alternative treatments should be recommended.

**P104: Comparative Key Malaria and Birth Delivery Practices Among Traditional and Skilled Birth Attendants in Esit Eket Local Government Area, Akwa Ibom State, Niger Delta Region, Nigeria**

Edueno Victor Bassey¹, Heyinwa Nkechi Bright¹, Bright C. Oji⁰¹
¹Primary Health Center, Ikot Abasi, Akwa Ibom State; ²Tropical and International Health Consultants, Ibadan; ³Ihepiego, Abuja, Nigeria.

**BACKGROUND:** Traditional (TBAs) and Skilled Birth attendants (SBAs) have played prominent roles in saving the lives of women during birth delivery. High maternal annual deaths led to the use of antenatal care clinic as the platform for promoting malaria in pregnancy prevention, while safe-motherhood promotes birth deliveries with the SBAs. TBAs are not recognized as SBAs and no specific roles assigned in the area of malaria prevention and control. However, women still patronize TBAs. This study investigates key malaria preventive and birth delivery practices among TBAs and SBAs in Esit Eket LGA, Akwa Ibom State, Nigeria.

**METHOD:** Study is a comparative exploratory cross section in design. Three FGD discussions were held with TBAs, SBAs and women that deliver with them between 18 – 48 years old. The study guide explored issues on socio-economic demographic data, key delivery practices, knowledge of malaria preventive methods and practices

**RESULTS:** The study revealed that the TBAs and SBAs malaria prevention practices included advice pregnant women to keep away from the sun, dirty environment, return home early from the farm, avoid mosquitoes, go to the hospital for a test and eat fruits and vegetables compared to the SBAs. The TBAs use of insecticide-treated-nets and intermittent-preventive-treatment of malaria during pregnancy. Knowledge gaps still exist among the SBAs, and TBAs malaria prevention practices, none mentioned IPTp while some of the SBAs mentioned the use of Chloroquine.

**CONCLUSION:** Reproductive health managers need to design training interventions to upgrade the knowledge of the SBAs and TBAs on malaria prevention and effective birth delivery practices for the TBAs and their clients in order to reduce maternal deaths during child birth.

**P105: Immune mediated selection of Plasmodium falciparum identical genetic variants via variant surface antigens**

¹² AK Bel, ¹ Å Diouf, ¹ K Mbi, ¹² RF Daniels, ¹ G Tullu, ¹ AE Zeituni, ¹² SK Volkman, ¹ AD Ahouidi, ¹ D Ndile, ¹² T Dieye, ¹ S Mboup, ¹ CA Long, ¹² DF Wirth
¹ Department of Immunology and Infectious Diseases, Harvard School of Public Health, 665 Huntington Avenue, Boston, MA, 02115, USA; ²Laboratory of Bacteriology and Virology, Le Dantec Hospital, and Laboratory of Parasitology, Cheikh Anta Diop University, BP 7325, Dakar, Senegal; ³Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA; ⁴Broad Institute of MIT and Harvard, 7 Cambridge Center, Cambridge, MA 02142, USA

**BACKGROUND:** As malaria transmission intensity has declined in some regions, Plasmodium falciparum parasite populations are displaying decreased genetic diversity. We have observed and monitored genetically identical parasite clusters from 2006-2012 in Thies, Senegal, and we have characterized these parasites to determine whether they are epigenetically and antigenically identical.

**OBJECTIVE:** The objective of the study is to test the hypothesis that the emergence, decline, or expansion of these populations is mediated or modulated by the human host immune system.

**METHODS:** We focus on one cluster of identical parasites that was present in 24% of clinical isolates in 2008 and declined to 3.4% in 2009. We studied the susceptibility of 2 representative common genetic signature (CGS) parasites to invasion inhibitory antibodies using Growth Inhibition Assays (GIA), and we measured infected RBC IgG reactivity by variant surface antigen (VSA) flow cytometry. We characterized the var genes expressed by CGS parasites by var Ups qRT-PCR and by sequencing using degenerate DBL domain primers.

**RESULTS:** We find that the CGS parasites are similarly susceptible to invasion inhibition by patient IgGs from 2008 and 2009, implying that invasion-blocking immunity is not the selective pressure against these parasites in 2009. By VSA flow, these parasites are recognized similarly by plasma IgG from 2008 and 2009, but reactivity against both is dramatically increased in 2009. Such findings could imply that VSAs present on infected RBCs are the target of immune responses that, while permissive in 2008, selected against these parasites in 2009. As PFEMP-1 is a dominant component of the VSA response, we investigated the role of var genes and we observed a surprising similarity in the var Ups class expression among CGS parasites, marked by a striking upregulation of UpsA var genes. To determine the identity and diversity of the var genes expressed, we cloned and sequenced var DBL domains. We observed that the CGS parasites expressed largely 2-cysteine containing DBL domains compared to non-CGS parasites that expressed 4-cysteine DBL domains.

**CONCLUSION:** Our work indicates that there is selection against common genetic signature parasites at the level of surface expression of VSAs and possibly implicates rosetting PFEMP-1 molecules in the phenotype.
P106: Reliability of Rapid Diagnostic Tests in The Diagnosis of Malaria amongst children in Two Communities in South West Nigeria.

Ibrahim S Bello, Akinjide O Ogundokun, Bridget Omosore
Department of Family Medicine, Obafemi Awolowo University Teaching Hospital, Ile Ife, Nigeria

BACKGROUND: Prompt treatment of malaria following adequate diagnosis help to reduce morbidity and mortality in children. Lack of resources and adequate manpower especially in primary healthcare facilities in many communities in Western make microscopic diagnosis which is the gold standard difficult. Rapid Diagnostic Test Kit despite its availability and ease of use has not been incorporated into primary healthcare because of paucity of data in the region as to its effectiveness leading to over-prescription of ACTs.

METHOD: A total of 132 children age range 1-9 years, mean age of (5 years) were screened for malaria routinely with rapid diagnostic test kits (RDTs HRP 2) at the general outpatient department of OAU Teaching hospital as well as at comprehensive health centre at Imesi Ile, South West Nigeria during the world malaria day exercise 2013. Needle prick was used to collect blood sample for thick and thin smear. Giemsa stain was used before microscopic examination was carried out on the slide.

RESULTS: From a total of 132 children who had rapid diagnostic test done, only 98 of the children had both RDTs and microscopy done. A total of 28(28.6%) children tested positive while 70(71.4%) children tested negative for malaria. In the Microscopy category, 27(27.6%) children tested positive while 71(72.4%) tested negative for malaria parasite. Out of the 27 children tested positive, RDT picked 25 as positive and 2 as negative, (sensitivity = 92.6%). While out of the 71 that tested negative for microscopy, RDT picked 68 as negative and 3 as positive (specificity = 95.8%). The positive predictive value and negative predictive values are 89.3% and 97.1%.

CONCLUSION: Rapid Diagnostic Test is an effective diagnostic tool for malaria amongst children in the study population. Primary and secondary health centres in the region should adopt Rapid Diagnostic Test in malaria diagnosis before administration of ACTs to avoid unnecessary treatment.

P107: Capacity of Private Sector Providers to Diagnose, Treat, and Report Malaria in an Elimination Setting: The Case of Swaziland

Adam Bennett1, Mbongiseni Mathobela2, Sarah Darthe1, Joseph Novotny3,4, Simon Kunene6
1 Center for Applied Malaria Research and Evaluation, Tulane University School of Public Health and Tropical Medicine, New Orleans, Louisiana; 2 National Malaria Control Programme, Ministry of Health, Manzini, Swaziland; 3 Clinton Health Access Initiative, Mbabane, Swaziland; 4 Global Health Group, University of California, San Francisco, United States of America

BACKGROUND: Swaziland is currently in the process of transitioning from malaria control to elimination, which requires the confirmed diagnosis of all suspected malaria cases, utilization of prompt and effective treatment, and case-based surveillance at health facilities. While the public health sector is well engaged in these activities, there is little knowledge of the capacity of private sector health providers to participate in malaria elimination efforts.

METHODOLOGY: Following service availability mapping (SAM) efforts, we conducted a survey of all known private, non-governmental (NGO), industrial, and mission facilities in Swaziland in June 2013 to assess capacity to diagnose, treat, and rapidly report malaria cases. Additionally we conducted a single focus group with seven private sector providers identified through the survey to explore barriers and potential solutions to strengthening private sector engagement.

RESULTS: Of 88 interviewed facilities, 25 (28.4%) reported at least one malaria diagnosis in the past six months. Fifty-nine (67.0%) facilities offered diagnostic testing for malaria, and of these 57 reported using rapid diagnostic tests (RDTs) for diagnosis. Only 21 reported confirming RDT results by microscopy; of these only 5 confirmed on-site whereas the majority used an external laboratory. Over half of facilities (56%) had the government-recommended front-line antimalarial in stock on the day of interview. Most facilities (77.0%) reported malaria cases to the health management information system (HMIS) on a monthly basis, but only 55% reported to the immediate disease notification system. Nearly all providers reported willingness to report confirmed malaria cases to both systems but cited lack of training as the primary reason for not doing so.

CONCLUSION: This assessment suggested a high degree of capacity and willingness within the private health sector to engage in malaria elimination activities. Greater outreach by the National Malaria Control Programme and inclusion of private health providers in government-led malaria trainings are needed to ensure that all malaria cases are rapidly identified, treated, and reported.

P108: Advancing drug registration for neglected tropical diseases in Africa-e.g-Malaria

Fabienne Benoist, Aurelia Aryy.
Novartis Pharma, AG ,Basel, Switzerland

BACKGROUND: There is increasingly more research and development of novel compounds and new formulations for the developing world, especially for tropical diseases. Consequently, African health authorities (HAs) are becoming more involved in the assessment of novel products. African HAs may have limited experience and lack the required resources for a thorough regulatory assessment. In some cases, African HAs do leverage prior assessments by regulatory authorities with robust regulatory expertise such as the US FDA, Swissmedic and EMA which is delaying the product availability for patients. Moreover, since registration dossiers for tropical diseases are not always submitted to these HAs, African HAs may be in the position to be the first to make an assessment. This presentation will focus on efforts to enable African HAs to perform a thorough assessment by leveraging of existing regulatory frameworks and initiatives and by support for evolving local regulatory processes and reform. Examples are given in the final presentation.

SUMMARY: Several regulatory mechanisms/systems have offered benefits, and helped develop some standardization in the assessment of drugs and devices for diseases specific to or predominant in the African population.

CONCLUSION /DISCUSSION: There is still a need for further capacity building and strengthening of the African HAs. Thus, there are limited resources in most HAs for effective clinical trials, pharmacovigilance, post-marketing surveillance and auditing, which is crucial in the heavily-counterfeited markets of some countries. There is also a need for a mechanism to allow for information exchange between the African HAs, hence benefitting from a reviews conducted by better-resourced authority's review.

Ethann Bessaens1, Otto Phanstiel2, Lyn-Marie Birkholz1
1Department of Biochemistry, Faculty of Natural and Agricultural Science, University of Pretoria, Pretoria, Gauteng, South Africa; 2Department of Medical Education, University of Central Florida, 6850 Lake Nona Blvd, Orlando, FL 32827, USA

BACKGROUND: The sustained control and elimination of malaria requires novel approaches to combat the emergence of artemisinin resistance. Polyamines are involved in a variety of cellular functions including cell differentiation and proliferation. The presence of polyamines in the asexual intraerythrocytic Plasmodium falciparum, is essential for parasite survival. DL-α-difluoromethylornithine (DFMO) elicits cytostatic effects on the parasite, attributable to compensatory mechanisms such as exogenous polyamine uptake. Consequently, a strategy focused on developing drug candidates that exploit the polyamine uptake mechanisms has emerged. In this study, a novel class of polyamine conjugates, shown to inhibit polyamine uptake in mammalian cancer cell lines, are being assessed for their ability to inhibit the in vitro proliferation of malaria parasites.

METHODS: The antiplasmodial activity of varied concentrations of anthracene-polyamine analogues in the presence or absence of DFMO was assessed on culture adapted intra-erythrocytic P. falciparum (3D7) using the malaria SYBR Green I growth fluorescence assay.

RESULTS: The third generation of anthracene-polyamine analogues do not inhibit the in vitro intra-erythrocytic proliferation of the parasite (50 μM). However, combination of these compounds with DFMO (1 mM) showed a significant 4-fold (P<0.001, n=2) or 1.5-fold (P<0.001, n=2) decrease in parasitemia, compared to DFMO (1 mM) treated controls, for triamide 444 and triamide 444, respectively.

CONCLUSION: The preliminary data indicates that the triamide 444 conjugate has a greater enhancing effect on the inhibition of parasite proliferation in combination with DFMO, perhaps owing to the conjugate’s longer polyamine functionality. The enhanced inhibitory effect of DFMO indicates a similar mechanism as that seen in mammalian cancer cell lines, whereby polyamine uptake is additionally inhibited. Further studies are ongoing to elucidate the potential benefit of the combination on the global threat of resistant malaria parasites.

P110: Modelling the effects of deltamethrin resistance on the resting behaviour of malaria vectors in North Cameroon: a cross-sectional study

Josiane Etang1,2, Fesuh Nono Betrand1,3, E-P. Ndong Nguema1, Parfait Awono-Ambene1, Henri Gwet1
1Laboratoire de Recherche sur le Paludisme, Organisation de Coordination pour la lutte contre les Endémies en Afrique Centrale, Yaoundé, Cameroon; 2Faculty of Medicine and Pharmaceutical Sciences, University of Douala, Cameroon; 3Ecole Polytechnique Yaoundé, Cameroon

BACKGROUND: The emergence of vector resistance to insecticides is a serious threat in the fight against malaria. In Cameroon, especially in the northern region, resistance to deltamethrin has been reported in An. gambiae s.l., the main malaria vector in the country. It is commonly believed that the development of resistance may induce a change in the resting behaviour and result in the failure of interventions. The aim of this study was to build a model on resting behaviour of A. gambiae s.l. carrying metabolic based resistance to deltamethrin insecticide, in response to the wide use of LLINs in North Cameroon.

METHODS: Data from a cross-sectional study involving the composition of vector species collected indoors and in exit traps and those on deltamethrin susceptibility were collected in 27 clusters in three health districts in the northern region of Cameroon. The Kruskal-Wallis rank sum test and the Chi-squared independence tests were used to identify relationships between variables. A two level ordinary logit model was used to relate resting behaviour and deltamethrin resistance, controlling the other factors. Analyses were done in EXCEL and in R.

RESULTS: Data including 374 A. gambiae s.l.collected indoors (81.55%) and at exit traps (18.45%) was used to build the behavioural model. The A. gambiae complex was represented by A. arabiensis (72.99%), A. coluzzii (7.21%), and A. gambiae s.s. (1.87%). A total of 272 (22.42%) of the 1211 A. gambiae s.l. collected was found resistant to deltamethrin. Model results showed an increasing chance for mosquitoes to remain indoors when the level of deltamethrin resistance increased. This increase was statistically significant for the A. arabiensis and A. coluzzii species (p-value<0.05), with remarkable variability observed for the A. arabiensis and very little variability for A. coluzzii. The effects of deltamethrin resistance on resting behaviour of these species decreased from unfed to freshly fed and finally to gravid.

CONCLUSION: Current model highly suggests behavioural change in A. arabiensis in the North Cameroon where there is a wide use of LLINs. This species commonly known to be exophilic was mostly found endophilic while developing deltamethrin resistance, leading to increasing malaria risk.

P111: Study on the Reproductive Potential of DDT Resistant Anopheles arabiensis sampled from Dendi Woreda, Central Ethiopia

T Beyene

BACKGROUND: Although its application is provisionally halted, utilization of DDT in Ethiopia dates back to the mid-1950s. However, resistance to DDT, in An. arabiensis; has been reported from different parts of the country. One of the strategies of insecticide resistance management is absence of insecticide selection pressure due to the assumption resistant genes tends to drift out of vector populations; through a life table study. This study envisaged to assess the status of the fecundity, fertility, developmental time and survival using the different life stages of field sampled DDT resistant female An. arabiensis in Ethiopia.

METHOD: The study was done on 80 indoor resting adult An. arabiensis collected from human dwellings and animal shelters in Dendi Woreda, Central Ethiopia. Eggs were oviposited in separate filter paper cups and counted under a dissecting microscope to determine fecundity. Fertility was determined using the number of operculated eggs hatched and the time of hatching. The developmental time was a record of time for each larval transformation to subsequent life stages. Survival rate were determined using percentage for a specific life stage transformation. Species specific Polymerase Chain Reaction (PCR) used to confirm the identity of An. arabiensis. Resistance status was checked using the standard WHO insecticide susceptibility test kit.

RESULT: The mean number for oviposited eggs was 144 and the hatching rate for the oviposited eggs was 116. Only 29.3% of larvae transformed to pupae and 23.3% of adults have emerged from their pupae stage. The larvae development time was comparable with other findings with a mean 12.8 ± 2.2 days. Male to female ratio was found with an almost equal proportion (1.32:1.35).

CONCLUSION: The reduced larvae survival rate and an equal or greater larval development time observed in this study are good prospects in malaria vector control strategies and managing insecticide resistance with regard to selection pressure.
P112: Plasmodium falciparum AP1m localizes to the cis-Golgi and is implicated in the transport of parasite proteins

Belinda C. Bezuidenhout1,2 and Thérèsa L. Coetzee1,3
1WITS Research Institute for Malaria (WIRM); 2Department of Molecular Medicine and Haematology, University of the Witwatersrand; 3National Health Laboratory Service, Johannesburg, South Africa

BACKGROUND: Plasmodium falciparum is the causative agent of the most severe form of malaria and insights into the molecular mechanisms by which it functions are critical. Numerous proteins are required for the invasion and remodelling of host erythrocytes, and it is postulated that newly synthesized proteins are transported from the Golgi network to their target destinations by specific interactions of a Yxxf motif in the C-terminal region of cargo proteins with the medium subunit (µ) of an adaptin protein (AP1) complex. The hypothesis is that a P. falciparum AP1µ is responsible for binding to invasion and/or host-cell remodelling proteins and transporting them to specific subcellular compartments within the parasite.

METHODS: Bioinformatic analysis of the putative P. falciparum AP1µ subunit, encoded by PF3D7_1311400, revealed a cargo-binding domain (PFAP1mBD), which was cloned and expressed as a recombinant GST-tagged protein. This was used to biopan P. falciparum phage display libraries to identify binding partners. Transgenic parasites were created by transfection of 3D7 parasites with a PFL0675c containing an armadillo repeat downstream of the binding domain. Since PFL0675c does not display the characteristics typical of AP1 cargo, it is postulated to be an accessory protein to the complex. Fluorescence microscopy showed that in vivo, the GFP-tagged PFAP1m localized to distinct foci on the nuclear periphery, coinciding with ERD2, a cis Golgi marker.

CONCLUSION: PFAP1m cargo proteins from the Golgi network to specific subcellular compartments within the parasite.

P113: Strengthening malaria research in Africa – The MCDC model

Amit Bhasin
Malaria Capacity Development Consortium1
1 Centre for Medical Parasitology, University of Copenhagen, Denmark, College of Health Sciences, Makerere University, Kampala, Uganda, College of Medicine, University of Malawi / Malawi - Liverpool - Wellcome Trust and the Bill & Melinda Gates Foundation, UNICEF/UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases, EDCTP, NIH’s Fogarty International Center, DfID, Danida and the Wellcome Trust’s African Institutions Initiative but there still remains a need to increase the number of African scientists to support these expanding malaria control programmes. This will become more important as these programmes become technically more demanding and their focus moves from control towards local and regional elimination programmes.

The research and capacity strengthening activities of the Malaria Capacity Development Consortium (MCDC), funded by the Welcome Trust and the Bill & Melinda Gates Foundation, and building on previous work of the Gates Malaria Partnership is highlighted in a short 10 – 15 minute film. This provides an overview of the work of MCDC in each of its five African partner institutions, addresses some of the challenges faced and uses improving research capacity in malaria in Africa as a model to drive change and support both individuals and institutions and the impact of the research being undertaken by students and post-doctoral fellows. The work of the consortium is framed within the wider context of the challenges faced by the malaria community and other capacity strengthening programmes.

P114: Submicroscopic infections of Plasmodium falciparum in symptomatic individuals of Franceville, southeastern Gabon

Claude Biteghé Bi Essone

INTRODUCTION: Malaria still remains a life threatening disease. Watered throughout year by two rainy seasons, malaria transmission is perennial in Gabon. P. falciparum represents more than 95% of all species from the country. Patients with P. falciparum submicroscopic infection (infection without circulating plasmodies by standard microscopy examination) are not detected and therefore remain untreated. PCR provides a powerful tool to diagnose this kind of infection allowing an accurate management of disease with regard to its eradication policy.

OBJECTIVE: To determine the prevalence of both patent and submicroscopic infections of P. falciparum in symptomatic patients.

MATERIALS AND METHODS: A cross-sectional study was carried out in Franceville, during two periods from May to July 2011 and from February to May 2012. A total of 595 symptomatic patients were enrolled, 250 in the first and 345 in the second period. A clinical examination and parasitological diagnosis by microscopy and PCR was carried out in all patients.

RESULTS: Of the 250 patients enrolled in the first study period, the prevalence was 24% (60/250) and 9.6% (24/250) for patent and submicroscopic infections respectively, a true prevalence of P. falciparum infection was 33.6% (84 / 250). In the second period of the study, a prevalence of 21.45% (74/345) for patent infections and 9.57% (33/345) for submicroscopic infections was obtained. The true prevalence of malaria due to P. falciparum was 31.01% (107/345). The prevalence of submicroscopic infection significantly decreases from 18.18% to 8.20% in February until May, while that of patent infection increases from 15.91% to 26.23% in the same period. Sixty-nine percent (69%) of the samples assessed by microscopy and PCR were negative for malaria.

CONCLUSION: The submicroscopic infections occur in symptomatic patients in an average of 9% of cases with a relative high prevalence at the beginning and the end of the rainy season.
P115: Anopheline fauna and its implication in the transmission of malaria in Pitoa health district, Northern Cameroon

Njeamboxay B.A.1, Bigoga J.D.1, Esemu L.1, Fodjo B., Tabue R.1, Tedjou A.1, Nanfack F.1, Leke R.F.1
1Molecular Parasitology and Disease Vector Research Laboratory, the Biotechnology Centre, University of Yaounde I

BACKGROUND: The northern part of Cameroon, because of its humid climatic conditions coupled to its marshy areas used for rice cultivation and a large number of stagnant pools resulting from the river Benoue, has been noted to be a zone heavily infested with mosquitoes. This is directly reflected in the high prevalence and an increasing incidence of clinical malaria in this area. In this study we aimed at examining the role of the Anopheles fauna in the transmission of malaria in Pitoa Health District in order to improve upon anti vector control measures.

METHODOLOGY: Mosquitoes were sampled by night collection (18:00-06:00) on human volunteers in twelve villages. They were identified at species level using various identification keys. Ovaries from female mosquitoes were dissected and parity status determined by observing tracheal distention. Plasmodium falciparum circumsporozoite antigen indices were estimated after the identification of CS antigen by ELISA.

RESULTS: A total of 2250 Anopheles mosquitoes were collected, with Anopheles gambiae s.l representing the principal Anopheles vector (77.2%). Anopheles funjeus (7.8%), Anopheles funestus (6.4%), and Anopheles paludis (5.1 %) were minor vector species. Averagely, individuals received 3.5 ib/p/n. However, the entomological inoculation rate was as high as 8.5 ib/p/n in some villages. Anopheles gambiae showed a parity index of 82.5%.

CONCLUSION: Anopheles gambiae remains the principal Anopheles vector species in Pitoa. This results show that existing anti vector control measures have to be reinforced and new methods implemented in order to scale down transmission in this zone.

P116: Transfusion does not significantly contribute to parasite clearance in artesunate-treated

Stephen Boateng

BACKGROUND: Exchange transfusion (ET) has remained a controversial adjunct therapy for the treatment of severe malaria. In order to assess the relative contribution of ET to parasite clearance in severe malaria, all patients receiving ET as an adjunct treatment to parenteral quinine or to artesunate were compared with patients treated with parenteral treatment with quinine or artesunate but who did not receive ET. ET was executed using a standardized manual isovolumetric exchange protocol.

METHODS: All patients in the Rotterdam Malaria Cohort treated for severe P. falciparum malaria at the Institute for Tropical Diseases of the Harbour Hospital between 1999 and 2011 were included in this retrospective follow-up study. Both a two-stage approach and a log-linear mixed model approach were used to estimate parasite clearance times (PCTs) in patients with imported malaria. Severe malaria was defined according to WHO criteria.

RESULTS: A total of 87 patients with severe malaria was included; 61 received intravenous quinine, whereas 26 patients received intravenous artesunate. Thirty-nine patients received ET as an adjunct treatment to either quinine (n = 23) or artesunate (n = 16). Data from 84 of 87 patients were suitable for estimation of parasite clearance rates. PCTs were significantly shorter after administration of artesunate as compared with quinine. In both models, ET did not contribute significantly to overall parasite clearance.

CONCLUSION: Manual exchange transfusion does not significantly contribute to parasite clearance in artesunate-treated individuals. There may be a small effect of ET on parasite clearance under quinine treatment. Institution of ET to promote parasite clearance in settings where artesunate is available is not recommended, at least not with manually executed exchange procedures.

P117: Insecticide resistance in Anopheles gambiae population from RD Congo

Thierry L. Bobanga1, Solange E. Umesumbu2, Billy S. Kunyu1, Olivier A. Fataki1, Alain S. Mandoko3, Celestin N. Nsibu1
1Department of Tropical Medicine, Faculty of Medicine, University of Kinshasa, DR Congo; 2Malaria National Control Program, Kinshasa, DR Congo; 3National Institute for BioMedical Research, Kinshasa, DR Congo; *Department of Pediatrics, University of Kinshasa, Kinshasa, DRC

BACKGROUND: Anopheles resistance in Africa is recorded in many countries. Control methods against malaria currently depend among other on deploying insecticide-treated nets, and vector resistance to insecticides is one of the main limitations for the use of these treated materials.

METHODS: The study was carried out from October 2010 to January 2011 in 9 sites

RESULTS: Any resistance to carbamates was observed. The An. gambiae populations from Butembo and Goma was fully susceptible to all insecticides (mortality rates >98%). Resistance to DDT was observed in all others sites, and highest was at Lubumbashi (4% mortality rate). Resistance to permethrin was observed in 7 sites and suspect resistance to deltamethrin was observed in one site (Lubumbashi). In many cases, prior exposure to synergists partially restored insecticide knockdown effect and increased mortality rates, suggesting a role of detoxifying enzymes in increasing mosquito survival upon challenge by pyrethroids.

CONCLUSION: The resistance is increasing in the DRC, detoxification enzymes are involved in this resistance in addition to the kdr gene. Vector control tools should reflect the resistance in the country.
P118: In vitro susceptibility of Plasmodium falciparum to antimalarial drugs five years after the change for treatment policy of uncomplicated malaria in Burkina Faso

Léa Bonkian

INTRODUCTION: Resistance to commonly used antimalarial drugs represents the major drawback and obstacle for controlling malaria in endemic countries. Burkina Faso has changed in 2005 its antimalarial drug policy for the treatment of uncomplicated malaria from Chloroquine to Arthemether-Lumefantrine and Amodiaquine + Artesunate. This study, conducted in Bobo Dioulasso, aims to study at comparing the in vitro sensitivity of the different ACT components with the results obtained various components of ACTs used in Burkina Faso and current antimalarial drugs after the implementation of effective use of ACT.

MATERIALS AND METHODS: The study was conducted from July 2009 to February 2010. Blood samples were collected from patients with a parasitemia between 4000 and 200000 trophozoïtes /µl and cultured in 5% CO₂ for 48 hours. These patients were treated at the inclusion.

RESULTS: A total of 40 blood samples were collected. We obtained, 2.78% resistant isolates to quinine, 6.06% to monodesethyl amodiaquine and 52.94% to chloroquine. The geometric mean IC50 of lumefantrin, dihydroartemisinin and piperaquin were respectively 30.61 nM, 1.31 nM and 8.38 nM.

CONCLUSION: At the end of this study, we conclude that five (05) years after the adoption of policy for use of ACT in the treatment of uncomplicated malaria in Burkina Faso, there is a lower rate of in vitro resistance to quinine. Regarding dihydroartemisinin, there is no great change in the geometric mean IC50 values. And finally, we have a good antiplasmodial activity for monodesethyl amodiaquine, lumefantrin and piperaquin.

P119: Study Of Hospital Based Malaria Cases In The Pediatric Department Of Korle Bu Teaching Hospital, Ghana

Cecilia. E. Lokor1, Seth. Amankwa2, David. K. Dosoo1, Felix. A. Botchway3,4

1Pathology Department, Korle Bu Teaching Hospital, Accra, Ghana, 2Chemical Pathology Department, University of Ghana Medical School, Accra, Ghana, 3Kintampo Health Research Centre, Kintampo, Ghana, 4Pediatric Department, Korle Bu Teaching Hospital, Accra, Ghana.

BACKGROUND: Malaria kills about one million children, under five years of age, each year worldwide, with nine out of 10 deaths occurring in sub-Saharan Africa. Rapid diagnosis is a prerequisite for the initiation of effective treatment and to reduce the mortality and morbidity of malaria. Microscopic examination of blood smears remains the gold standard for the diagnosis of malaria, but it is time-consuming and requires skilled microscopist. The most promising new malaria diagnostics are the serological dipstick tests. This study was carried out to determine the incidence of malaria in the pediatric department of Korle Bu Teaching Hospital from January 2011 to October 2011, and to compare available diagnostic tests for malaria.

METHODS: 978 suspected cases of malaria (507 males and 471 females, aged 1 day - 12 years), attending the Outpatient Department and admitted as inpatients in the Emergency Room of the Pediatric Department of Korle Bu Teaching Hospital were included in this study. 1.0 ml of blood sample was collected into EDTA bottle from all febrile cases clinically suspected of malaria, before starting any treatment. Thick and thin smears were prepared, stained and examined. Subsequently, the blood samples were subjected to antigen detection using the First Response Malaria pLDH/HRP 2 Combo Test according to the manufacturer’s instructions. The results were tabulated and analyzed statistically.

RESULTS: 51 cases out of 978 suspected cases were positive for malaria, with an incidence of 5.2%. Out of these 40 (78.4%) were positive for Plasmodium falciparum, 5 (9.8%) were positive for Plasmodium malariae, 2 (3.9%) were positive for Plasmodium ovale, and 4 (7.8%) were positive for both Plasmodium falciparum and Plasmodium malariae. The First Response Malaria pLDH/ HRP 2 Combo Test detected 51 positive cases compared with the blood smear study, which detected 41 cases. 36 cases were detected both by the First Response Malaria pLDH/ HRP 2 Combo Test and blood smear study. 15 cases were positive by the First Response Malaria pLDH/ HRP 2 Combo Test, but not by the blood smear study. 5 cases detected to be positive by the blood smear study were found to be negative by the First Response Malaria pLDH/ HRP 2 Combo Test. 937 cases were negative both by the First Response Malaria pLDH/ HRP 2 Combo Test and the blood smear study. Among 51 positive cases, 35 were males with a percentage of 68.6% as compared to females (31.4%). The sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic efficiency of the First Response Malaria pLDH/ HRP 2 Combo Test when compared to microscopy, were 87.5, 96.8, 90, 98.9, and 96, respectively.

P120: Stage-specific transcriptional profiling of Plasmodium falciparum gametocytes.

Mariëtte E. Botha, Janette Reader, Lyn-Marie Birkholtz
Department of Biochemistry, University of Pretoria

BACKGROUND: Malaria transmission requires the production of dormant male and female gametocytes in the human host. Mosquito blood meal ingestion stimulates gametocytes to produce gametes which mate and develop into infectious sporozoites, responsible for transmission to humans. Novel malaria eradication strategies should focus on global elimination of asexual erythrocytic, as well as the longevous mature stage IV/V gametocytes and reproduction of liver stages, thereby preventing transmission. It is imperative that the maturity of gametocytes is confirmed prior to performing gametocidal assays so that mature stage IV-V parasites are assessed as end-point. In this study, stage-specificity was initially evaluated microscopically, followed by quantitative Real-Time PCR (qRT-PCR) and flow cytometric (FACS) analysis.

METHODS: Sexual parasite production was ensured through removal of asexual parasites by N-acetyl glucosamine (NAG) treatment. Periods of NAG treatment were varied to obtain early and late stage gametocytes and development was monitored microscopically. Diagnostic qRT-PCR was used to confirm stage-specificity by transcript changes, using primers for the asexual stages and gametocyte-specific genes. Normalisation to Seryl tRNA synthetase and fold-change comparisons for asexual parasites and gametocyte stages were performed. Flow sorting of SYBR Green I stained parasites was preceded by gametocyte enrichment using the MidiMACS™ and LS columns. Excitation of cells was at 497 nM and emission detected using a band-pass filter of 515 - 545 nM. Sorting was performed by population gating on the basis of FITC fluorescence. Parasites (0.5 - 1x10⁶) were collected in PBS and analysed microscopically.

RESULTS: Gametocytes were visible from day 3-4 and stage IV-V observed microscopically after day 13-15. Preliminary results suggested that transcript levels of gametocyte specific genes were significantly higher in gametocytes than asexual stages and this correlated with literature. A comparison of stage-specific transcript expression levels and microscopic data is currently underway. A maximum enrichment of 85% was obtained, as determined with microscopy and verified using FACS. Several populations were observed, possibly indicating different stages or sexes of gametocytes. Two distinct populations were morphologically determined to be stage IV-V gametocyte cultures with forward-side scatter plots. Future analysis includes microscopic analysis of the gated, sorted populations and correlation thereof with qRT-PCR data.
P121: Prevalence of glucose-6-phosphate dehydrogenase deficiency, HbS and HbC living in children under 5 years-old efficiency living in a high and seasonal Plasmodium falciparum transmission area of Burkina Faso.

E.C. Bougouma1, A.Tiono1, V Mangano1, I.Soulama1, A.Ouedraogo1, A. Diarra1, A.Gansané1, M.Lankouande1, A.T. Konaté1, The MalariaGEN Consortium1, O. Nébié1, K Rockett2, D. Modiano and S.B.Sirima3
1Centre National de Recherche et Formation sur le Paludisme, Ouagadougou, Burkina Faso, 2Department of Public Health Sciences, Sapienza University of Rome, Italy, 3A global network for investigating the genomic epidemiology of malaria; Malaria Genomic Epidemiology Network. *Welcome Trust Centre for Human Genetics, University of Oxford, Oxford, United Kingdom

BACKGROUND: Resistance to P. falciparum is an important adaptive trait in human populations. The aim of this study was to assess the role of Glucose-6-phosphate dehydrogenase deficiency in relation to the Hbs and HbC haemoglobinopathies in children under five-years-old children in a malaria-endemic setting.

METHODS: The study was carried out in rural villages close to Ouagadougou, Burkina Faso. A cross-sectional survey was performed during the high malaria transmission season in 2005 (Sept to Nov). Samples were collected from 490 children to determine malaria status and for DNA extraction. DNA was assayed for Hbs, HbC and the most common African G6PD deficiency polymorphisms, G6PD (A376G), and G6PD (G202A) using the Sequenom iPLEX platform.

RESULTS: The prevalence of Hb genotypes was: AA, 68.2%; AC, 19.8%; AS, 9%; CS, 0.4%; and SS, 0.4%. Individuals with either Hbs or HbC accounted for 31.8% of the study group. G6PD genotype prevalence was 13.2% (A376G) and 41.9% (G202A) male hemizygotes; 14.7% (A376G) and 1.3% (G202A) female hemizygotes and 53% (A376G) and 25.4% (G202A) for female heterozygotes. Results show that the prevalence of G6PD (376 and 202) deficiency was significantly higher (P<0.001) in normal hemoglobin subjects (54.1% for G6PD376 and 20.7% for G6PD 202) compared with Hbs/HbC subjects (47.9% for G6PD 202 and 8.3% for G6PD 376).

P. falciparum prevalence was: AA, 87.7%; AC, 79.2%; AS, 11.6%; G6PD202, 19.6% and G6PD376, 54.1%. For individuals with G6PD deficiency alleles and Hbs/HbC, malaria prevalence was 54.1% for AS+G6PD376, 19.6% for AS+G6PD202, 44.4% for AC+G6PD376 and 16.7% for AC+G6PD202, compared with the control group (86.0%).

We found that Hbs alone results in a reduced parasite density compared with possession of either G6PD SNP, Also HbS, HbC or the G6PD SNPs all occur with reduced parasite prevalence (P<0.001). However, we did not observe any difference for malaria prevalence, or haemoglobin levels among the different groups.

CONCLUSION: Our cross-sectional survey data show that individuals who possess either Hbs or HbC are more likely to also have a G6PD deficiency allele than people with normal Hb. As expected we see that the Hb and G6PD polymorphisms result in a decrease in parasite prevalence (Hbs > HbC > G6PD). This effect is not lost when the alleles occur together and without increasing clinical malaria or Hb reduction. Further work is required to investigate this interaction that may help maintain the balancing selection of both gene variants.

P122: Stratification and eco-epidemiological types of malaria in relation to vector control

Allan Schapira1,2 and Konstantina Boutsika1,2
1Swiss Tropical and Public Health Institute, Socinstrasse 57, P.O. Box CH-4002 Basel Switzerland; 2University of Basel, Petersplatz 1, CH-4003 Basel, Switzerland

BACKGROUND: Stratification is necessary for malaria control programmes to distinguish areas of no malaria risk, non-endemic areas with malaria risk and endemic areas. Endemic areas may be further classified according to the intensity of transmission and/or other relevant characteristics. Classification according to eco-epidemiological types can be helpful as a supplement to use of malaria epidemiological data for stratification. Such classification must take into account biogeographical region, local ecology, anthropic factors and health systems.

METHODS: Relying on published research and grey literature we reviewed earlier classifications of malaria based on ecology.

RESULTS: We found that all malaria in the world could be assigned to one or more of the following ecotypes: savanna, plains and valleys; forest and forest-fringe; foothill; mountain-fringe and northern and southern fringes; desert-fringe; coastal and; urban. Only two of these ecotypes are well defined and have relatively constant implications for control within certain biogeographical regions: forest environments in the Indo-malay and the Neo-tropic and urban malaria, which has different implications in Africa and in the Indian sub-continent.

CONCLUSIONS: Decision-making on vector control should be based not only on stratification, which may or may not be related to an ecotype classification, but also on past experience in the area under consideration and in areas with similar conditions, knowledge of entomological, human ecological and other determinants and an assessment of the resources available and likely to be available in the future as well as the level of ambition of the programme for each particular area. The ecotype classification above nevertheless provides a universally applicable framework and will be used to inform simulation modeling of malaria in different biogeographical regions.

P123: Reduced prevalence of malaria infection in children living in houses with window screening or closed eaves on Bioko Island, Equatorial Guinea

John Bradley1, Andrea M Rehman1, Christopher Schwabe2, Daniel Vargas3, Feliciano Mont1, Immo Kleinschmidt2
1Tropical Epidemiology Group, London School of Hygiene and Tropical Medicine, London, UK; 2Medical Care Development International, Malabo, Equatorial Guinea; 3Medical Care Development International, Malabo, Equatorial Guinea

BACKGROUND: Previous studies have demonstrated that mosquitoes are less likely to enter houses which are screened or have closed eaves. There is, however, little evidence about the effect on malaria infection that changes in housing may have. Bioko Island has seen major changes in housing in recent years due to the economic boom associated with petro-chemical revenues. This study examines the impact of protective housing improvements on malaria infection on the island.

METHODS: Data from the annual malaria indicator surveys between 2009 and 2012 were examined to assess trends in housing characteristics and their effect on malaria infection in household members. Odds ratios were adjusted for socio-economic status of the household.

RESULTS: 22726 children between the ages of 2 and 14 were tested for P. falciparum infection over the 4 survey years. Prevalence of infection in those living in houses with open eaves was 23.0%, compared to 18.8% for those living in houses with closed eaves (OR = 0.81, 95% CI 0.67 – 0.98). The prevalence of infection for children in screened houses was 9.1% compared to 20.1% for those living in unscreened houses (OR = 0.44, 95% CI 0.27 – 0.71). The proportion of houses with closed eaves was 66.0% in 2009 and 74.3% in 2012 (test for trend p = 0.01). The proportion of screened houses remained unchanged over time at 1.3%.

DISCUSSION: As a malaria control intervention, house modification has the advantages that it is not affected by the growing problem of insecticide resistance; it protects all household members equally and at all times while indoors; and it offers protection against a number of vector borne diseases. The study provides evidence in support of efforts to regulate or encourage housing improvements which impede vector access into residences as part of an integrated vector control approach to complement existing measures which have been only partially successful in reducing malaria transmission in some parts of Bioko.
P124: Changing patterns of malaria and management of fever in southern Senegal (2000-2012)

Philippe Brasseur, Malick Badiane, Moustafa Cisse, Cheikh Sokhna, Jean-François Trape, Michel Vaillant, Piero Olliaro

1UMR 198, Institut de Recherche pour le Développement, Dakar, Sénégal; 2District Médical d’Oussouye, Sénégal; 3Programme National de Lutte contre le Paludisme, Dakar, Sénégal; 4Methodology and Statistics Unit, Centre de Recherche Public (CRP)-Santé, Luxembourg, 5UNICEF/UNDP/WB/WHO Special Programme for Research and Training in Tropical Diseases (TDR), Geneva, Switzerland

BACKGROUND: Malaria is reportedly decreasing in different epidemiological settings, but local long-term surveys are limited. At Mlomp dispensary in south-western Senegal, an area of moderate malaria transmission, year-round, clinically-suspected malaria was treated with monotherapy as per WHO and national policy in the 1990s. Since 2000, there has been a staggered deployment of artesunate-amodiaquine after monotherapy as per WHO and national policy in the 1990s. Since 2000, there has been a staggered deployment of artesunate-amodiaquine after parasitological confirmation; this was adopted nationally in 2006.

METHODS: Data were extracted from clinic registers of the dispensary for the period between January 2000 and December 2012 and analyzed.

RESULTS: Over the 13-year study period, the risk of malaria decreased about 26-times (from 0.18 to 0.007 episodes person-year), while anti-malarial treatments decreased 94-times (from 0.66 to 0.007 treatments person-year). Consultations for fever decreased 5-times (from 0.73 to 0.15 visits person-year). Anti-malarial treatments were given in 83% of patients with a negative thick smear (87.9% in 2000-2006 and 27.6% in 2007-2012), and in 89.3% of those not tested (99.3% in 2000-2006 and 49.4% in 2007-2012). This was paralleled by changes in the age profile of malaria patients so that the risk of malaria is now almost uniformly distributed throughout life, while in the past malaria used to concern more children below 16 years of age.

CONCLUSIONS: This study provides direct evidence of malaria risk decreasing between 2000 and 2012 and becoming equal throughout life where transmission is moderate. Infection rates are no longer enough to sustain immunity. Temporarily, this coincides with deploying artemisinin combinations on parasitological confirmation, but other contributing causes are not identified.

P125: Improving malaria case management by health care providers in antenatal clinics in Akwa Ibom State of Nigeria

William R Brieger, Bright C Orji, Jhpiego, Emmanuel Otolorin, Jhpiego

1Department of International Health, The Johns Hopkins Bloomberg School of Public Health, Baltimore, USA; 2Nigeria

BACKGROUND: In Nigeria approximately 11% of maternal deaths are caused by malaria in pregnancy (MIP). Use of Long Lasting Insecticide-treated Nets (LLINs), intermittent preventive treatment (IPTp) and prompt and effective case management have been recognized as key interventions to control MIP. Of these three MIP interventions, case management is the less well developed with fever in pregnancy often being treated presumptively as malaria, possibly leaving pregnant women to die from other illnesses. The use of rapid diagnostic tests (RDTs) to confirm malaria before treatment provides an opportunity for earlier recognition of febrile illnesses not due to malaria and appropriate treatment for those that are due to malaria. This study sought to learn whether the introduction of RDTs into antenatal care (ANC) would influence the pattern of malaria fever and malarial case management in Akwa Ibom State, Nigeria.

METHODS: The study reviewed record cards of pregnant women attending government owned ANC clinics before and after introducing and training health staff on parasitological diagnosis of malaria using RDTs. The ANC client cards were drawn from first non-follow-up visits where a complaint of ‘fever’ was recorded. Data extraction was conducted between February 2010 and March 2011 by trained nurses/midwives across six primary health care centers in two Local Government Areas of the state.

RESULTS: At baseline 597 cards were reviewed, and 472 at endline. At baseline presumptive malaria treatment took place among 506 (84.8%) of the febrile women using ACT (23%), Quinine (32%) and other antimalarials (30%). At endline 361 (76%) of febrile women were tested with RDTs, with 71 (20%) of tests being positive. All RDT+ women received an antimalarial, with 76% getting either ACT or quinine as recommended. Among the 290 RDT+ women, 28% were given an antimalarial drug. In contrast 60% of RDT- women received an antibiotic, although most of them had complained of a respiratory illness.

CONCLUSIONS: The review of records did show that nursing and midwifery staff at government clinics could in a relatively short time period adopt the use of RDTs. They did improve their prescribing of appropriate antimalarials, but still were using some inappropriate ones and did treat a small proportion of RDT- women for malaria. Continued follow-up and supervision will be needed to ensure that correct malaria diagnostic and treatment guidelines are fully practiced.

P126: Persistence of Malaria in Pregnancy as Rwanda Targets Pre-Elimination

William Brieger, Corine Karema, Beata Mukarugwiro

1Department of International Health, The Johns Hopkins Bloomberg School of Public Health, Baltimore, USA; 2The Malaria and Other Parasitic Diseases Unit, Rwanda Ministry of Health, Kigali; 3Jhpiego, Kigali, Rwanda

BACKGROUND: Through universal coverage of long-lasting insecticide-treated nets and access to artemisinin-based combination treatment Rwanda has achieved a national malaria prevalence estimated at 1.4% among children aged 6-59 months and 0.7% among women aged 15-49 years (2010 DHS). Slide positivity rates at health centers have dropped over 85% since 2005, and yet malaria persists. Pregnant women remain vulnerable. While Rwanda no longer practices IPTp, it is interested in offering the best malaria protection to pregnant women. In order to plan appropriately, there was need for a malaria in pregnancy prevalence study.

METHODS: Pregnant women were studied at first ANC registration in 38 health centers in two districts each of low, moderate and relatively higher malaria transmission areas (as determined by health information system laboratory reports) using microscopy, rapid diagnostic test (RDT) and polymerase chain reaction (PCR). Ethical clearance was provided by the ethical review board within the Ministry of Health. ANC staff were trained to obtain data during normal client visits. RDTs were performed by the ANC staff. They also prepared blood slides to be analyzed in health center laboratories and PCR papers that were analyzed at the Johns Hopkins Bloomberg School of Public Health. Information on parity, age, bednet use, anemia, HIV status and fever were normally collected for ANC records and also recorded on project data forms.

RESULTS: Among 3,781 women studied, malaria prevalence with microscopy was 1.6%, RDT was 2.4%, and PCR was 5.6%. Negative tests were associated with LLIN use the night before. Positive tests were associated with anemia, but none of the other variables. The highest positivity for all three tests (4.5%, 6.9% and 12.5% respectively) was in the designated high prevalence districts on the eastern border of the country.

CONCLUSIONS: Results show that even with low apparent levels of malaria, health services need to continue to protect pregnant women and their unborn children in Rwanda through consistent use of LLINs and identification and tracking women with anemia. Cross-border collaboration will also be needed to prevent reintroduction of the disease as the country moves towards elimination.
P127: Repeated mass distributions and continuous distribution of LLINs: modelling sustainability of health benefits depending on case management

Olivier J.T. Briët1,2 & Melissa A. Penny1,2

1 Department of Epidemiology and Public Health, Swiss Tropical and Public Health Institute, Basel, Switzerland; 2 University of Basel, Basel, Switzerland.

BACKGROUND: There is increased global interest in the problem of sustaining the gains of recent malaria control successes with long lasting insecticidal nets (LLINs) and improved case management (CM).

RESEARCH QUESTION: This simulation study examined malaria transmission and disease burden dynamics in the presence of sustained interventions with LLINs and CM. The study aimed to determine optimal LLIN distribution rates and how benefits of disease averse could be sustained.

METHODS: Dynamic simulations of malaria in humans and mosquitoes were run using the OpenMalaria modeling platform studying a range of transmission settings, CM coverage (probability of treatment) and LLIN distribution rates.

RESULTS AND DISCUSSION: In the short term, LLIN distributions are beneficial over the entire spectrum of pre-intervention transmission settings. Repeated distribution of LLINs results in sustained reduction in malaria transmission. However, even though initially malaria disease burden is reduced the burden will gradually increase because of the resulting reduction in the acquisition of immunity and eventually stabilize to a new level. This might lead one to conclude that with a long term perspective, distributing LLINs might not be cost effective in high transmission settings. However, the analysis revealed a strong interaction between CM and LLINs, and the direction of this interaction depends on the pre-intervention transmission setting. Improved CM increased the cost-effectiveness of LLINs at higher transmission settings compared to a scenario with low CM coverage. Nevertheless, in very high transmission settings, distributing LLINs may not be cost effective in the long term. However, the great majority of the African population lives in areas where the sustained combination of both CM and LLINs is cost effective. The effects of changes in LLIN distribution rate on cost effectiveness were relatively small compared to effects of transmission setting and case management.

CONCLUSIONS: Long term planning for malaria control using combinations of malaria vector control and CM interventions will need to take both the pre-intervention potential for transmission, and existing CM levels into account.

P128: Application of a CDC bottle bioassay-based rapid diagnostic tool for assessing insecticide resistance intensity in Zambian anophelines.

William G. Brogdon1, Musapa Mulenga2, Mulakwa Kamulwo1, Patricia M. Kasoma2, Claudia Corredor-Medina1

1Entomology Branch, Division of Parasitic Diseases and Malaria, Center for Global Health, Centers for Disease Control and Prevention, Atlanta, Georgia, USA; 2Zambia Integrated Systems Strengthening Program, Lusaka, Zambia and National Malaria Control Centre, Lusaka, Zambia

BACKGROUND: Up to now, practical field application of the WHO paper-based and CDC bottle-based bioassays have focused on measuring the frequency of insecticide resistance by measuring mosquito survival when exposed to diagnostic or discriminating dosages of insecticides for a specified time. With either method it is possible to use a range of dosages that will permit calculation of resistance ratios, the long established method for assessing resistance intensity. Unfortunately, precise direct calculation of a resistance ratio in the field requires a prohibitive number of mosquitoes, especially where it is desired to assess resistance tendencies in wild caught mosquitoes that are more representative of the epidemiologically significant mosquito population that is actively acquiring or transmitting malaria infections.

METHODS: A bottle bioassay-based intensity assay includes a series of bottles treated with multiples of the diagnostic dose. We have thus far used one, two, five and ten times the diagnostic dosage of deltamethrin, bendiocarb and DDT at the diagnostic times for each insecticide to measure resistance in field-collected Anopheles gambiae s.s. from the Zambian Copperbelt districts and Anopheles funestus from districts in the eastern provinces of that country. Indoor resting mosquitoes were captured by aspiration and tested using this method. Every mosquito from the bioassays was preserved in RNAlater for species identification and kdr detection using PCR techniques.

RESULTS: Collections of Anopheles gambiae s.s. from the Zambian Copperbelt contained up to seven percent of mosquitoes that survived five times the diagnostic dosage of deltamethrin and fifty-five percent that survived exposure to ten times the diagnostic dosage of DDT at the diagnostic times of 30 and 45 minutes, respectively. These collections showed complete susceptibility to bendiocarb and lambda-cyhalothrin. Collections of Anopheles funestus from the eastern Zambian provinces of Chipata, Chadiza and Katete contained up to forty percent of individual mosquitoes that survived ten times the diagnostic dosage of deltamethrin while showing complete susceptibility to DDT, lambda-cyhalothrin and bendiocarb.

The instances of higher intensity resistance were both focal and associated with such evidence of control failure as heavy malaria incidence occurring at the local health clinic or observation of bloodfed anophelines resting in residences with recently deployed bed nets. Continued observation of high intensity resistance foci for both anophele species revealed a rapid return to deltamethrin susceptibility following insecticide class rotations for one season.

P129: Determination of erythrocyte invasion mechanisms of Plasmodium falciparum isolates in Ghanaian children

H Brown

BACKGROUND: Morbidity and mortality associated with Plasmodium falciparum infection are caused by invasion of erythrocytes by the malaria parasite, a process which is mediated by multiple receptor-ligand interactions. Antibodies against some parasite ligands have been shown to significantly inhibit parasite growth in vitro, demonstrating the these interactions may be good targets for the development of an effective blood stage vaccine. The aim of this study is to investigate the erythrocyte receptors used by P. falciparum isolates in Ghana.

METHODS: P. falciparum field isolates were collected from children aged 2-14 attending hospitals in three ecologically distinct zones in Ghana: Accra, Navrongo, and Kintampo. Erythrocyte invasion assays were performed to test the ability of the parasites to invade erythrocytes treated with neuraminidase, trypsin and chymotrypsin which selectively remove receptors from the erythrocyte surface. In addition, antibodies against two recently identified receptors, basigin (BSG) and complement receptor 1 (CR1) were used to determine the dependence of the isolates on these pathways. Two to four assays were performed in duplicate on each isolate.

RESULTS: All clinical isolates tested so far were capable of invading neuraminidase treated erythrocytes with invasion efficiencies of 40-80% relative to untreated erythrocytes, suggesting that these field isolates utilize sialogic acid independent pathways for erythrocyte invasion. Invasion efficiency varied between 5 to 75% relative to untreated erythrocytes in both trypsin and chymotrypsin treated erythrocytes, with the majority being highly sensitive to both enzymes. Sensitivity to trypsin represent the contribution of glycoporphin A and C and CR1, while sensitivity to chymotrypsin indicates the contributions of glycoporphin B and CR1 Furthermore, for nearly all field isolates tested, antibodies against CR1 significantly inhibited invasion efficiency of neuraminidase treated erythrocytes, but did not significantly inhibit invasion levels in untreated erythrocytes. However, anti-BSG antibodies significantly inhibited invasion in both untreated and neuraminidase treated erythrocytes to a similar extent.

CONCLUSION: Preliminary results suggest that the majority of field isolates in Ghana express sialogid acid independent invasion phenotype. The most common invasion phenotype among clinical isolates among Ghanaian clinical isolates was neuraminidase-resistant, trypsin- and chymotrypsin-sensitive, suggesting that CR1 plays a major role in erythrocyte invasion.

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P130: Stakeholder Engagement and the Malaria Decision Analysis Support Tool (MDAST)

Zachary Brown1, Randall Kramer2, Clifford Mutero1, Dohyeong Kim4, Marie Lynn Miranda3, Birkinesh Amenesewa2, Adriane Lesser2

1Organisation of Economic Cooperation & Development, Paris, France; 2Duke Global Health Institute, Duke University, Durham, North Carolina, USA; 3University of Pretoria, Pretoria, South Africa, & International Centre for Insect Physiology and Ecology, Nairobi, Kenya; 4Department of Economic, Political, and Policy Science, University of Texas at Dallas, Dallas, Texas, USA; 5School of Natural Resources and Environment, University of Michigan, Ann Arbor, Michigan, USA; 6WHO Regional Office for Africa, Harare, Zimbabwe

The sustainability of advances in the fight against malaria in Africa has been confronted with a number of challenges arising in overlapping biological, economic, and social dynamics. Decision support systems (DSS) can help address these challenges, by facilitating evidence-based evaluation of alternative malaria control portfolios. One type of DSS – model-based DSS – is particularly useful for national malaria control programs (NMCPs) because it permits the analysis of the potential effects of novel intervention combinations, and allows program managers to explore the potential consequences of proposed policy measures across a range of different contexts and time horizons. However, the specialized capacity required to develop a model-based DSS can limit the role of intended users in its development, and therefore their ability and willingness to implement it.

The Malaria Decision Analysis Support Tool (MDAST) is a model-based DSS combining scientifically peer-reviewed models and results from field studies into a flexible software package developed for use by NMCP managers and staff. The purpose of MDAST is to enable users to (a) explore the possible consequences of alternative policy combinations along a number of outcome dimensions (e.g., malaria burden, costs, and possible externalities), (b) provide a consistent framework for advocating alternative policy approaches, and (c) evaluate the sustainability of alternative policy combinations over longer time horizons (e.g. 10 to 20 years) than is often considered in NMCP planning. The creation of MDAST explicitly focused on stakeholder recruitment and consultation. The development process included a review of the scientific literature, an iterative process of user consultation, software development and refinement, and consideration of potential implementation barriers. While MDAST can generate a variety of model-based outputs based on user-defined scenarios and policy combinations, the key results from the project relate to the successful use of MDAST by its intended audience. During workshops in all three partner countries in the MDAST project (Kenya, Tanzania, and Uganda), NMCP stakeholders successfully posed and analysed the implications of a range of alternative policy portfolios through hands-on use of MDAST. In addition, stakeholders were highly positive about MDAST in anonymous surveys on the tool’s comprehensibility, usability, applicability, and credibility.

P132: Comparison of Age-Specific Malaria Mortality Rates in the KEMRI/CDC Health and Demographic Surveillance System in Western Kenya, 2003–2010

Meghna Desai1,2, Ann M. Buff3, Sammy Khagayi4,5, Annemieke van Eijk4, Laurence Slutske1, John Vulule4, Frank Odhiambo4,5, Kayla Laserson4, Mary J. Hame4

1Kenya Medical Research Institute/Centers for Disease Control and Prevention (KEMRI/CDC) Research and Public Health Collaboration, Kisumu, Kenya; 2Division of Parasitic Diseases and Malaria, Centers for Disease Control and Prevention, Atlanta, GA, USA; 3KEMRI Center for Global Health Research, Kisumu, Kenya; 4Liverpool School of Tropical Medicine, Liverpool, UK; 5CDC, New Delhi, India

BACKGROUND: Malaria control interventions have been scaled-up in Kenya during the past decade. Most malaria interventions and burden assessments have focused on young children. Modeling of malaria burden in adults has produced highly variable estimates. To measure progress, accurate malaria burden estimates across age groups are necessary. We determined age-specific malaria mortality rates in western Kenya, an area of high malaria prevalence (38%, 2006), HIV prevalence (15% in adults, 2003–2004) and insecticide-treated net use (41%, 2006).

METHODS: We collected data from 140,000 persons in a health and demographic surveillance system from 2003–2010. Deaths were captured via community informant reporting and thrice yearly surveillance visits. Standardized verbal autopsies were conducted; probable cause of death was assigned by InterVA-4, a probabilistic method for verbal autopsy interpretation. Annual malaria mortality rates per 1,000 person-years (PY) were generated by age group. Trends from 2003–2010 were analyzed using Poisson regression.

RESULTS: In children <5 years, the malaria-specific mortality rate (MMR) decreased from 13.2 to 3.7 per 1,000 PY, a mean reduction of 20% annually from 2003 to 2010. In children 5–14 years, MMR remained stable from 0.46 to 0.47 per 1,000 PY. In adults 215 years, MMR decreased from 1.5 to 0.42 per 1,000 PY for a mean reduction of 20% annually. From 2003 to 2010, the proportion of all malaria deaths by age group decreased in children <5 years from 69.8% to 61.4%, increased in children 5–14 years from 4.3% to 14.3% and decreased slightly in adults 25.9% to 24.3%.

CONCLUSIONS: Malaria mortality rates in young children and adults have been confronted with a number of challenges arising in overlapping biological, economic, and social dynamics. Decision support systems (DSS) can help address these challenges, by facilitating evidence-based evaluation of alternative malaria control portfolios. One type of DSS – model-based DSS – is particularly useful for national malaria control programs (NMCPs) because it permits the analysis of the potential effects of novel intervention combinations, and allows program managers to explore the potential consequences of proposed policy measures across a range of different contexts and time horizons. However, the specialized capacity required to develop a model-based DSS can limit the role of intended users in its development, and therefore their ability and willingness to implement it.

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Katta Bruxvoort1,2, Charles Festo3, Admirabilis Kalolella1, Frank Mayaya1, Matt Cairns3, S. Patrick Kachur2, Catherine Goodman2, and David Schellenberg5

1Ifakara Health Institute, Dar es Salaam, Tanzania; 2London School of Hygiene and Tropical Medicine, London, UK; 3U.S. Centers for Disease Control and Prevention, Atlanta, USA

BACKGROUND: As artemisinin -based combination therapies (ACTs) become more widely available in the public and private sectors, patient adherence is a critical step in ensuring treatment effectiveness and reducing the development of antimalarial resistance. Some studies have reported high adherence to ACTs obtained from public health facilities (90-100%), while other studies have reported adherence less than 50%. Most studies rely on self-reported data obtained through questionnaires, which can be prone to recall bias and social desirability bias.

METHODS: This study was conducted in 72 drug shops and 36 public health facilities in Mtwara, Tanzania. Patients of all ages who indicated an intention to purchase ACTs at drug shops or who were prescribed an ACT in public health facilities were given age or weight-appropriate blister packs of artemether-lumefantrine (AL). We used a mixed supply of regular AL blister packs and identical-looking smart blister packs containing electronic tags that recorded the day and time each tablet was dispensed. Patients receiving AL were followed up at home a minimum of 68-72 hours after drug purchase, and consenting patients or their caregivers were administered a detailed questionnaire about when and how each dose of AL was taken. Patients were asked to present their blister packs for a tablet count and download of timestamp data from the smart blister packs.

RESULTS: We interviewed 1476 patients who obtained AL. Using self-reported data, we will report the percent who completed all doses of AL at appropriate time intervals. We will also report the percent of patients with data available from smart blister packs and the percent that completed all doses of AL at the appropriate times. We will assess the concordance between the two methods and discuss the advantages and disadvantages of each.

CONCLUSION: Most data on adherence is based on patient self-report. Given the implications of adherence for ACT effectiveness, it is important that methods of measuring adherence are better understood and improved. Smart blister packs that report the exact time of tablet removal have potential to reduce bias and to provide more accurate results, however operational challenges must be addressed.
decreased dramatically from 2003 to 2010 in western Kenya, but older children have not benefited. Almost 40% of malaria deaths occur in older children and adults. Malaria deaths in adults might be overestimated due to HIV-associated mortality. Our data support current strategies to reach older children and adults and inclusion of all age groups in malaria control interventions, including universal coverage with insecticide-treated nets.

P133: Antiplasmodial potential of the African mistletoe: Agelanthus dodoneifolius
Polh and Wiens

BACKGROUND: The wide spread resistance of malaria parasites to conventional anti-malarials have stimulated the search for new drug entities especially those with new modes of action. Preparations of Agelanthus dodoneifolius have been used in the traditional Nigerian medicine to treat malaria and this practice has remained till date without scientific validation. This study aimed to authenticate the antimalarial potential of this plant by evaluating its antiplasmodial activities.

METHODS: The antiplasmodial property of the water extract of Agelanthus dodoneifolius was evaluated in vivo and in vitro against Plasmodium berghei berghei and clinical isolates of Plasmodium falciparum, respectively.

RESULTS: There was a dose-dependent inhibition of parasites in the in vivo antiplasmodial tests likewise, the in vitro screening demonstrated a strong and concentration-dependent activity (21.54 µg/ml < IC50 < 50 µg/ml) of the extract against the clinical isolates of Plasmodium falciparum. The phytochemical analysis revealed the presence of tannins, saponins, sterols, glycosides, phenols, anthraquinones, terpenes, reducing sugars and resins. It also showed a strong free-radical scavenging activity on 2, 2-diphenyl-2-picrylhydrazyl. The oral median lethal dose (LD50) in mice was estimated to be greater than 5000 mg/kg.

CONCLUSION: Our results evidence that Agelanthus dodoneifolius may contain biologically active principles those are relevant in the treatment of malaria, thus supporting further studies of its active components.

P134: Post-translational modifications regulating expression of TNF in human monocyte derived dendritic cells exposed to natural hemozoin

BACKGROUND: In order for the human host to successfully control a Plasmodium falciparum infection, a balance between pro and anti-inflammatory responses must be achieved. The immune response is to a great extent driven by a distinct cytokine milieu that is under the influence of genetic as well as epigenetic factors. Various studies have shown that monocyte derived dendritic cells (moDCs) release pro-inflammatory cytokines such as TNF in response to certain malaria-related stimuli and exhibit altered maturation and differentiation. This study aims to assess the regulatory basis of the previously reported effects with focus on chromatin modifications.

METHODS: Monocytes were isolated from peripheral blood mononuclear cells (PBMC) from healthy Swedish and Italian donors. Cells were cultured in presence of IL-4 and GM-CSF to generate moDCs. At day 5, cells were harvested and exposed to natural hemozoin (nHz) and LPS. Latex beads were used a control for phagocytosis. Unstimulated cells were used as a negative control. The presence of H3K9 acetylation was determined using chromatin immunoprecipitation (ChIP). Supernatants were used for measuring protein level of TNF by ELISA.

RESULTS: Preliminary ChIP data suggest increased H3K9 acetylation in the promoter region of TNF in moDCs exposed to nHz, with even higher acetylation in cells exposed to LPS. No upregulation was observed in cells after contact with latex beads or in unstimulated cells. ELISA data suggests increased levels of TNF in cell supernatants from cells exposed to nHz as compared to the negative controls. Transcriptional analysis regarding TNF mRNA levels is ongoing.

CONCLUSION: The ongoing study indicates that nHz induces early epigenetic changes, which suggest that H3K9 acetylation might be involved in regulating the cytokine response in moDCs. Further experiments are aimed to verify results, extend the study and examine additional cytokines and chemokines.

P135: An affordable, quality-assured community-based system for high resolution entomological surveillance of vector mosquitoes that reflects human malaria infection risk patterns

Prosper Chaki

BACKGROUND: More sensitive and scalable entomological surveillance tools are required to monitor low levels of transmission that are increasingly common across the tropics, particularly where vector control has been successful. A large-scale larviciding programme in urban Dar-es-Salaam, Tanzania is supported by a community-based (CB) system for trapping adult mosquito densities to monitor programme performance.

METHODOLOGY: An intensive and extensive CB system for routine, longitudinal, programmatic surveillance of malaria vectors and other mosquitoes using the Ifakara Tent Trap (ITT-C) was developed in Urban Dar-es-Salaam, Tanzania, and validated by comparison with quality assurance (QA) surveys using either ITT-C or human landing catches (HLC), as well as a cross-sectional survey of malaria parasite prevalence in the same housing compounds.

RESULTS: Community-based ITT-C had much lower sensitivity per person-night of sampling than HLC (Relative Rate (RR) [95% Confidence Interval (CI)] = 0.079 [0.051, 0.121], P < 0.001 for Anopheles gambiae s.l. and 0.153 [0.137, 0.171], P < 0.001 for Culicines) but only moderately differed from QA surveys with the same trap (0.536 [0.406,0.617], P = 0.001 and 0.747 [0.677,0.824], P < 0.001, for An. gambiae or Culex respectively). Despite the poor sensitivity of the ITT per night of sampling, when CB-ITT was compared with QA-HLC, it proved at least comparably sensitive in absolute terms (171 versus 169 primary vectors caught) and cost-effective (153US$ versus 187US$ per An. gambiae caught) because it allowed more spatially extensive and temporally intensive sampling (4284 versus 335 trap nights distributed over 615 versus 240 locations with a mean number of samples per year of 143 versus 141). Despite the very low vectors densities (Annual estimate of about 170 An gambiae s.l bites per person per year), CB-ITT was the only entomological predictor of parasite infection risk (Odds Ratio [95% CI] = 4.43[3.027,7. 454] per An. gambiae or An. funestus caught per night, P =0.0373).

DISCUSSION AND CONCLUSION: CB trapping approaches could be improved with more sensitive traps, but already offer a practical, safe and affordable system for routine programmatic mosquito surveillance and clusters could be distributed across entire countries by adapting the sample submission and quality assurance procedures accordingly.

P136: Cause specific mortality in an area with different malaria transmission intensity in Korogwe district, north-eastern Tanzania


INTRODUCTION: Although death records are useful for planning and monitoring health interventions, such information is lacking in most
developing countries. Verbal autopsy (VA) interviews are alternatively used to determine causes of death. We conducted a study to determine all causes and cause-specific mortality in a demographic surveillance area which has been prepared for assessment of different malaria control interventions including vaccines.

**METHOD: The data reported in this study were collected from January 2006 to December 2012 in 14 villages. The study villages were stratified into three strata (3 in lowland semi-urban, 7 in lowland rural and 4 in the highland areas) based on the level of malaria transmission intensity. Standard verbal autopsy questionnaire were administered to parents/ close relatives of the deceased. Two physicians independently reviewed the responses and discordant results were reviewed by a third physician, and final cause was based on two clinicians with similar assessments. In cases, where none of the two had similar assessments, a panel of three clinicians was used for the common consensus.**

**RESULTS: A total of 1,429 deaths in 48,994 person years of follow-up (crude mortality rate of 29.2/1000, 95%CI =27.7 – 30.7) were recorded between January 2006 and December 2012. A total of 378 (26.4%) of the deceased were infants. Only 29.5% of these deaths occurred at health facilities. A total of 964(67.5%) verbal autopsies were conducted and 800(83%) of them had probable cause of deaths determined. Communicable diseases (CDs) caused 53.7% of the deaths while non-communicable was responsible for 33.7%. Malaria accounted 23.5% of all deaths; from which 50.0% were under-fives. There was a decreasing trend of overall mortality and CDs (specifically malaria) across the follow-up period.**

**CONCLUSION: Malaria was the main cause of deaths in infants and under-fives while HIV/AIDS was the main cause in young-olds. NCDs caused majority of the deaths in adults over 45 years of age.**

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**P137: Introducing RDTs into the retail sector in Uganda: a qualitative evaluation alongside a randomised controlled trial**

**Eleanor Hutchinson**, 1 Clare I R Chandler, 1 Miriam Kayendeke,2 Sham Lal, 1 Pascal Magnusson, 1 Sian Clarke1 and Anthony K Mbonye2

1ACT Consortium, London School of Hygiene and Tropical Medicine, London, UK 2Department of Community Health, Ministry of Health, Uganda / School of Public Health, Makerere University, Kampala, Uganda; 3Centre for Medical Parasitology, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark

**BACKGROUND: The introduction of rapid diagnostic tests (RDTs) for malaria in the drug retail sector has been anticipated to improve access to diagnostics in the context of poorly functioning, underfunded and fractured public health services. Diagnostics should enable targeting of malaria treatment in the face of escalating treatment costs and the poor diagnosis of other diseases. This study aimed to evaluate the social impact of RDTs for different actors involved in seeking and providing care for febrile patients.**

**METHODS: 13 focus group discussions were undertaken with 54 drug shop vendors and 54 patients who had been part of a randomised controlled trial comparing the impact of RDT introduction with routine practices. A further 8 FGDs were held with 71 health workers from facilities in the surrounding area. In each FGD, participants were asked for their experiences and views on the practices of drug shop vendors with and without RDTs.**

**RESULTS: Our results demonstrate the powerful symbolic value of RDTs and the ability to associate their user with the formal health service. Using RDTs appears to raise the status and authority of the drug shop vendor, making the drug shop appear to be part of the regulated health system and an attractive option to patients seeking good quality care. This finding may be particularly pronounced in the context of this trial, where RDTs were recognised as a Ministry of Health intervention, which appeared to confer legitimacy to vendors. Health workers were less keen on the use of RDTs by vendors who they felt should remain outside of formal health services.**

**CONCLUSIONS: While RDTs are often presented as a simple means of confirming malaria diagnosis, in this presentation we demonstrate the complexity of their introduction into drug shops in Uganda. Our analysis suggests that the appeal of RDTs to national and global policy makers, as a pragmatic solution to the overuse of antimalarial drugs, is seen as the reverse at community level, where RDTs represent an ideology of investment by the state into securing regulated, quality health care for its citizens.**

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**P138: Malaria transmission and the impact of control efforts in Southern Africa**

**Mike Chaponda1, Mbanga Muleba1, William Moss2**

1Tropical Diseases Research Centre, Department of Clinical Sciences, P.O Box 71769, Ndola, Zambia; 2Johns Hopkins Bloomberg School of Public Health, Baltimore, USA

**BACKGROUND: The burden of malaria has decreased dramatically in parts of sub-Saharan Africa within the past several years. In Southern Africa for example, the burden of malaria declined in Zambia coincident with the use of ACTs, distribution of LLIN and targeted IRS. While it is tempting to attribute this decrease solely to intensified control efforts, the epidemiological situation is more complex. The reductions in the burden have not been achieved uniformly. Understanding the reasons for sustained malaria transmission in the face of current control efforts, why malaria control efforts have not been successful in some epidemiological settings, and the epidemiological patterns following resurgence, are critical to achieving further malaria control and possible elimination.**

**OUTCOMES:**

**Outcome 1:** Measure spatial and temporal changes in malaria parasitemia, gametocytemia and serological responses to guide targeted, risk-based combinations of malaria control strategies that are cost-effective and acceptable to the community in three regions of Southern Africa with different levels of malaria transmission and control.

**Outcome 2:** Characterize the biomics, seasonal entomological inoculation rate, and the feeding and resting behavior of *Anopheles arabiensis*; measure insecticide susceptibility and identify mechanisms of insecticide resistance; and determine the population genetic structure of *An. arabiensis* in regions of Southern Africa with different levels of malaria transmission and control.

**Outcome 3:** Obtain a high-resolution profile of the genetic diversity of *Plasmodium falciparum* isolates and establish how genetic diversity is impacted by intervention history, vector differences, climate differences and other factors; establish and monitor changes in parasite population genetics over time; and determine the level and distribution of parasite clonal diversity within individuals residing in three regions of Southern Africa with different levels of malaria transmission and control. The project is still ongoing and results will be disseminated after completion.

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**P139: Population Genetics of Plasmodium falciparum Surviving Artemisinin Treatment in Children Taking Part in an Efficacy Clinical Trial in Kisumu County, Western Kenya**

**Lorna Jemose1, Luiser Ingasia1, Bidii Ngalah1, Angela Omondi1, Dennis Juma1, Nancy Jelagat1, Ndegwa Mutwiiri1, Peninna Muiruri1, Redemptah Yeda1, Agnes Cheruiyot1, Charles Okudo1, Hosea Akaka1, Ben Andangalu1, Wallace Bulimo1,2, Edwin Kamau1**

1Global Emerging Infections Surveillance (GEIS) Program, United States Army Medical Research Unit-Kenya - Kisumu-Nairobi, Kenya. 2Institute of Tropical medicine and Infectious disease (ITROMID) – Jomo Kenyatta University of Agriculture and Technology, Kenya Medical Research Institute (KEMRI) - Nairobi, Kenya

**INTRODUCTION:** The genetic basis for artemisinin resistance is beginning to be understood with most data coming from Southeast Asia. In order to establish how widely applicable the identified genetic markers are in predicting artemisinin resistance, comparable data is needed from other regions where artemisinin is the drug of choice for
P140: β-hematin formation inhibitory activity of novel Ursolic acid derivatives and their effect on in-vitro growth of Pfalciparum

Harveer S. Cheema, Komal Kalani, Santosh K. Srivastava, Anirban Pal, Mahendra P. Darokar

CSIR-Central Institute of Medicinal and Aromatic Plants, Near Kukrail Picnic Spot, P.O. CIMAP, Lucknow-226015

BACKGROUND: Malaria is one of the most serious infectious diseases killing about 655,000 people each year worldwide. The situation is further aggravated since tolerance of this parasite increasing toward artemisinin and its derivatives an only effective therapy. Digestion of hemoglobin is a complex process and thought to be initiated by the action of proteases releasing heme which is oxidized to form hematin and further polymerization leading to the formation of inert crystals of hemozoin (β-hematin) which is an essential for parasite growth and development.

METHODS: P. falciparum (clone NF-54) was cultivated in human O- red blood cells using method described by Trager and Jensen. Parasite growth was determined by parasite lactate dehydrogenase (pLDH) assay. β-hematin formation inhibitory activity of ursolic acid and its derivatives were evaluated by ferriprotoporphyrin IX (FPPIX) binimerization inhibition assay. Highly synchronized ring, trophozoites and schizonts stage culture with same parasitaemia were incubated with derivative M7 (100 μM) for 12h to study blood stage specific action. Microscopic observations were recorded at 12 intervals up to 72 h.

RESULTS: Ursolic acid and its derivatives observed to inhibit anti-plasmodial activity (IC50: range 10-100 μM) against asexual blood stages of Plasmodium falciparum. Two derivatives M6 and M7 showed 52.2±0.32 % and 93.0±0.11 % inhibition of β-hematin formation respectively at 1 mM concentration as compare to chloroquine 90.8±0.67%. Further, derivative M7 was observed to block the parasite life cycle progression at late ring or trophozoite stages.

CONCLUSION: β-hematin formation inhibitory potential of derivatives M3, M5, M6, M7 and M9 and ursolic acid strongly correlated with their respective antiplasmodial activity. Therefore, it is concluded that ursolic acid and its derivatives exert antiplasmodial activity by inhibiting β-hematin formation. These observations supported previous reports of ursolic acid and its derivatives interfering with β-haematin formation process in Pfalciparum.

P141: Dynamics of Plasmodium falciparum Malaria and Allele frequencies in a Holoendemic Area with indistinct Seasonal Transitions

Agnes Cheruliyot1,2, Hoseah M. Akala1, Angela O. Achieng1, Fredrick L. Eyase1, Dennis Wekesa1, Luiser Ingasia1, Charles Okudo1, Duke Omari1, Eunice A. Awiti1, Catherine Muruki1, Redemptah Yeda1, Ben Andagal1, Edwin Kamau1

1Global Emerging Infections Surveillance (GEIS) Program, United States Army Medical Research Unit-Kenya (USAMRU-K), Kenya Medical Research Institute (KEMRI) - Walter Reed Project, Kisumu and Nairobi, Kenya; 1Department of biomedical sciences, Maseno University, Maseno, Kenya

BACKGROUND: The renewed malaria control and elimination efforts requires a better understanding of how the climate and seasonal weather patterns impact the frequency of Plasmodium falciparum genetic polymorphism. Of interest, is the stability of the drug resistance profile of parasites collected overtime. Such information would be critical in focusing the limited resources available in the fight against malaria. In this study, we analyzed the genetic polymorphisms in two critical drug resistance markers and parasitemias of samples collected from Kisumu District Hospital for 53 months from March 2008 to August 2012. Observations were correlated to the climatic conditions including humidity, rainfall and temperature of when the samples were collected.

METHODS: Parasitemia was established by microscopy for P. falciparum malaria positive samples. DNA was extracted from whole blood using Qiagen DNA Blood Mini Kit. Single nucleotide polymorphism (SNP) identification at positions K76T and N86Y of the PfMDR1 genes respectively were performed using real-time PCR and/or sequencing. Climatic variables data was obtained from Tuntiempo, as daily measurements of temperature in degrees centigrade (°C), percent humidity and precipitation in milliliters (ml). Stata 12 software was used for data analysis.

RESULTS: A total of 869 field isolates, 2008 (169); 2009(161); 2010 (216); 2011 (223); and 2012 (126) showed large variations in frequency from months to month in PfCRT 76 and PfMDR1 86 codons. These polymorphisms did not show correlation with the climatic factors. The mean percent parasitemia was 2.6±1.01, (CV = 115.86%). PfCRT K76 allele showed marginal association with elevated parasitemia. Humidity significantly correlated with parasitemia, (Spearman rank coefficient -0.022, p = 0.045).

CONCLUSION: This study shows that the drug resistance genotype profile of P. falciparum parasite changes drastically from month to month. This data highlights the need to redefine how cross-sectional data is collected in the analysis of drug resistance prevalence in a study site. Interestingly, low humidity appears to delay malaria clinical symptoms in western Kenya, which is a holoendemic area. The lack of correlation between genotypes and climatic changes emphasizes the cost of genotypic investment in sustaining the resistance allele in parasite population.
P142: Microsatellites Polymorphism within Pfne-1 and Pfmdr-6 Genes in association with in vitro antimalarial drug Sensitivity in Kenyan Plasmodium falciparum isolates

Jelagat Cheruiyot1, Fredrick Eyase2, Luiser Ingasia1, Angela Omondi1, Dennis Juma1, Joseph Ndewa1, Joan Mativo1, Agnes Cheruiyot1, Redemptah Yeda1, Charles Okudo1, Paul Angienda2, Hoseah Akala1, Ben Andagalu1, Edwin Kamau1

1Global Emerging Infections Surveillance (GEIS) Program, United States Army Medical Research Unit-Kenya (USAMRU-K), Kenya Medical Research Institute (KEMRI) - Walter Reed Project, Kisumu and Nairobi, Kenya
2School of Biological/ physical sciences, Department of Zoology, Maseno University, Kenya

BACKGROUND: Drug resistance continues to undermine malaria control, threatening malaria elimination efforts. Artemether-lumefantrine (AL) and Quinine (QN) are the first-line treatment for uncomplicated malaria and faecal falciparum malaria respectively in Kenya. Resistance to artemisinin has now been confirmed in Southeast Asia and QN monotherapy clinical failure, although rare, has been reported as well. Studies in Kenyan coast have reported parasite reduced sensitivity to QN and artemisinin, indicating an urgent need for surveillance of first line drugs in Kenya. In this study, we determined the correlation between in vitro drug sensitivity (QN and DHA [active metabolite of artemisinin]) and microsatellite polymorphism within P. falciparum sodium/hydrogen exchanger-1 (pfne-1) and multidrug resistance -6 (pfmdr-6) genes.

METHODS: Samples were collected from western, highlands and coastal regions of Kenya. In vitro drug sensitivity to QN and DHA were established using standard culture methods. DNA was extracted and polymorphisms in pfne-1 and pfmdr6 genes were determined by sequencing. Associations between the in vitro QN and DHA sensitivity [phenotypic] and the polymorphisms of the pfne-1 and pfmdr6 gene [genotypic] were established.

RESULTS: The IC50 (Interquartile range) for QN and DHA were 226.1 (77.7 - 997.5) nM and 10.0 (4.2 - 20.9) nM respectively where n=43. Ten Pfhne-1 genotypes were observed, 4 of these were new. The DNNND repeats varied from 1 to 3, the DDNDHNDHNDND repeats varied from 1 to 2. There was positive correlation between QN susceptibility and increase in number of DNNND, DDNDHNDHNDND repeats. Strains with profile above 503.8 nM, P-0.013). There was no significant difference in the association between in vitro DHA sensitivity and Pfne-1 ms 4760 microsatellite motif. Positive correlation between increased N motif of pfmdr-6 and QN susceptibility was observed.

CONCLUSION: Positive association between QN IC50 and polymorphisms of DNNND and DDNDHNDHNDND repeats in Pfne-1 ms4760 and N repeat of pfmdr-6 could be important markers of QN resistance. Continued surveillance of these markers is important in tracking trends in P. falciparum susceptibility to QN. However, these markers need to be validated through in vivo efficacy studies.

P143: The pharmacokinetics of piperaquine: A literature review

Farai Chigutsa, Lesley workman, Karen Barnes

World-wide antimalarial resistance network (WWARN)

BACKGROUND: Dihydroartemisinin-piperaquine is of comparable efficacy to other artemisinin-based combination therapies, and has been recommended as a first-line treatment option for uncomplicated malaria. In light of emerging resistance to artemisinins it is important to review available information, to assess whether current dosage recommendations achieve the drug exposure needed to ensure successful treatment. This review focusses on the pharmacokinetic properties of piperaquine in patients with uncomplicated malaria.

METHODS: A literature search using PUBMED was conducted to identify studies where patients with uncomplicated malaria were treated with dihydroartemisinin-piperaquine, and concentrations of piperaquine reported. Data was extracted systematically from published studies using a predefined data dictionary.

RESULTS: Data were retrieved from 16 studies conducted between 2000 and 2012. Three studies were conducted in Africa and 13 in Asia. Piperaquine reached maximum concentrations between 3.04 and 5.07 hours after administration. Elimination half-lives (t1/2) between 13.5 and 28 days were reported. Oral clearance (Cl/F) ranged between 0.4 and 1.8 L/hr/kg, and the volume of distribution ranged between 164 and 686 L/kg. A plasma concentration on day 7 (C7) below 30ng/ml showed a greater risk of recurrence (hazard ratio=6.6[95%CI:1.9-23]; P=0.003). In women, total doses of 51.2mg/kg piperaquine were associated with C7 below 30ng/ml, while children had lower day 7 concentrations, higher CI/F and increased risk of treatment failure compared to adults given the same body-weight adjusted doses. There was no significant difference in the total drug exposure in pregnancy (p=0.1), but the t1/2 was significantly shortened. Bioavailability of piperaquine was significantly increased upon co-administration with moderate to large amounts of fat (16.7-53.4g).

CONCLUSIONS: Current literature indicates that dosing recommendations for young children need to be reviewed in light of the increased risk of treatment failure, while body-weight adjusted dosing for women should be maintained above 51.2mg/kg. Additionally, co-administration of piperaquine with a moderate amount of fat may be essential for optimal exposure. Finally, current dosages for pregnant women may need to be reviewed in light of a shortened post-treatment prophylactic phase which might affect cure rates.

P144: Incidence of Plasmodium falciparum infection in asymptomatic rural Nigerian populations

Grace I. Olashinde1,2, Olubanke O. Ogunlana1,2, Shalom N. Chinenu1,2, Emeka E.J. Iweala1, Israel S. Afolabi1,2 and Donnici E. Azuhi1,2

1Department of Biological Sciences, College of Science and Technology, Covenant University, PMB 1023 Ota, Ogun State, Nigeria  2Department of Economics and Development Studies, College of Development Studies, Covenant University, PMB 1023 Ota, Ogun State, Nigeria; 3Covenant University Public Health and Wellbeing Research Group (CUPHWERG)

In spite of several interventions, malaria still remains a major parasitic disease and the most prevalent human disease in many tropical and subtropical regions of the world. Patients and asymptomatic carriers constitute malaria parasite reservoir. Different methods are routinely used to diagnose malaria infection. This study compared three diagnostic methods used to examine the incidence of Plasmodium falciparum infection amongst healthy subjects in two rural communities in Nigeria. Blood samples were collected from 200 asymptomatic subjects (44% males, 66% females) in Iyesi and Ilogbo communities in Ondo-Ondo State, Nigeria. ACON (HRP-2/Aldolase) method. There was a notable reduction in the detection of asymptomatic malaria among the study subjects by the RDT kits. Although Rapid Diagnostic Tests are less time consuming, this study has shown that Microscopy with the detection of malaria parasite in the blood remain the Gold standard for detecting malarial infection. The incidence of P. falciparum infection in the asymptomatic subjects was higher in females than in the males.
P145: Improving Payment of Indoor Residual Spray (IRS) Teams Using Mobile Money Transfer

Brian Chimwa1, Kasubika Chibuye1, Chadwick Sikaala1
1Zambia Integrated Systems Strengthening Program, Lusaka, Zambia; 2National Malaria Control Centre, Lusaka, Zambia

BACKGROUND: Mobile money transfer is a payment service used to replace cash payment systems with mobile electronic payments. Zambia has used mobile money transfer since 2012 to pay spray teams. The Zambia Integrated Systems Strengthening Program (ZISSP) with financial support from the President’s Malaria Initiative, has been supporting the Zambia National Malaria Control Center to conduct Indoor Residual Spraying (IRS) in 20 districts. To pay the 870 spray operators, ZISSP staff is expected to carry huge amounts of cash, a risk not only to staff but to ZISSP which entrusts such funds to staff to administer.

THE PROBLEM: We explored whether mobile money transfer is a viable means for paying IRS spray teams and whether this has advantages over traditional payment methods, particularly regarding the risk of theft as a result of carrying huge amounts of cash.

METHOD: Spray operators and supervisors activated mobile money services on their mobile phones. ZISSP maintained a register of each person’s details and gave this information to Mobile Payment Solution, the company providing the technological platform for transfer of funds to the mobile service provider’s designated local agents (mostly shopkeepers). Payment to individual payees was made through the mobile service provider’s agents and the payee’s mobile wallet was credited with the amount indicated in the payment schedule.

RESULTS: In the first round of spraying, 870 spray operators and 162 supervisors were paid using mobile money transfer and 455 spray operators and 94 supervisors were paid during the second round. During round one, several challenges were experienced including problems with the agents having insufficient cash on hand and spray operators forgetting their personal identification numbers. These were markedly reduced in round two. Spray teams and employees increased their confidence in the system.

CONCLUSION: The mobile money transfer addresses the risks to spray teams and employees having to work with large cash payments in the field. Payees can redeem their cash in their own time without disturbing spray sessions. Employees could then focus on technical issues instead of having to carry and guard their cash. The mobile money transfer has potential for wide use and acceptance.

P146: Enhanced surveillance for malaria in Lusaka, Zambia: A case study

Zunda Chishita1, Anna Winters1, John Miller1, Daniel J. Bridges2, Mulakwa Kamuliwo3, Sichitamba Chibesa-Wamulume4, Moonga Hawela5, Jacob Chirwa1, Matthew Burns6, Benjamin Winters1, Clara Mbwill6, Timothy Nzangwa1, Kathrine R. Tan1, Allen S. Craig6

BACKGROUND: Lusaka, the capital city of Zambia, has reported very low malaria prevalence in national surveys while still reporting high incidence rates of malaria through routine passive surveillance at Ministry of Health (MOH) clinics. The enhanced malaria surveillance program was initiated to develop a better understanding of the true malaria burden in Lusaka district.

METHODS: In the first phase of the program, retrospective malaria data were collected from all public health facilities in Lusaka district. The second phase was initiated through monthly clinic visits to collect the latest malaria data recorded in clinic logbooks. These visits were also used as an opportunity to provide feedback to health facility staff on data collected the previous month. Additionally, laboratory blood slides, the gold standard for malaria diagnosis in Zambia, were collected and re-examined for quality assurance.

RESULTS: Several improvements were observed in the malaria data captured throughout the enhanced surveillance program; a marked reduction from more than 200,000 clinical (versus laboratory confirmed) malaria cases reported in 2010 to just over 100,000 in 2012 was noted. An increase in the malaria testing rate from 12.2% in 2010 to 19.5% in 2012 together with a concomitant reduction in the number of antimalarial courses dispensed from 242,140 courses dispensed in 2010 to 177,134 and 119,790 courses in 2011 and 2012 respectively was also observed.

CONCLUSIONS: Enhanced surveillance appears to have exerted a positive influence on the reporting and management of malaria in the district with improvement in adherence to case definitions and provision of treatment to only diagnostically confirmed cases. Anecdotal evidence suggests that the programme has reduced the perception of high malaria burden in the district and helped reinforce best clinical practice.

P147: Can deltamethrin in Indoor Residual Spraying reduce malaria transmission in a context of very strong and multi-resistance of Anopheles gambiae vector to insecticides? Experimental hut studies

Mouhamadou Chouaibou

BACKGROUND: Vector control remains a fundamental part of the global strategy to prevent malaria transmission. Each of the following bellow can be used alone or in combination as a vector control method: (i) Indoor Residual Spraying (IRS) of houses which reduces transmission by reducing the survival of malaria vectors entering houses or sleeping units, (ii) Insecticide Treated Materials (ITMs) and/or larviciding. Vector control should rely on a rational decision-making process for the optimal use of resources for vector control. This rational decision-making should take into account the specific local information that could enhance the efficacy of the control method adopted.

METHODS: We carried out this study to check the effectiveness of deltamethrin IRS in a context of very high multi-resistance to insecticides in malaria vectors. The study was held in standardised experimental huts. Two huts were treated with deltamethrin at the recommended dosage of 25mg/m² and two others non-treated served as control. Four volunteers who gave informed consent were hired to sleep in each hut and collect mosquitoes. The effects of each treatment on mosquitoes was assessed relative to the control huts in terms of the percentage reduction in the number of mosquitoes caught in a treated huts compared to the control huts, excito-repellency, bloodfeeding rate and overall mortality after 24 hours.

RESULTS: As results, Deltamethrin treated huts failed to deter mosquitoes from entering the huts. The oxophily induced was 1.3 fold higher than the one observed in untreated hut, the overall mortality almost 1.9 fold elevated and the bloodfeeding 1.1 fold lesser than in untreated huts. Furthermore, 8.6% of personal protection and 9.6% and mass effect protection were observed.

DISCUSSION AND CONCLUSION: Our highlights the problem of the most important parameter among bloodfeeding and mortality. As there is no effective deterrent effect, the mosquito can bite and die after and thus, the objective of vector control is only partially achieved as the person bitten can be sick of malaria. Our study provides futher evidence that pyrethroid resistance in malaria vectors is a real threat and can drastically impact control measures based on insecticides compounds. Deltamethrin in the context of high and multi-resistance to insecticides in malaria vectors provide a low but non neglected protection against mosquito bites.
P148: Efficacy and tolerancy of Artemisia annua lemonade in treatment of Plasmodium Falciparum Malaria of children between 6 months to 10 years at Bahouoc and Bangang Fokam (Cameroon).

Rosine D. Chougouko K.1, Wilfried Ketchizzo L.1 Jonas Kouamouo2, Roger Moyou S.2.
1Faculty of Pharmacy, Université des Montagnes, Bangangté, Cameroon.
2IMPM and Faculty of Medicine and Biomedical Science, University of Yaoundé I, Cameroon.

INTRODUCTION: Artemisia annua has been reported to be effective against uncomplicated Plasmodium falciparum malaria in adults but no clinical trial has been done on children because of the bitter taste of tea, so we use a lemonade form to evaluate the efficacy, tolerability, and acceptability of in children aged between 6 months to 10 years in the Bangangté health district in Cameroon.

METHOD: The WHO protocol for undertaking clinical trial for antimalarial drugs was followed. Bahouoc and Bangang-Fokam health centers were used for the study. Amongst other criteria, parasite density > 1000/µl of blood was enrolled. The informed consent document was administered. The drugs were prepared by infusion of 5g of dried leaves of A.annua in 1L of water sweetened with 200 mg of Stevia rebaudiana and given from day 0 to day 6 at the dose of 16.66ml/BW/24h 4 times every 30 minutes. Each patient was followed up to day 28, with the assessment of the parasitological and hematological parameters.

RESULTS: A total of 153 patients were screened, 62 met enrolment criteria 53 were followed up to day 14 but 49 patients completed the trial. Results showed that the geometric mean parasite density (GMPD) of enrolled children was reduced from 1,860 to 35 per µl of blood on day 2 and completely cleared by day 3. They were no ETF. Analysis on day 14 revealed 3 cases of late parasitological failures (6%) and 36 cases (73.48%) of CPAR. Patients had a mean increase in hematocrit from 31.6% to 36.0% on day 2 and 38.0% on day 28. A lot of 30 children from 6 months to 1 year accepted. Both tea and lemonade from A. annua growing in Cameroon was also rich in essential oil. As a continuation of their work we decided to study the larvicidal, antifungal and antibacterial properties of the essential oil. The WHO standard protocol was used to determine their larvicidal properties on Anopheles gambiae L1, L2 larval (KISUMU reference strain). We tested the antibacterial activity on eight bacterial strains, while the antifungal activity was experimented on yeasts. The effectiveness of each organism was calculated by ratio MBC/MIC for bacterial strains and MFC/MIC for yeasts.

RESULTS: The extraction yield of the essential oil was 0.19%. The physico-chemical tests revealed a density of 0.90, a saponification index of 25.0; an acid index of 5.30 and an ester index of 19.7. The larvicidal properties on Anopheles gambiae larval at stage 3 and 4 were noted with a LD50 = 452.8 ± 0.19 ppm and a LD90 = 900 ± 0.46 ppm. Strong antibacterial activity against six bacterial strains among the eight tested was observed. Vibrio cholerae showed the greatest sensitivity to A. annua essentials oil with a MIC < 0.3 mg/ml, followed by Staphylococcus aureus, Salmonella enteritidis, Escherichia coli with a MIC = 10 mg/ml, then, Klebsiella pneumoniae, Proteus mirabilis, with MIC = 20mg/ml. Shigella flexneri presented a low sensitivity with MIC = 80 mg/ml. Meanwhile, Pseudomonas aeruginosa was not sensitive to the essential oil of A. annua. Candida albicans was sensitive with MIC = 10 mg/ml. A ratio MBC/MIC and MFC/MIC was calculated for each sensitive bacterial strain, and Candida albicans.

CONCLUSION: At the end of the study, we can conclude that essential oil extracted from Artemisia annua from Cameroon have larvicidal properties and presented fungicidal activities against Candida albicans and bacterial activities against gram positive as well as gram negative bacteria.

P149: Antibacterial, Antifungal and Larvicidal Activity of the Essential Oil Extracted by Hydro-Distillation from Artemisia annua Grown in West-Cameroon

Rosine D. Chougouko K.1, Pierre R. Fotsing K.2, Jonas Kouamouo1,2, Bibiane Donum T.1,2, Roger Moyou S.1 and Lazare Kaptué1,2.
1Laboratory of Chemistry, Université des Montagnes, 2Laboratory of Microbiology, Cliniques Universitaires des Montagnes, 3Faculty of Medicine and Biomedical Science, University of Yaoundé I.

INTRODUCTION: Artemisia annua, a plant originating from China and belonging to the family Asteraceae is known to have many medicinal properties related to the diversity of its chemical compounds. Chougouko and al in 2010 showed that A. annua growing in Cameroon was also rich in essential oil. As a continuation of their work we decided to study the larvicidal, antifungal and antibacterial properties of the essential oil contained in a variety growing in western Cameroon.

METHODS: Essential oil were obtained by vapodistillation, from dried leaves of A. annua. Standards of quality control from the pharmacopoeia allowed us to evaluate the physico-chemical characteristics of those essential oil. The WHO standard protocol was used to determine their larvicidal properties on Anopheles gambiae L1, L2 larval (KISUMU reference strain). We tested the antibacterial activity on eight bacterial strains, while the antifungal activity was experimented on yeasts. The effectiveness of each organism was calculated by ratio MBC/MIC for bacterial strains and MFC/MIC for yeasts.

RESULTS: The extraction yield of the essential oil was 0.19%. The physico-chemical tests revealed a density of 0.90, a saponification index of 25.0; an acid index of 5.30 and an ester index of 19.7. The larvicidal properties on Anopheles gambiae larval at stage 3 and 4 were noted with a LD50 = 452.8 ± 0.19 ppm and a LD90 = 900 ± 0.46 ppm. Strong antibacterial activity against six bacterial strains among the eight tested was observed. Vibrio cholerae showed the greatest sensitivity to A. annua essentials oil with a MIC < 0.3 mg/ml, followed by Staphylococcus aureus, Salmonella enteritidis, Escherichia coli with a MIC = 10 mg/ml, then, Klebsiella pneumoniae, Proteus mirabilis, with MIC = 20mg/ml. Shigella flexneri presented a low sensitivity with MIC = 80 mg/ml. Meanwhile, Pseudomonas aeruginosa was not sensitive to the essential oil of A. annua. Candida albicans was sensitive with MIC = 10 mg/ml. A ratio MBC/MIC and MFC/MIC was calculated for each sensitive bacterial strain, and Candida albicans.

CONCLUSION: At the end of the study, we can conclude that essential oil extracted from Artemisia annua from Cameroon have larvicidal properties and presented fungicidal activities against Candida albicans and bacterial activities against gram positive as well as gram negative bacteria.

P150: Validation of pediatric galenic form of antimalarial with Artemisia annua.

Rosine D. Chougouko K., Gaele A. Chuijpet N., Dalia Fomemkong F., Jonas Kouamouo, Jean-Michel Tekam, Lazare Kaptué.
Faculty of Pharmacy, Université des Montagnes, Bangangté, Cameroon.

INTRODUCTION: Malaria is a parasitic endemic, origin of numerous deaths, especially with children under five years. The chemio-resistance of various antimalaria has lead to the development of ACT, very expensive. So, some people use Artemisia annua herb in which artemisinin is extracted. Chougouko and al have proved that A. annua tea is as efficient as ACT after 7 days of treatment. But few children have taken part in that study because of bitter. We initiated a different form: Lemonade that will be acceptable by children.

METHODS: We have validated the artemisinin stability in tea and lemonade conserved for 7 days at different temperature, also quantity of water use to the infusion, by dosage of artemisinin in aqueous extracts in different concentrations: 0.25; 0.5; 1; 2.5; and 5g/L of water by TLC- Densitometry. We evaluated acceptability of lemonade by children between 6 months and 5 years by administration in 7 days. We have studied acute toxicity as well as the sub-acute toxicity agent (transaminase, creatinin and serum urea) from tea and lemonade on mice and rats by administration a extract in order of 5g to 10g/kg.

RESULTS: Lemonade and tea conserved for 7 days lose 20 % to 34% of their amount of artemisin per day. The extraction of artemisinin al in 2010 showed that A. annua growing in Cameroon was also rich in essential oil. As a continuation of their work we decided to study the larvicidal, antifungal and antibacterial properties of the essential oil contained in a variety growing in western Cameroon.

METHODS: Essential oil were obtained by vapodistillation, from dried leaves of A. annua. Standards of quality control from the pharmacopoeia allowed us to evaluate the physico-chemical characteristics of those essential oil. The WHO standard protocol was used to determine their larvicidal properties on Anopheles gambiae L1, L2 larval (KISUMU reference strain). We tested the antibacterial activity on eight bacterial strains, while the antifungal activity was experimented on yeasts. The effectiveness of each organism was calculated by ratio MBC/MIC for bacterial strains and MFC/MIC for yeasts.

RESULTS: The extraction yield of the essential oil was 0.19%. The physico-chemical tests revealed a density of 0.90, a saponification index of 25.0; an acid index of 5.30 and an ester index of 19.7. The larvicidal properties on Anopheles gambiae larval at stage 3 and 4 were noted with a LD50 = 452.8 ± 0.19 ppm and a LD90 = 900 ± 0.46 ppm. Strong antibacterial activity against six bacterial strains among the eight tested was observed. Vibrio cholerae showed the greatest sensitivity to A. annua essentials oil with a MIC < 0.3 mg/ml, followed by Staphylococcus aureus, Salmonella enteritidis, Escherichia coli with a MIC = 10 mg/ml, then, Klebsiella pneumoniae, Proteus mirabilis, with MIC = 20mg/ml. Shigella flexneri presented a low sensitivity with MIC = 80 mg/ml. Meanwhile, Pseudomonas aeruginosa was not sensitive to the essential oil of A. annua. Candida albicans was sensitive with MIC = 10 mg/ml. A ratio MBC/MIC and MFC/MIC was calculated for each sensitive bacterial strain, and Candida albicans.

CONCLUSION: At the end of the study, we can conclude that essential oil extracted from Artemisia annua from Cameroon have larvicidal properties and presented fungicidal activities against Candida albicans and bacterial activities against gram positive as well as gram negative bacteria.

E Chukwuemeka

BACKGROUND: Knowledge of malaria and its practice are important determinants of malaria burden in rural communities of Africa. Information on these will aid efforts at control. This study was aimed to determine the knowledge and practice of malaria and the factors that influence them among artisans and traders in a rural community.

METHODS: A cross-sectional survey involving 39 artisans and 61 traders was conducted between May and June 2013. A pretested interviewer administered structured questionnaire was used to obtain the relevant information.

RESULTS: Of the 100 (39 artisans and 61 traders) interviewed, (64.1%) of Artisans and (62.3%) traders associated mosquito bite with the cause of malaria. only 2 (5.1%) of the artisans recognized malaria parasite as a cause of malaria. However, 30.8% of artisans and 27.7% of traders felt that non mosquito causes such as exposure to sunlight, oily food, eating bad food, drinking bad water, etc. caused malaria. More traders (50.8%) visited the lab/health centre for treatment while more of the artisans (64.1%) went to the local patent medicine dealers (chemist). Use of anti-malaria ranked highest among treatment methods for artisans (87.2%) and traders (86.9%), followed by herbal remedies, 9.8% of artisans and 7.7% of traders. Keeping the surrounding clean ranked highest among methods of preventing malaria for artisans (46.2%) and traders (50.8%). Use of insecticide treated nets (ITNs) was low with (5(12.8%) artisans and 10(16.4%) of traders. Artisans, 15(38.5%) and traders 18(29.5%) did nothing to prevent mosquito bites. There was a significant link between level of education and recognized symptoms of malaria and also methods of preventing malaria. P<0.05.

CONCLUSION: Though knowledge of role of mosquitoes in malaria transmission and use of anti malarial were high. It was observed that there was poor practice in the prevention of malaria and protection from mosquito bites. Comprehensive health messages targeted at correcting misconceptions about malaria transmission and prevention are recommended for impact on appropriate health seeking behavior.

P152: Meliaceae Plants and Vector Control of Malaria III: Larvicidal studies of Ekebergia senegalensis A Juss and Cedrela odorata Linn

Chukwuma, Obiadima C1,2, Edith O. Ajayeban1,2

1,2 Department of Pharmacognosy, 1,2 Institute of Advanced Medical Research & Training, University of Ibadan, Ibadan, Nigeria.

BACKGROUND: Plant species have been known to produce chemical factors and metabolites of value used as pest control that target specific organisms. As part of the objective of possible utilization of indigenous plant extracts as pest control measures, two Meliaceae plants identified as pest control were fractionated and tested for larvicidal activity against the fourth instars larva of Anopheles gambiae; the primary vector of malaria in sub-Saharan Africa.

METHODS: Dried powdered leaves of the two medicinal plants were extracted by maceration in methanol. The most active extracts from Ekebergia senegalensis and Cedrela odorata were fractionated into hexane, chloroform and ethyl acetate solvents by liquid-liquid partitioning. Larvae of An. gambiae were collected and reared in well water. Toxicity was evaluated by exposing 4th instar larvae to different concentrations (62.5-1000 µg/mL) of extracts, larval mortality was recorded after 24 h of exposure and LC50 values determined using the non-linear regression analysis.

RESULTS: The hexane fraction of E. senegalensis displayed an absolute mortality at 0.63 mg/mL with an LC50 value of 0.81 mg/mL; also hexane soluble fraction of C. odorata had inhibitory toxicity on mosquito larvae with an LC50 value of 0.83 mg/mL. Results were compared to those of larvae exposed to N,N-diethyl-3-methylbenzamide, the reference insecticide and untreated groups. The hexane fractions of both plants displayed good activities compared to the reference insecticide with an LC50 value of 1.09 mg/mL. Ekebergia senegalensis extract had a very impressive larvicidal activity recording the highest mortality rate when exposed to the larvae.

CONCLUSION: Though a battery of plants from different families and ethnomedicines have been reported as useful larvicides on An. gambiae, only a few botanicals have moved from the laboratory to field use. Ekebergia senegalensis crude extract showed promising activity in mosquito control, commercial utilization should be feasible, and contribute significantly to malaria control.

P153: Understanding the processes influencing Plasmodium falciparum genetic diversity

Thomas S. Churcher1, Hua Liu2, Paul Parham1, Francois Baloux4

1 Department of Infectious Disease Epidemiology, Imperial College London; 2 Current address alex_liu@hotmail.com 3 Centre for Health Economics and Medicines Evaluation, Bangor University; 4 Genetics, Evolution & Environment, University College London.

BACKGROUND: Parasite genetic diversity is increasingly being used to investigate and monitor malaria though it is unclear how the complex population dynamics of the disease will influence study results.

METHODOLOGY: Coalescence methods are combined with an extension of the basic Ross-Macdonald population dynamics model to investigate how aspects of the life-cycle will influence parasite genetic diversity. F-statistics for the parasite within the human and mosquito are derived and sensitivity analyses of static and dynamic versions of the model show how these statistics change with different control interventions.

RESULTS: Results indicate that variables such as the mosquito life-expectancy and the mean number of oocysts per mosquito have a large impact on parasite genetic diversity. These parameters are seldom recorded during data collection and are likely to vary geographically and with the introduction of control interventions, complicating the interpretation of genetics surveys.

CONCLUSION: Genetic analysis of the malaria parasite is becoming more accurate and economic. Increasingly realistic models merging population dynamics with population genetics will be required to interpret this new raft of epidemiological information.

P154: Delivery of Seasonal Malaria Chemoprevention (SMC) to children under 10 yrs of age in Senegal.

B Cire

METHODS: SMC was delivered to 180,000 children under 10 yrs of age in 3 districts by community health workers who visited each household on designated days in September, October and November 2010. To include older children, visits were arranged outside school hours. Dates of doses were recorded on a record card kept by the family and in a village register. Coverage was estimated from administration records and independently from a cluster sample survey in which socioeconomic status and other household characteristics and ownership of insecticide treated bednets, were also recorded. Detailed data on resource use associated with SMC delivery were collected at district level and from each of the 46 health posts involved to estimate the incremental costs of implementing SMC at scale. To determine the scope for integrating SMC delivery into other health programmes, CHWs were asked to keep diaries recording their activities and sources of income.

RESULTS: SMC delivery took 2 to 6 days each month, health posts employed from 4 to 68 CHWs, each CHW treating an average of 99 children each day coverage was high 80%-90% of eligible children receiving SMC each month. Coverage was similar in all age and socioeconomic groups. Wastage rates of tablets were 9% (sulfadoxine-pyrimethamine) and 13% (amodiaquine). Refusal rates were 0.6% of children. Including
children 5-9 years old doubled the number of children to be treated but increased by only 13% the number of households to be visited and did not substantially increase the time required for delivery.

**DISCUSSION:** In areas where the burden of malaria remains high in older age groups of children, SMC could be extended to include a wider age range of children without substantially increasing the time needed for delivery.

**P155: Congenital malaria in Bobo-Dioulasso (Burkina Faso): a case series report**

**Mamoudou Cisse, Abdoulaye Hama Diallo, Guékoun Lougue, Adama Zida, Robert Tínga Guiguémè
1 Centre MURAZ, Ministry of Health, 01 BP 390 Bobo-Dioulasso, Burkina Faso 2 Unité de Formation et de Recherche en Sciences de la Sante, University of Ouagadougou**

**BACKGROUND:** Congenital malaria is poorly reported in recent literature from Africa. Prophylactic strategies such as intermittent preventive treatment during pregnancy (IPT_{p}) using sulfadoxine-pyrimethamine (SP) were reported to reduce significantly maternal parasitaemia and hence the risk of congenital malaria. We investigated in a cross-sectional study in Bobo-Dioulasso (South west of Burkina) the prevalence of congenital malaria.

**METHODS:** A cross-sectional study was implemented in two primary health facilities of Bobo-Dioulasso where IPT_{p} was the national policy for malaria prevention during pregnancy. Maternal baseline was recorded among women delivering in these 2 centres. Samples of maternal, placental and cord blood were taken and stained with Giemsa and examined for malaria parasites.

**RESULTS:** Overall, 193 pregnant mothers were included in the study. Three neonates were found to harbour *falciparum* malaria resulting in prevalence of 1.5% (3/195) for congenital malaria. Analyses of maternal baseline revealed that the three mothers of those newborns had received IPT_{p} during pregnancy (2 doses of SP), two were primigravids and only one mother was sleeping under ITN during pregnancy. No history of blood transfusion was reported in any of the mothers. At admission, one mother was found to be febrile (39°C) and her baby had also fever at birth (38°C). All the three mothers had both peripheral and placental *falciparum* parasitaemia. Among the three newborns infected by *falciparum* malaria, parasites densities were of 200, 472 and 1,120 trophozoites/µl, respectively. Birth weights were of 1430g, 2000g and 2500g, respectively. The three newborns were successfully treated with quinine and only one mother was sleeping under ITN during delivery. No infection, and 90% power to detect a 40% reduction in prevalence in each intervention compared to control. A year of baseline data collection was done in 2012 to obtain estimates of the outcomes in each cluster before intervention. Intervention will start in July 2013, for 2 years, and results will be available in 2015.

**P156: Randomized trial of spatially targeted malaria control to virtually eliminate malaria in areas of low incidence and patchy transmission in Senegal**

**Badara Cissé,1 El Hadji Ba,2 Abdoulaye Diallo,1 Oussmane Sy, Fassiatou Tairou,1 Ousmane Faye,2 Matt Cairns,2 Jean Gaudart,1 Jean-Louis Ndialy1, Cheikh Sokhna1, Boniface Mutombo2, Jules-François Gomis,3 Catherine Pitt,2 Babacar Faye,1 Jean-François Trape,2 Colin Sutherland,2 Fatou B Fall,2 Brian Greenwood,1 Oumar Gaye,2 Paul Milligan1,2 Université Cheikh Anta Diop, Dakar, Sénégal; London School of Hygiene and Tropical Medicine, UK;3Institut de Recherche pour le Développement, Dakar, Sénégal;4Ministère de la Santé et de la Prévention, Sénégal;5Université de Marseille Provence;6MACEPA, Poth, Senegal.**

**BACKGROUND:** In Central Senegal scaling-up of malaria control measures has reduced malaria incidence but transmission persists in hotspots which provide a continuing source of infection and sustain transmission. New strategies are needed to eliminate the disease. The purpose of this trial is to evaluate a targeted malaria control strategy using indoor residual spraying (IRS) and chemotheraphy, delivered by district health staff to villages reporting clinical cases. We will also determine whether chemotheraphy should be delivered to all members of the community (Mass Drug Administration, MDA) or only persons who have been tested and are known to be infected (Mass Screening and Treatment, MSAT).

**METHODS:** 40 health-posts, each serving about 8000 people, will be randomized. In 15 health-post areas, all households in target villages will receive IRS with Actellic 300CS, (pirimiphos-methyl formulated in capsules to provide a long-lasting effect), in July, followed by MDA with dihydroartemisinin-piperquine (DHA-PQ) to all persons in the household in September and again in October. In another 15 health-posts, the interventions will be done in a similar way, but instead of MDA, all persons in the household will be screened using a malaria RDT and those who test positive will be treated with DHA-PQ. 10 health-posts will serve as controls, with no targeted intervention. In all arms, persons diagnosed with malaria at health facilities will be treated with artemether-lumefantrine and provided with a long-lasting insecticide-treated bednet. Intervention will be delivered over two years. The primary outcomes will be the incidence of malaria, and the prevalence of parasitaemia just after the main peak period of transmission in each year, from a stratified survey in targeted and non-targeted areas.

**RESULTS:** The study is designed to have at least 90% power for non-inferiority of MSAT compared to MDA in reducing the prevalence of infection, and 90% power to detect a 40% reduction in prevalence in each intervention compared to control. A year of baseline data collection was done in 2012 to obtain estimates of the outcomes in each cluster before intervention. Intervention will start in July 2013, for 2 years, and results will be available in 2015.

**P157: Antibodies to crude Plasmodium falciparum Blood Stage Antigens in a Cohort of Children Living in Mutengene, Cameroon**

**Clarisse Njua-Yafi, Judith Anchang-Kimbé, Tobias Apinjoh, Regina Mugri, Hanesh Chi, Roland Tatata, Emmanuel Nkock Eric Achidi Malaria Research Laboratory, Faculty of Science, University of Buea, Buea, Cameroon**

**BACKGROUND:** Decades of research have shown that naturally acquired antibodies are important for protection against blood stage malaria parasites. Previous studies demonstrated an association between cytophilic antibodies with protection against *Plasmodium falciparum* malaria. In other studies, IgG2 antibodies to some *P. falciparum* antigens have been associated with protection, indicating that the role of IgG subclasses in malaria protection still needs to be elucidated.

**METHODOLOGY:** The study was carried out in Mutengene, a semi-urban community located in Fako division of South West Cameroon. A cohort of 357 children aged ten years and below recruited from randomly selected households were followed for 12months. The incidence of malaria was determined by bi-monthly morbidity surveys and blood sampling every trimester for malaria parasitaemia examination and antibody ELISAs.

**RESULTS:** Participants positive for malaria parasites increased from 18% at enrolment(dry season) to 19.3% 6 months later(rainy season) and decreased to 12% 12 months later(dry season, P=0.036. IgG seropositive individuals decreased significantly(P<0.01) from 47.2% at enrolment(dry season) to 37.1% 6months later(rainy season) and 33.2% 12months later(dry season). The proportion of IgM seropositive individuals slightly decreased from 78.8% at enrolment(dry season) to 76% 6months later but significantly(P<0.01) increased to 88.5% 12months later. Mean IgG and IgM ODs were significantly(P<0.01) higher in the 5-10years age group compared to the <5years age group. With the exception of IgG1, the mean ODs for IgG2, IgG3 and IgG4 were significantly(P<0.01) lower in the dry season compared to the rainy season. However, all participants were seronegative for IgG2 and IgG3 and IgG4 seropositivity increased respectively from 72.4% & 22.4% at enrolment to 75% & 44.8% 6months later and decreased to 47.7% & 15.6% 12months later(P<0.01).

**DISCUSSION AND CONCLUSION:** Changes in the proportion of malaria parasitaemia positive cases was accompanied by corresponding changes
P158: A cluster-randomised trial introducing rapid diagnostic tests among community medicine distributors: impact on treatment and referral of patients in two areas of high and low transmission in Uganda

Richard Nyomugyenyi1, Sham Lai1, Kristian Schultz Hansen2, Clare Chandler2, Pascal Magnusson2, Siân Clarke3
1Vector Control Division, Ministry of Health, Kampala, Uganda; 2ACT Consortium, London School of Hygiene and Tropical Medicine, London, UK; 3Faculty of Health and Medical Sciences, University of Copenhagen, Denmark

INTRODUCTION: Community-based treatment increases access to ACTs to populations beyond the reach of formal health services. WHO recommends universal access to malaria diagnostics, and rapid diagnostic tests (RDTs) are the only feasible means of diagnosis at community level. Evidence regarding adherence to RDT results by community medicine distributors (CMDs) and impact of diagnostic testing on referral remain limited.

METHODS: A cluster-randomised trial examined the impact and cost-effectiveness of RDT use by CMDs on proportion of children receiving appropriate ACT treatment in two contrasting settings of high and low transmission in rural Uganda. 120 communities (379 CMDs) were randomised to one of two arms: ACT treatment following RDT testing (intervention arm) or to presumptive treatment (control arm). All CMDs were trained how to give ACTs, rectal arsunate pre-referral treatment, and to recognise signs requiring referral to a health facility. Data on diagnosis, treatment, and referral were recorded by CMDs in treatment registers. Patient compliance to referral was assessed through record linkage.

RESULTS: Over 12,000 children were seen by CMDs in the high transmission setting; 2507 in low transmission setting. Adherence to RDT results by CMDs exceeded 85% in both settings. In the high transmission area, 44% of children seen by CMDs using RDTs were treated with an ACT compared with 99% in control arm, reducing ACT treatment by 55%. In the low transmission area, <10% of children in RDT arm were treated with an ACT vs. 94% in control arm, reducing ACT prescription by 87%. Referral was more common in RDT arm compared to control, with non-severe signs of illness accounting for >50% of referrals, in both settings. Referral completion by patients was low in both sites, but was higher where CMDs used RDTs (13% vs. 4% presumptive treatment) in the high transmission site.

CONCLUSIONS: Training CMDs in use of RDT-based diagnosis was feasible; CMDs adhered to RDT result and malaria treatment guidelines, thus improving rational use of ACTs. CMDs using RDTs were more likely to refer children than those using presumptive diagnosis. Although referral completion by patients remains a challenge, use of RDTs was also associated with more children completing referral.

P159: Management and leadership training for public health managers: Preliminary evaluation results from a capacity building initiative to support health systems strengthening in Zambia

Sian Clarke

BACKGROUND: For health systems to function effectively, WHO argues that effective governance and leadership is essential. However, curricula on building practical management and leadership skills are scarce in the Zambian health education system, particularly for medical doctors who often hold key leadership positions in the ministry of health.

OBJECTIVE: To build leadership and management capacity of middle management in the Zambian health sector.

METHODS: Between October 2011 and June 2013, we developed and implemented a three pronged strategy of lectures, case studies and mentorship to train 468 managers in the Zambian health sector. Course content targeted behaviours on problem solving, leadership and project management, strategic information management and human resource and financial management. Each participant completed a self-administered survey at the start and end of the programme to measure changes in self-rated management and leadership skills. Knowledge retention tests were completed by each participant pre and post each workshop.

RESULTS: Preliminary results from 262 trainees who completed the program indicate a significant average increase in their management and leadership capacity self-perception index from 7.3 to 8.8 (CI=1.505 - 1.512; p=0.05) and understanding of key management concepts – 20% (CI=19.98% - 20.54%; p=0.05). Post training, trainees felt more prepared to meet management and leadership challenges in their current and future positions and felt more committed to their career under their organization (to 41% and 26% respectively).

CONCLUSION: These findings support our hypothesis that management and leadership training for adults can be effective in changing self-perceptions of management capability and leadership efficacy.

P160: The impact of intermittent parasite clearance on malaria, anaemia, and cognition in schoolchildren in an area of highly seasonal transmission in Mali

Sian Clarke1, Saba Rouhani2, Seybou Diarra2, Modibo Bamadio2, Rebecca Jones2, Diarahara Traore1, Matthew Jukes1, Josselin Thuillez1, Moussa Sacko1, Natalie Roschnik2, Simon Brooker1
1 London School of Hygiene and Tropical Medicine, London, UK; 2 Save the Children, Mali; 3 Programme National de Lutte contre le Paludisme, Ministry of Health, Mali; 4 Room to Read, London, UK; 5 Centre d’Economie de la Sorbonne, Paris, France; 6 Institut National de Recherche en Sante Publique, Bamako, Mali

BACKGROUND: An increasing number of studies have shown that school-age children, who previously were not the traditional targets of malaria control, can benefit from targeted malaria interventions, with potential gains for both health and education. In Mali, an estimated 80% of school children at the end of malaria transmission season harbour malaria parasites despite high levels of LLIN coverage following a national campaign of universal coverage. This calls for additional control measures in this age group, and we evaluated the impact of intermittent parasite clearance in schools (in which a treatment dose is given irrespective of infection status) on the health and cognitive performance of schoolchildren in southwest Mali.

METHODS: A cluster-randomized controlled trial was conducted in 80 primary schools in Sikasso, Mali where the majority of schoolchildren already slept under insecticide-treated nets. Children in intervention schools received a single treatment dose of AS-SP (artesunate; sulphadoxine/pyrimethamine combination) at the end of November 2011; administered in school by teachers over three consecutive days. RDTs were completed by each participant pre and post each workshop. The intervention was estimated to cost $2.72 per child treated.

CONCLUSIONS: These results add to the growing body of evidence on the impact of asymptomatic malaria infection on cognitive performance in schoolchildren. Intermittent parasite clearance in schools has potential as an efficacious and cost-effective control strategy to improve the health and education performance of school-aged children. This approach is particularly suited to areas of seasonal transmission where a single annual treatment can be given at the end of the transmission season.
P161: Targeting epigenetic histone post-translational modifications in *Plasmodium falciparum* gametocytes

Nanika Coetsee, Nabila Ismail, Janette Reader, Bianca Verlinden, Prof. LM Birkholtz
Department of Biochemistry, University of Pretoria, Pretoria, Gauteng, South Africa

**BACKGROUND:** The lethal *P. falciparum* parasite, responsible for the significance of malaria worldwide, has developed resistance against many of the currently used therapeutics. The efficacy of these drugs are compromised and novel antimalarial agents, which act against new targets with new modes of action are needed urgently. Histone PTM’s play important roles in regulating gene expression during the intra-erythrocytic development (IDC) of *P. falciparum*. The precise extent of this mechanism of gene expression regulation in the sexual stages of *P. falciparum* still awaits discovery. Targeting the enzymes responsible for the PTM’s can be a way to target the development of parasites such as *P. falciparum*. This can be exploited in antimalarial drug discovery to determine the effect of various leading LSD1 and HDAC inhibitors on the asexual and sexual stages of the parasite’s life cycle.

**METHODS & RESULTS:** A method needs to be developed to isolate histone proteins from an adequate volume of the *P. falciparum* gametocytes. Gametocytes have been cultivated in *vitro* in small culture volumes that would not be satisfactory for histone isolations. Previous studies have shown the successful isolation of intact histone proteins from the asexual IDC stages of the parasite by saponin lysis and acid extraction. These methods need to be optimized for the mature stage V gametocytes and possibly applied to isolate stage-specific histone proteins from the various gametocyte stages. This will enable us to determine the histone codes for the various gametocyte stages of *P. falciparum* that will be analysed to identify any differences in the gametocyte epigenome compared to that of the IDC. The epigenome of the isolated *P. falciparum* gametocytes will be analysed by Mass Spectrometric analysis.

**CONCLUSION:** In optimizing a method for the cultivation of an increased volume of *P. falciparum* gametocytes and subsequently successfully isolating intact histone proteins for further analysis, it will be possible to study the epigenome of these life cycle stages of the parasite to elucidate novel targets in antimalarial drug development.

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P162: Cross cutting challenges for monitoring IRS in disease control programmes.

M Coleman

Indoor residual spraying (IRS) is the cornerstone intervention for many vector born diseases. The global scaling up of IRS activities, the huge financial investment and the anticipated outcome of saving lives have fuelled a demand for improved, more accurate and timely monitoring systems. Even with large scale roll out programmes the expected impact of IRS is not always seen. This can be due to many reasons but low coverage of targeted areas and application of insecticide on surfaces at concentrations too low to be effective have be investigated.

Key challenges remain the unit to use and method to determine coverage and the quickest, easiest, most cost effective and informative way of determining insecticide active ingredient on sprayed surfaces. The availability of quality data to prompt reactive responses and remedial action, in time, can assist vector control programmes and benefit the communities they aim to protect.

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P163: The operational impact of pyrethroid insecticide resistance on malaria control in Malawi.

Michael Coleman, Janet Hemingway, Immo Kleinschmidt, Themba Mzilahowa, Helen Irving, Miranda Ndula, Andrea Rehman, John Morgan, Kayla G. Barnes, Charles S Wondji

**BACKGROUND:** The impact of insecticide resistance on insect-borne disease programs is difficult to quantify. Controlling malaria in high-transmission settings is heavily dependent on effective vector control reducing disease transmission rates. Pyrethroids are currently the dominant insecticides used for malaria control, and their failure will adversely affect our ability to control malaria.

**METHODS:** Insecticide resistance was monitored in Malawi using standard WHO diagnostic assays. The presence of insecticide resistance mechanisms were determined using standard WHO biochemical assays. Geneotyping for kdr and Ace-1 was carried out and QPCR analysis of P450 genes CYP6P9a and CYP6P9b. Cross-sectional household surveys were carried out at each sentinel site on a random sample of 140 1- to 4-y-old children over 3 years.

**RESULTS:** In the LLIN-only sites the reduction in prevalence from 2009 to 2010 was 53.2% (CI 40.4–65.5%) to 40% (23.4–59.1%), whereas the reduction in the IRS plus LLIN sites was from 54.9% (42.3–66.8%) to 38.6% (30.2–47.8%) Significant pyrethroid resistance (8-66% survival) was detected in over 3,000 F1 An. funestus tested. While there was evidence of carbamate resistance (24-45% survival) no resistance to organophosphate or DDt was recorded. Biochemical assays indicated that this resistance was due to elevated levels of monooxygenase and transcription profiling determined that CYP6P9a and CYP6P9b were elevated.

**DISCUSSION:** The selection of insecticide resistance did not result in surge in malaria transmission, as judged by annual point prevalence surveys in 1- to 4-y-old children. This is true in areas with long-lasting insecticide-treated nets (LLINs) alone or LLINs plus pyrethroid-based insecticide residual spraying (IRS). However, in districts where IRS was scaled up, it did not produce the expected decrease in malaria prevalence. It is feasible that this reduction is due to the control of secondary vectors in this area such as An. gambiae which are less resistant. As resistance increases in frequency from this low initial level, there is the potential for vector population numbers to increase with a concomitant negative impact on control efficacy.

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P164: Non-invasive Pulse Oximetry Predicts Death in Ugandan Children with Malaria

Andrea Conroy1,2, Michael Hawkes1,2, Sophie Namasopo3, Chandy John4, Bob Opoka5, Kevin Kain1,4,5

1Department of Medicine, University of Toronto, Toronto, Canada; 2Sandra A. Rotman Laboratories, McLaughlin-Rotman Centre for Global Health, Toronto, Canada; 3Department of Paediatrics, Jinja Regional Referral Hospital, Uganda; 4University of Minnesota, USA; 5Department of Paediatrics and Child Health, Mulago Hospital and Makerere University, Kampala, Uganda; 6McLaughlin Centre for Molecular Medicine, Toronto, Canada; 7Tropical Disease Unit, Toronto General Hospital, Toronto, Canada.

**BACKGROUND:** In resource limited settings implementation of simple and inexpensive point-of-care diagnostic devices could be used to identify children at-risk of poor outcomes and direct resources accordingly. We hypothesize that non-invasive pulse oximetry will predict mortality in Ugandan children admitted to hospital with malaria.

**METHODS:** Since February 2012 febrile children between 2 months and 5 years have been enrolled in a prospective observational study at Jinja Regional Referral hospital and followed to determine disease outcome. At admission, pulse oximetry is performed to measure oxygen saturation (SpO2) and the perfusion index (PI, the ratio of pulsatile blood flow to
non-pulsatile static blood flow in peripheral tissue) (Masimo Pulse CO-oximeter). Receiver operator characteristic (ROC) curves were used to assess the predictive ability of the markers among children with malaria.

**RESULTS:** Between February 2012 and April 2013, 1677 febrile children were enrolled with known disease outcomes. The mean age was 1.7 years with 76% of children diagnosed with malaria based on local expert microscopy and/or positive 3-band RDT (pLDH/HRP2). The mortality rate in children with malaria was 3.1%. The area under the ROC curve (AUC) for SpO2 was 0.69 (95% CI, 0.59-0.80; p<0.0001), and a SpO2 less than 92% was 97% sensitive and 37% specific in predicting mortality. The PI had an AUC of 0.68 (0.57-0.78; p<0.0001), and a PI less than 0.20 was 98% sensitive and 17% specific in predicting mortality. Initiation of appropriate life-saving measures (oxygens, fluids) in children with low SpO2 or a low PI resulted in marked patient improvement. Venous lactate ≥5.5mmol/L had an AUC of 0.80 (95% CI, 0.71-0.90; p<0.0001) and a sensitivity and specificity of 81% and 77%. When combining risk factors (hypoxia, SpO2 <92%; hyperlactatemia, ≥5.5mmol/L; low perfusion, PI<0.20) into a risk score, the mortality rate was 1.4% for one risk factor present and rises to 6.2% and 18.2% for 2 and 3 risk factors respectively.

**CONCLUSIONS:** These data suggest that pulse oximetry may be useful in the triage and treatment of children with severe malaria. Additional advantages of pulse oximetry are low operating costs and real-time patient monitoring.

**P165:** Differential salivary gland protein expression in insensitive acetylcholinesterase resistant *Anopheles gambiae* mosquitoes.


**BACKGROUND:** Management of insecticide resistance is a major concern to preserve the benefits of vector control against malaria. Of particular concern, mutation in the acetylcholinesterase encoding ace-1 gene conferring cross-resistance to organophosphate and carbamate insecticides was reported in *Anopheles gambiae* populations in Central and West Africa. This mutation is associated with a genetic cost revealed through alterations of some life history traits but physiological and behavioural changes in insects baring the ace-1R allele are poorly known. Comparative analysis of the salivary gland contents between susceptible and ace-1R resistant strains was conducted to characterize factors that could be involved in modifications of blood-meal digestion, trophic behaviour or pathogen infection obtained by different Cox models. In these models, MIP was compared the estimates of the effect of MIP on time to first malaria infection obtained by different Cox models. In these models, MIP was compared to the ace-1R resistance. Further functional studies and insect behaviour experiments could confirm the cost of this mutation on blood-feeding and pathogen transmission abilities in the resistant mosquitoes.

**RESULTS:** Differential analysis revealed differences in abundance for eleven proteins, most of them down-regulated in the resistant strain. Among them Saglin, a salivary-gland 1 related protein, receptor of *Plasmodium falciparum* TRAP binding domain that plays a role in sporozoite invasion displayed a lower expression in the resistant strain. Conversely, a protein known to be preferentially expressed in adult female salivary glands was up-regulated in the resistant strain. Glutathion- S transferase was also down regulated in the resistant strain.

**CONCLUSIONS:** This work is the first analysis of the impact of ace-1 resistant allele on the siolome composition of *An. gambiae*, the primary vector of human malaria in Africa. It reveals differentially regulated proteins involved in pathogen interaction, signal transduction, protein-protein interaction, insect metabolism or protection against oxidation. These data contribute to the understanding of the profound alterations linked to the ace-1 resistance. Further functional studies and insect behaviour experiments could confirm the cost of this mutation on blood-feeding and pathogen transmission abilities in the resistant mosquitoes.

**P166:** Importance of adequate local spatiotemporal transmission measures in malaria cohort studies: application to the relation between placental malaria and first malaria infections in infants


1,3,4 Institut de Recherche pour le Développement (IRD), UMR216, Mère et Enfant Face aux Infections Tropicales, Paris, France; 2,3,4,5 Centre d’Etudes et de Recherche sur le Paludisme Associé à la Grossesse et à l’Enfant (CERPAGE); 3Centre d’Etudes et de Recherche sur le Paludisme Associé à la Grossesse et à l’Enfant (CERPAGE); 4Institut de Recherche pour le Développement (IRD), Maladies Infectieuses et Vecteurs, Ecologie, Génétique, Évolution et Contrôle (MIVEGEC, UM1-CNRS 5290-IRD 224), Montpellier, France

**BACKGROUND:** According to several studies, infants whose mothers had a malaria-infected placenta (MIP) at delivery are at increased risk of presenting a first malaria infection. Immune tolerance caused by the intrauterine contact with parasite could explain this phenomenon, but it is also known that infants highly exposed to anopheles infected with Plasmodium are at greater risk of contracting malaria. Consequently, local malaria transmission has to be taken into account to demonstrate the immune tolerance hypothesis.

**METHODS:** From data collected between 2007 and 2010 on 545 infants followed up from birth to 18 months in South Benin, the authors compared the estimates of the effect of MIP on time to first malaria infection obtained by different Cox models. In these models, MIP was either adjusted on 1) “village-like” time-independent exposure variables, or on 2) spatiotemporal exposure prediction (EP) derived from climatic, environmental and behavioral local factors.

**RESULTS:** Only the use of EP improved the model’s goodness of fit (BIC) and led to clear conclusions regarding the effect of placental infection, whereas the models using the village-like variables were less successful than the univariate model.

**CONCLUSIONS:** This demonstrated clearly the benefit of taking adequately into account the transmission in malarial cohort studies.
P167: Accessing the Effective use of Mosquito bed nets amongst women in Rural Areas in the North West region of Cameroon

P Cynthia

Improvements in preventive and care-seeking behaviors to reduce maternal mortality in rural Africa depend on the knowledge and attitudes of women and communities. Surveys have indicated a poor awareness of maternal health problems by individual women (Mikey R. et al. 2006). The aim of this Study was to find out the knowledge, attitudes and practice of rural women towards the proper use of mosquito bed nets. This study was conducted in some five villages in Momo division of the North West region where in 350 women were recruited for the study using convenient random sampling technique. Questionnaires in-depth interview was used to collect data for this study. From the result obtained from studies, 45 % of the women reported have owned a mosquito bed nets while 55% said they have never owned one. Out of those who had owned these nets only 23% of them confess to actually using these bed nets while 77% sad they don’t use it always or have them in their boxes or are using them for other purposes. Interesting was the fact that out of the 350 women, only 48% of them actually understood the importance of mosquito bed nets. Amongst those who receive nets but are not using them, most of them gave reasons ranging from difficulties handing the nets to overheat while sleeping under these nets. Some also said they have bad dreams when they sleep under the nets. This study therefore suggests that more education is necessary before the distribution of bed nets amongst the rural population. Also The localities of the population including the type of houses and beds they use should be considered when educating them on mosquito bed nets use.

P168: In vivo antiplasmodial activities of Cochlospermum planchonii, Phyllanthus amarus and Cassia alata tested individually or in combination

O. Daï1, R.S. Verbangana2, B. Koama1, Z. Kabré2, S. Tamboura2, Z. P. Dakuyo2, M. Sekhoacha4, M.G. Matsabisa1, M. Traoré1, G.A. Ouedraogo2 and J.B. Ouedraogo2

1Direction Régionale de l’Institut de Recherche en Sciences de la Santé (IRSS-DRO/CNRST), BP 545 Bobo-Dioulasso, Burkina Faso; 2Centre Muraz, BP390, Bobo-Dioulasso, Burkina Faso; 3Pharmacie de la Comoé, Phytofla, BP 293 Banfora, Burkina Faso; 4Indigenous Knowledge System Unit of the Medical Research Council (IKS/MRC) in Cape Town, South Africa; 5Université Polytechnique de Bobo-Dioulasso, Laboratoire de Recherche en Science de la Santé et Biotechnologie Animale (LARESBA), BP 1091 Bobo-Dioulasso, Burkina Faso.

INTRODUCTION: A standardized remedy known as “Saye” is widely used for malaria treatment by the community. Saye is a combination of 3 plants including Cassia alata L. (Fabaceae), Cochlospermum planchonii Hook.f. (Cochlospermaceae), Phyllanthus amarus Schumach. et Thonn. (Euphorbiaceae).

OBJECTIVE: This study aimed to assess the antiplasmodial activities of Saye or the combination Cassia alata + Phyllanthus amarus + Cochlospermum planchonii (2:2) and 1,2 Phyllanthus amarus + Cochlospermum planchonii (2:1) on the suppression of parasitemia and the percentage reduction of parasitemia.

METHODS: A standardized decoction of each of the three plants was prepared and tested individually or in combination on NMRI mice infected with Plasmodium berghei (ANKA strain).

RESULTS: The results showed that the combination Cassia alata + Phyllanthus amarus (2:1) at doses of 50, 100, 250 and 400 mg/kg bw failed to inhibit the parasites in treated mice. But the combination Phyllanthus amarus + Cochlospermum planchonii (2:1) at dose of 50 mg/kg bw exhibited 63.8% of parasites reduction. None additive activity was observed with higher doses (100 to 400 mg/kg bw). In the groups treated with Saye or the combination Cassia alata + Cochlospermum planchonii (2:2) the percentage reduction of parasitemia was 43.6% and 50.0% respectively.

CONCLUSION: The preliminary results have shown a higher activity of Saye or the combination Cassia alata + Cochlospermum planchonii (2:2) comparatively to the other combined extracts.

P169: Complexity in mating and swarming systems in natural populations Anopheles gambiae s.s. in Burkina Faso: occurrence of parous, blood-fed, semi-gravid and gravid females within the swarms

Dabié K.R.1, Sawadogo S.P.1, Gibson G.2, Costantini C.2, Diabaté A.1

1IRSS/Centre Muraz, 01 BP 390, Bobo-Dioulasso, Burkina Faso; 2Natural Resources Institute /University of Greenwich, Central Avenue Chatham Maritime Kent, ME447B, UK; 3Institut de Recherche pour le Développement (IRD), UMR 224- MIVEGEC, Montpellier, France.

BACKGROUND: The control of malaria vector based mainly on the use of insecticide is challenging since two decades the emergence of resistance in vector populations and needs new alternatives. The advanced knowledge in the genomic and molecular genetic of Anopheles gambiae Giles s.s., the main malaria vector in sub-Saharan Africa, increased the prospects of the use of transgenic or sterile males mosquitoes. Gathering basic information on the swarming and mating behaviour would help to better inform any control strategy based on the release of such modified mosquitoes.

MATERIALS AND METHODS: Across four-year longitudinal surveys from 2006 to 2009, we investigated the swarming and mating systems in natural populations of An. gambiae s.s. in two sites, Vallée du Kou (VK7) and Soumousso, two rural areas of south-western Burkina Faso. Individual mosquitoes and pairs collected in copula were sampled from swarms in both sites using insect sweep-nets during four consecutive days per month. Females collected in copula or in solo were observed for their physiological status (blood-fed, semi gravid and gravid) and then dissected to establish their insemination and parity rates.

RESULTS: Out of 978 females collected within 250 swarms in VK7 and 210 females from 70 swarms in Soumousso, including pairs and females in solo, 5% (49/978) and 5.7 % (12/210) were respectively composed by gravid, semi gravid and blood fed females. Their insemination rate revealed that 51% and 66% of them were inseminated respectively in VK7 and Soumousso. We also estimated the parity rate, which reached an average of 4.3% in VK7 for 7.5% in Soumousso indicating that although in low proportion, parous females were participated to the swarming system.

CONCLUSIONS: As it was assumed that Anopheles female mates once during her lifetime, the presence within the swarms of females considered as presumably “inseminated” appears intriguing and address the question of the possibility of remating of anopheline females. These results could have relevant consequences for any control strategy based on the release of transgenic or sterile males.
P170: Analysis of the genitalia rotation in the male Anopheles funestus (Diptera: Culicidae)

Yael Dahan

INTRODUCTION: Anopheles funestus is a major malaria vector in Africa. Insecticide resistance has developed in populations of this species in several African countries, prompting the need to develop additional vector control methods such as the sterile insect technique (SIT). This technique requires an understanding of those underlying physiological events that lead to sexual maturity of An. funestus males, the rotation of their genitalia in particular. The aim of this study was to quantitatively describe genital rotation in An. funestus males as it is an essential function of sexual maturation.

METHODS: The genital rotation of adult wild caught An. funestus males (maintained at 23 ± 1°C post emergence) and laboratory colonised An. funestus males (maintained at either 18 ± 1°C, 23 ± 1°C or 29 ± 1°C post emergence) was monitored in males that were 2 to 48 hours old.

RESULTS: Genital rotation of all the males reached its final rotation stage (135 to 180° rotation) 36 hours post emergence at 23 ± 1°C in laboratory colonised An. funestus males. These males had a comparable rotation rate as wild caught An. funestus at the same temperature setting. A temperature change (either 18 ± 1°C or 29 ± 1°C versus 23 ± 1°C) significantly influenced the genital rotation rate such that this rate increased with increasing temperature.

CONCLUSION: The information from this study enhances our understanding of An. funestus male biology and is important in terms of applying the sterile insect technique because the rate of sexual maturation in males has implications for the timing of sterile male release.

P171: Effect of fungal surface on the prevalence of malaria in students Yaounde, Cameroon

Eno Anna Aret 1, Tonye III Daves Stéphane 1 Et Kechira Frederick Agem 2
1 Département de Biologie et de Physiologie Animale, Université de Yaoundé I, Yaoundé, Cameroun; 2 Faculté de Médecine et des Sciences Biomédicales, Université de Yaoundé I, Yaoundé, Cameroun.

BACKGROUND: Despite all the novel research, management and control measures put in place, malaria remains one of the leading infectious diseases, world wide. The main aim of this study was to establish a relationship between superficial mycoses and malaria prevalence.

METHODS: 50 pupils were randomly recruited with consent of their parents, and 3 drops of blood were bled from them for malaria identification by microscopy and 50 Bioline Malaria Antigen test. Skin scrapings were also collected from those identified with superficial mycoses.

RESULTS: Global sample 21 (42 %) cases were positive for Plasmodium: 18 (52.94 %) from pupils with healthy skin and 3 (18.75 %) from 16 pupils with superficial mycoses. However, 7 (43.7 %) Trichophyton spp., 6 (37.5 %) Candida spp., and 3 (18.8 %) Malassezia spp. were identified from skin samples.

CONCLUSION: The mycoses fungi inhibited malaria prevalence with toxins that they secrete.

P172: Risk factors for malaria transmission in Engela District, of the Ohangwena region of Northern Namibia

J. Auala1, H. Sturrock2, I. Kleinschmidt1, I. Du Preez2, R. Bock1, R. Gosling2, S. Katokie1, D. R. Mumbengegwi2
1 Multidisciplinary Research Centre, Science, Technology & Innovation Division, University of Namibia, Windhoek, Namibia; 2 Malaria Elimination Initiative, Global Health Group, University of California, San Francisco, USA; 3 Faculty of Epidemiology and Population Health, Dept. of Infectious Disease Epidemiology, London School of Hygiene and Tropical Medicine, London, United Kingdom.

BACKGROUND: Malaria transmission in Namibia has declined dramatically from 477,786 in 2000 to 1546 cases in the 2012/13 malaria season. Namibia has adopted a policy of malaria elimination by 2020 (zero local transmission). This presents new challenges as interventions that were successful in bringing down malaria cases may no longer be appropriate at low transmission settings. New tools and interventions are required to move to no local transmission of malaria. This study was conducted to determine the risk factors for malaria transmission in a low transmission setting by following up and interviewing, all malaria cases in Engela district in the Ohangwena region.

METHODS: All RDT confirmed malaria cases reported from the 17 clinics in the Engela district were recruited for this study. Four surrounding households were also selected and recruited into the study and this constituted a neighbourhood. All fever individuals testing malaria positive by RDT were visited at their homes; and interviewed about malaria risk factors such as use of mosquito nets, indoor residual spraying of sleeping structures, presence of mosquito breeding sites and travel history.

RESULTS AND CONCLUSION: Twenty two of malaria were reported including 4 death cases. One hundred and ninety four households were visited from 46 neighborhoods. Out of the 22 cases reported, 12 individuals had a history of travelling to Angola close to the time in which they were diagnosed with malaria. Forty six percent of households reported having a breeding site close to the sleeping structures. Thirty three percent of the houses did not have mosquito nets and almost 76% percent of all sleeping structures had a space between the roof and the wall where mosquitoes can pass freely at night. Only 16.5% of structures were not sprayed, 78.4% were not sprayed.

CONCLUSION: Importation of malaria is a major risk factor for malaria transmission in Engela district as persons travelling are potential reservoirs of the disease in their communities. The low usage of mosquito nets and low IRS coverage, poses a real challenge to stopping malaria transmission. Health check-ups following travelling to malaria-endemic areas should be made a priority.

P173: Anti-malarial properties of novel symmetrical, terminally alkylated polyamine analogues against Plasmodium falciparum

Marna de Beer1; Pachaiyappan Boobalan 2; Patrick Woster2, Lyn-Maria Birkholzt 1
1 Department of Biochemistry, University of Pretoria, Private Bag x20, Pretoria, 0003; 2 Medical University of South Carolina, 70 President St, Charleston, SC 29403, USA

BACKGROUND: The malaria parasite, Plasmodium falciparum, displays genetic plasticity and therefore increasingly develops resistance to currently used anti-malarial drugs. Innovative strategies to develop novel anti-malarial drugs to eradicate the disease are therefore of utmost importance. Recently, alkylated (bis)urea and (bis)thiourea polyamine analogues were shown to have potent nM anti-malarial activity against various strains of P. falciparum parasites 1. These symmetrical, terminally alkylated polyamine analogues inhibit the parasite's nuclear division in a cytotoxic and irreversible manner and is highly selective (>7000 fold) against P. falciparum compared to HepG2 cells2. A third generation (bis)urea and (bis)thiourea symmetrical, terminally alkylated polyamine analogues with a 3-5-3 carbon backbone were generated and analysed for their ability to inhibit in vitro proliferation of P. falciparum parasites.

METHODS AND RESULTS: Anti-malarial activities (IC50) ranging from 28 to 793 nM were obtained against various strains of P. falciparum parasites including, chloroquine sensitive (3D7) and resistant (HB3) lines and antifolate resistant (W2) lines, using the Malaria SYBR Green I-based assay. The leading compounds proved to be >3000 fold more selective toward P. falciparum compared to the mammalian HepG2 cells, as measured by their selectivity indices. The analogues prevented parasitic growth within 24 h of exposure by inhibiting nuclear division and therefore asexual development seen from flow cytometric analysis.
The leading compounds reacted both additively and synergistically when tested with the known anti-malarial, DFO, and one compound lead to irreversible parasite toxicity after only 12 h treatment.

CONCLUSION: Terminally alkylated (bis)urea and (bis)thiourea polyamine analogues pose as an enticing structurally novel and distinct class of potential anti-malarials with potent activities and against P. falciparum parasites. In vivo activity determination is currently underway.

P174: Implications of health provider compliance on out-of-pocket expenditure during care-seeking for fever in South East Tanzania

Inez Mikkelsen-Lopez1, Fabrizio Tediosi2, Gumi Abdallah1, Mustafa Njozi1, Baraka Amuri1, Rashid Khalib, Fatuma Manzi1, Don de Savigny2
1Swiss Tropical and Public Health Institute, Basel, Switzerland, 2University of Basel, Basel, Switzerland, 3The Ifakara Health Institute, Tanzania

BACKGROUND: Health system provider failures including drug stockouts and consequent health provider behaviour are key barriers to effective malaria case management. We have quantified how these influence household expenditures during care-seeking for fever in the Ulanga District in South East Tanzania.

METHODS: We combined weekly ACT stock data for the period 2009-2011 from six health facilities in the Ulanga District in Tanzania, together with household data from 333 respondents on the cost of fever care-seeking events in Ulanga during the same time period to establish how health seeking behaviour and expenditure might vary depending on ACT availability in their nearest health facility.

RESULTS: Irrespective of ACT stockouts, more than half (58%) of respondents sought initial care in the public sector, the remainder seeking care in the private sector where expenditure was 19% more expensive. Over half (54%) of respondents who went to the public sector reported incidences of non-compliant behaviour by the attending health worker (e.g. charging those who were eligible for free service or referring patients to the private sector despite ACT stock), which increased household expenditure per fever episode from USD 0.34 to USD 1.76. ACT stockouts were considered to be the result of non-compliant behaviour of others in the health system and increased household expenditure by 21%; however we lacked sufficient statistical power to confirm this finding.

CONCLUSION: System design and governance challenges in the Tanzanian health system have resulted in numerous ACT stockouts and frequent non-compliant public sector health worker behaviour, both of which increase out-of-pocket health expenditure. Interventions are urgently needed to ensure a stable supply of ACT in the public sector and increase health worker accountability.

P175: Extended surveillance for malaria in the Eastern and Northern provinces of Sri Lanka through focused laboratory diagnostic services

Fernando Deepika

INTRODUCTION: Following the end of the civil conflict the ability of the Anti Malaria Campaign to carry out malaria diagnostic services in the Eastern and Northern Provinces of Sri Lanka was hampered due to a breakdown in health infrastructure and a large number of vacancies in the preventive and curative cadres. Tropical and Environmental Diseases and Health Associates (TEDHA), a private sector organization was called upon to assist in intensified surveillance in these provinces to achieve the goal of malaria elimination in Sri Lanka by 2014.

METHODS: TEDHA set about training two categories of laboratory personnel who would be involved in the laboratory component of surveillance. Fever Surveillance Assistants were trained towards receptivity of patients suspected to have malaria and preparation of blood smears by finger prick method. Parasitology Surveillance Assistants were trained to stain and examine blood smears for malaria parasites. Hospitals to establish laboratories were identified in areas where government services were lacking, and refurbishments carried out where needed.

RESULTS: Following the above process, 50 fully functional laboratories have been established and are actively carrying out malaria diagnosis by microscopy by a staff of 112 persons. Quality assurance procedures are in place with field monitoring taking place regularly. From January 2010 till 31st December 2012, TEDHA has screened 584,081 blood smears for malaria. It is noteworthy that hospital based laboratories have now been extended to outreach through mobile malaria clinics and hospital village clinics.

CONCLUSIONS: The adequacy of TEDHAs training of laboratory personnel and their performance in the current level of malaria diagnosis is a positive solid indicator of the processes adopted. TEDHA has contributed in a number of ways to supplement the national malaria elimination efforts in the Northern (Mannar district ) and Eastern Provinces (Ampara, Batticaloa and Trincomalie districts), based on the fact that the private sector has the flexibility and capacity to reach populations and provide services which would require longer time commitments to be accomplished by government or civil society. The success of this public-private partnership is an example that can be used in other countries attempting malaria elimination.

P176: Cotrimoxazole prophylaxis versus mefloquine intermittent preventive treatment to prevent malaria in HIV-infected pregnant women: two randomized controlled trials

Lisa Denoue-Ndam1, Dijimon-Marcel Zannou1, Camille Fourcade1, Clément Taron-Brocard1, Raphaël Porcher1, Felix Atadokpé1, Didier K. Komongui2, Lucien Dossou-Gbete1, Aldric Afangnhoun2, Nicaise T. Ndam3, Pierre-Marie Girard3, Michel Cot1
1UMR 216, Institut de Recherche pour le Développement and Université Paris Descartes, Paris 75006, France; 2Centre de Traitement Ambulatoire, Centre National Hospitalier Universitaire Hubert Koutoukou Maga, Cotonou, and Faculté des Sciences de la Santé, Université d’Abomey-Calavi, Benin; 3Inserm U717, Hôpital Saint-Louis, Paris 75011, France; 4Service de Médecine Interne, Hôpital d’Instructions des Armées, Cotonou, Benin; 5Service de gynécologie, Hôpital de la Mère et de l’Enfant Lagune Homel, Cotonou, Benin; 6Clinique Louis Pasteur, Porto-Novo, Benin; 7Centre de Traitement Ambulatoire, Hôpital de zone de Suru Léré, Cotonou, Benin; 8Service des Maladies Infectieuses et Tropicales, Hôpital Saint-Antoine, APHP; INSERM U707 and Université Pierre et Marie Curie, Paris 75012, France.

BACKGROUND AND OBJECTIVE: Malaria in pregnancy is responsible for serious consequences that are increased by HIV infection. Cotrimoxazole (CTX) prophylaxis given to prevent HIV-related opportunistic infections is assumed to protect against malaria. Alternatively, an intermittent preventive treatment (IPTp) is recommended. For the first time, the efficacy of CTX prophylaxis versus mefloquine (MQ) IPTp was evaluated in HIV-infected pregnant women.

METHODS: PACOME gathered two randomized, open-labelled, non-inferiority trials in Benin (registration number: NCT00970879, www.clinicaltrials.gov). In the CTX mandatory (CM) trial, women below 350 CD4 cells/mm3 received daily CTX alone, or associated with MQ IPTp (N=292). In the CTX not mandatory (CNM) trial of women with CD4>350/ mm3, CTX prophylaxis was compared to MQ IPTp (N=140). Microscopic placental malaria was the primary endpoint.

RESULTS: At delivery, one woman in each trial receiving CTX alone had placental malaria, versus none in the MQ groups. Non-inferiority of CTX was demonstrated in the CM trial. Moreover, PCR-detected placental malaria was markedly reduced in the MQ+CTX group. In the CMN trial, sample size was insufficient to conclude. No serious adverse event related to the drugs was found, though dizziness and vomiting of moderate intensity were reported by half of women receiving MQ.

CONCLUSIONS: This study demonstrated that CTX used alone provides sufficient protection against malaria in HIV-infected pregnant women. Additionally, MQ IPTp was highly efficacious with no serious adverse events reported. It thus appears an alternative in the next future, when
P177: Study of antibody and cellular responses to *Plasmodium vivax* variant vir proteins during pregnancy


*Barcelona Centre for International Health Research (CRESIB)-Hospital Clinic, Universitat de Barcelona, Barcelona, Spain; **Papua New Guinea Institute of Medical Research (PNG IMR), Goroka, Papua New Guinea; ***International Center for Genetic Engineering and Biotechnology (ICGEB), New Delhi, India; 1Instituto Superior de Sanitá (ISS), Rome, Italy; 2Centro Internacional de Vacunas (CIV) / Instituto de Inmunología, Cali, Colombia; 3Universidad del Valle de Guatemala (CES-UVG), Guatemala, Guatemala; 4Fundación de Medicina Tropical Dr. Heitor Vieira Dourado (FMT-HVD), Manaus, Brazil; 5Sardar Patel Medical College (SPMC), Bikaner, India; 6University of Melbourne, Melbourne, Australia; 7School of Tropical Medicine, London, United Kingdom; 8Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain; 9Fundación de Hematología y Hemoterapia do Amazonas (HEMOAM), Manaus, Brazil; 10Walter and Eliza Hall Institute (WEHI), Parkville, Australia.

BACKGROUND: VIR antigens may be considered as promising candidates for a *Plasmodium vivax* malaria vaccine. We set out to characterize naturally-acquired cellular and antibody responses to VIR proteins in pregnant and non-pregnant women heavily exposed to malaria.

METHODS: This work is part of a multicenter cohort study (PregVax) conducted in pregnant women in five *P. vivax* endemic countries: Brazil, Colombia, Guatemala, India, and Papua New Guinea (PNG) funded by European Commission (under grant agreement FP7-HEALTH-201588).

RESULTS: Antibody responses were detected to all VIR antigens tested, with the highest responses found in PNG. The lowest levels of antibodies in this study at baseline, sixth and twelfth months. At baseline 7.2% women in the control versus 6.1% women in the intervention group were positive for malaria. 74.7% of the pregnant women in the control and 56.1% in intervention villages slept under LLIN the previous night of the survey. At the baseline survey, pregnant ladies in the intervention arm (32.2%) were more likely to be febrile than pregnant ladies in the control arm (18.9%). Utilization of LLIN steadily improved in the intervention villages than the control village at the six and twelve months. LLIN was increased by 32.3 percentage point (95% CI: 20.0-44.5) at sixth month and by 43.2 percentage point (95% CI: 30.0-56.4) at twelfth month of the intervention. Fever in the last two week before the survey significantly declined, with an effect size of -26.7 (95% CI: -39.4,-14.0) at twelfth month of the intervention.

CONCLUSION: The study highlights a marked increase in LLIN utilization following the training of household heads, which in turn lead to significantly lowering of febrile episodes and severe anemia in the intervention group. Ministry of health and other partners working on malaria in pregnancy should consider scaling up the intervention in other areas.

P178: Utilization of insecticide-treated bed nets among pregnant women and its effect on fever and malaria: A cluster randomized controlled trial

Kebede Deribe

BACKGROUND: Malaria infection during pregnancy is a major public health problem in tropical and subtropical regions throughout the world. Malaria and severe anaemia may result in maternal death or adverse pregnancy outcomes. The objective of the study was to determine the effect of training of household heads on utilization of long-lasting insecticide treated bed nets (LLITN) on the prevalence of malaria parasitaemia and anaemia in pregnant women in rural southwest Ethiopia.

METHODS: Twenty-two (11 intervention and 11 control) villages were included in a cluster randomized trial in southwest Ethiopia. The intervention consisted of tailored training of household heads about the proper use of LLITN and community network system. Burden of malaria and anaemia in pregnant women were determined at the baseline, six and twelve months. Pregnant women with malaria and anemia were treated based on the national protocol. Burden of malaria and anemia between the intervention and control villages was compared using the complex logistic regression model by taking into account the clustering effect.

RESULTS: A total of 442, 233 and 192 pregnant women were included in this study at baseline, sixth and twelfth months. At baseline 7.2% women in the control versus 6.1% women in the intervention group were positive for malaria. 74.7% of the pregnant women in the control and 56.1% in intervention villages slept under LLIN the previous night of the survey. At the baseline survey, pregnant ladies in the intervention arm (32.2%) were more likely to be febrile than pregnant ladies in the control arm (18.9%). Utilization of LLIN steadily improved in the intervention villages than the control village at the six and twelve months. LLIN was increased by 32.3 percentage point (95% CI: 20.0-44.5) at sixth month and by 43.2 percentage point (95% CI: 30.0-56.4) at twelfth month of the intervention. Fever in the last two week before the survey significantly declined, with an effect size of -26.7 (95% CI: -39.4,-14.0) at twelfth month of the intervention.

CONCLUSION: The study highlights a marked increase in LLIN utilization following the training of household heads, which in turn lead to significantly lowering of febrile episodes and severe anemia in the intervention group. Ministry of health and other partners working on malaria in pregnancy should consider scaling up the intervention in other areas.
and Tano (24-659 ib/p/m) depict relatively high transmission of malaria in Ahafo.

CONCLUSIONS: The survey shows areas to intensify vector control activities. It also demonstrates that transmission in Asutifi and Tano is high even before the commencement of mining operations. The study has built a platform for future vector control studies and interventions.

P180: Indoor Residual Spraying in Ghana; baseline insecticide susceptibility studies to select insecticides for spraying in ten districts in Ghana

Dominic Dery

BACKGROUND: Of options for vector control, Indoor Residual Spraying with appropriate insecticides is a key intervention which can reduce vector populations in a given area and interrupt transmission. Insecticides susceptibility of Anopheles vectors in order to recommend appropriate insecticides for spraying operations was initiated in ten selected districts in Ghana.

METHODS: October 2012-January 2013, immature Anopheles were collected and reared to adults. Mortality from tarsal contact of females was assessed with 11 insecticides in four chemical classes: i) organochlorines, ii) organophosphates, iii) carbamates and iv) pyrethroids. Four replicates of 25 unfed Anopheles gambiae females, aged 3 days, were exposed to insecticide impregnated papers for 1 hour (Fenitrothion was 2hrs). Number knocked down was recorded every 10 minutes and mortality 24 hours exposure. Pyrethrum Spray Collections (PSC) were performed in 60 randomly selected rooms in each district.

RESULTS: Of 2,038 PSC collections, species compositions were An. gambiae s.l (64%), funestus (22%), Culex species (9%), Aedes (4%), Pharaohensis (1%) and few Mansonia and rufipes species. Sporozoite rate of 0.036 was computed and Malalithion identified as appropriate insecticide for spraying in seven districts, Fenitrothion in three districts and Propoxur in one district. Of 483 Polymerase Chain Reactions, An. gambiae s.s was prevalent (475) and few arabiensis (5) detected in savannah arid communities in the northern districts. The M-form was dominant (143) with no hybrids detected. The S-form was detected across the country though in low numbers (21). Few kdr susceptible strains were detected (14) but majority were homozygous kdr(5) (120) resistant species and heterozygous were moderate in number (32).

CONCLUSIONS: An organophosphates is appropriate for Indoor Residual Spraying in Ghana. Rotation of insecticides is recommended as it offers a practical solution for resistance management. Though reported that kdr mutation is widespread in West Africa, results contrast observation that, frequency within S-form is much higher and the distribution is more widespread than within the M-form.

P181: Malaria vector transmission indices and insecticide susceptibility profile of Anopheles gambiae in Birim area in Ghana

Dominic B. Dery1, KwakuPoku Asante1, Kwaku Gbaa Feli2, Robert Addah1, George Adjie1, Emmanuel Mahama1, Seth Owusu-Ayee1

1Kintampo Health Research Centre, Clinical Laboratory, Ghana Health Service, Box 200, Kintampo, Brong-Ahafo Region, Ghana

BACKGROUND: Malaria vector studies are essential prior to commencement of mining activities. A baseline survey in Birim North and South districts was conducted before active mining operations in the area.

METHODS: Rooms were randomly selected in communities to collect mosquitoes. Larva and pupa Anopheles mosquitoes were collected between March-June 2012 in Okairom, Mamamso, Suedru and Aduasa. Malaria vectors were checked for presence of circumsporozoite (CS) antigens of P. falciparum by ELISA. Polymerase Chain Reactions (PCRs) were done to confirm species, identify molecular forms and kdr genotypes. Susceptibility assays were performed with Permethrin, Deltamethrin, DDT and Bendiocarb.

RESULTS: A total of 165 CDC light trap-nights caught 3,651 Anopheles vectors made up of mainly gambiae (93%) and funestus (7%) species and few rufipes (5) and pharaohensis (2) species. A total of 9,803 non-malaria vectors were caught composed of Mansonia species (22%), Phlebotomus (39%), Culex (39%) and Aedes (1%) species. Individuals in Birim receive 1-7 infective bites from Anopheles vectors monthly with high infective bites encountered in October and February. Only An. gambiae s.s were detected from 195 analyzed by PCR. No An. arabiensis were identified by PCR. Equal numbers of M and S molecular forms (47 each) were confirmed by PCR. Only two kdr(10) genotypes were detected in samples from two communities. An gambiae were susceptible to Deltamethrin and Permethrin (92%, 84% mortality respectively) but resistant to DDT and Bendiocarb (38%, 63% mortality respectively).

CONCLUSIONS: Malaria transmission is high in Birim over a seven-month period (Sep-Mar) and low-to-significantly low over three months (Apr-Jun). Pyrethroids are effective in killing An. gambiae vectors in most communities in Birim which is evident in low (only two) kdr genotypes detected in the area.

P182: A need for external quality assurance of malaria microscopy in research

Mehul Dhorda on behalf of Mehul Dhorda1, Francine Ntoumi1,4, Omar Gaye5, Karen I Barnes1,2, Carol Sibley1,8, Philippe J. Guerin1,9, Bernhards Ogutu10

1WorldWide Antimalarial Resistance Network; 2Malaria Group, Centre for Vaccine Development, University of Maryland School of Medicine, Baltimore, USA; 3Congoles Foundation for Medical Research, Faculty of Health Sciences, University Marien Ngouabi, Brazzaville, Republic of Congo; 4Institute for Tropical Medicine, University of Tubingen, Tubingen, Germany; 5Central Africa Network on Tuberculosis, HIV/AIDS and Malaria; Faculty of Medicine, University of Dakar, Dakar, Senegal; 6Division of Clinical Pharmacology, Department of Medicine, University of Cape Town, Cape Town, South Africa; 7Tropical Medicine, University of Washington, Seattle, USA; 8Centre for Tropical Medicine, Nuffield Department of Clinical Medicine, University of Oxford, Oxford, UK; 9Keny Medical Research Institute/United States Army Medical Research Unit, Kenya, Malaria Diagnostics Centre, Kisumu, Kenya.

Microscopy continues to be a mainstay of malaria clinical research, especially in surveillance of antimalarial resistance, as well as in malaria drug and vaccine trials. The definition of artemisinin resistance currently depends entirely on accurate microscopy. The quality of microscopy is highly dependent on the proficiency of the microscopists, and high quality microscopy is difficult to implement and sustain. Lack of standardisation in methods for malaria microscopy and for quality assurance are major impairments to comparability of results. This is being addressed by an ongoing initiative to develop quality assurance standards for the more stringent requirements of research malaria microscopy. The adoption of these standards by malaria researchers worldwide would greatly benefit from the establishment of a broadly accessible, international, external quality assurance (EQA) programme to support the development of a network of pre-qualified research laboratories with demonstrable capacity to produce high quality microscopy results. Such an EQA programme would contribute to the development of local and regional centres of excellence and hence to significant improvements in the quality of malaria microscopy for clinical research and surveillance. It would also promote standardisation of methods. Ensuring that data collected across diverse contexts is comparable is essential for pooled data analyses, which have the power to address questions that can seldom be answered in individual studies.
P183: Assessment of two strategies to prevent malaria during pregnancy in Mali (Koro)

Hamadou Dialkite

We conducted a study to evaluate two strategies to prevent malaria during pregnancy in a sahelian area (Koro) from September 2006 to April 2007. From the 424 women enrolled at delivery, 74, 2% were younger than 20 years old, 41, 6% were primi-secundigravidae. The proportion of women reported the use of IPT with SP was 57, 5% with 22,9% using two doses. Insecticide treated net use was reported by 47, 2%of women. The prevalence of anemia was 56, 3% and that of severe anemia was 10, 8%. The prevalence of maternal and placental parasitemia was 29, 7% and 31, 2% respectively singleton low birth weight and stillbirth were found in 7, 6%and 10, 8% of new born baby.

P184: Malaria in Dakar’s health facilities: a common disease

Sarah Mahdjoub-Assaad1,2, Abdoulaye Diallo1,2, Jean-Yves Le Hesran1,2

1 Institut de Recherche pour le Développement, UMR 216 - Mère et enfant face aux infections tropicales, Faculté des sciences pharmaceutiques. 4, avenue de l’Observatoire 75270 Paris Cedex 06, France; 2Université Paris Descartes, Sorbonne Paris Cité, Faculté des sciences pharmaceutiques. 4, avenue de l’Observatoire 75270 Paris Cedex 06, France.

INTRODUCTION: The Dakar region is described as a low malaria transmission area. However, the use of anti-malarial drugs is reported to be high suggesting a high incidence of clinical attacks. To understand this discrepancy, it was necessary to evaluate the malaria burden in urban health facilities, one year after the use of rapid diagnostic tests (RDTs) was introduced for diagnosis of malaria.

METHODOLOGY: The study was conducted in seven public health facilities in the Dakar region. Information on outpatient consultations over one year during the period 2008-2009 were obtained from clinic registers. All consultations with fever, and all consultations without fever were introduced for diagnosis of malaria.

RESULTS: The proportion of positive RDT’s was higher during the rainy season in all health facilities (for example 40% in Ouakam and 54% in Yoff in October 2008); in some health facilities the proportion was high in the dry season (for example 40% in Sandial in March 2008). However, our data show also that malaria treatment is still often based on presumptive diagnosis without parasitological confirmation. Fever cases without signs of respiratory illness should be tested by RDT; the proportion of such cases where a test was performed was low in some health facilities and varied depending on season; the use of RDTs was more common during the rainy season. The proportion of fever cases treated with an anti-malarial during the year ranged from 14% to 64%, and the majority of these were treated with artemisinin-based combination therapy.

CONCLUSION: This study showed that malaria was frequently diagnosed during the rainy season in health facilities, but also it suggested the presence of malaria in the dry season. However, real incidence of malaria cases in Dakar needs to be assessed using measurement of parasitaemia. Furthermore, an evaluation of RDT’s in routine practice is necessary.

P185: Purchasing patterns of antimalarial drugs at private pharmacies in Dakar, Senegal

Abdoulaye Diallo1,2, Sarah Mahdjoub-Assaad1,2, Assane Diop1, Jean-Yves Le Hesran1,2

1 Institut de Recherche pour le Développement, UMR 216 - Mère et enfant face aux infections tropicales, Faculté des sciences pharmaceutiques. 4, avenue de l’Observatoire 75270 Paris Cedex 06, France; 2Université Paris Descartes, Sorbonne Paris Cité, Faculté des sciences pharmaceutiques. 4, avenue de l’Observatoire 75270 Paris Cedex 06, France; 3Institut de Recherche pour le Développement-Université Cheikh Anta Diop, UMR151 - Laboratoire, Populations, Environnement et Développement - Route des Pêres Maristes – BP 1386, Dakar, Sénégal

INTRODUCTION: Dakar is located in a low malaria endemic area. Since 2007, any suspected malaria attack must be treated with an artemisinin-based combination therapy (ACT) after a positive diagnostic test. Nevertheless, use of anti-malarial drug is reported to be high and self-medication is common. Our objective was to evaluate the accessibility of anti-malarial drugs and to identify practices of customers in management of malaria–like fever at private pharmacies in urban Dakar, three years after the implementation of ACT and rapid diagnostic tests.

METHODOLOGY: A survey was conducted in 10 pharmacies of Dakar area from October to December 2009. Information about prescriptions, origin of the prescription, type of anti-malarial prescribed and type of presentation, the name of anti-malarial purchased, and the cost were collected by pharmacy workers from persons seeking anti-malarial treatment.

RESULTS: A total of 3145 purchases were recorded during the study period, among which 56% (1759/3145) did not have a prescription. The proportion with a prescription varied between pharmacies. 31 different brands of antimalarial were purchased, 6 common brands made up 64% of anti-malarial purchases. Among those without a prescription, Maloxine® was the most preferred drug, representing 22% of the quantity purchased. Overall only 3.2 % of persons seeking treatment without prescription asked for advice at pharmacy workers about the choice of drug. For drugs purchased with a prescription, Artequin® represented 15% of prescriptions; 7 drugs (Artequin®, Coartem®, cofantrine®, duo cotexin®, malacur®, Fansidar®, co-Arsucam®) accounted for 67% of prescriptions. Prices of drugs varied depending on the drug components and the manufacturer.

COMMENTS-CONCLUSION: Our results showed that self-medication with anti-malarial drugs was common in Dakar. It showed also that treatment without artemisinin (Fansidar) was still being used despite the national guideline that malaria should be treated with ACT. Anti-malarials are widely available in pharmacies which do not follow national guidelines with regard to malaria treatment. Self-medication using drugs from pharmacies should be monitored more widely and measures should be taken to promote use of national guidelines in private pharmacies.

P186: Identification of natural anti-arial immune responses during repeated administration of three artemisinin-based combination therapies

Nouhoum Diallo1, Antoine Dara1, Amadou Togo1, Bakary Fofana1, Bakary Sidibe1, Demba Dembelé1, Sekou Toure1, Kassoum Sanogo1, Issaka Sagara1, Abdoulaye Djimde1, Ogobara K Koumbo1, Pierre Drulhe2

1Malaria Research and Training Center, University of Science technical and technology of Bamako, Mali; 2Biomedical Parasitological Laboratory, Pasteur Institute of Paris

BACKGROUND: From July 2005 to June 2007 to Bougoula, Mali we conducted a prospective study comparing three artemisinin-based combination therapies (ACTs) AR-L (artemether-lumefantrine), AS-AQ (artesunate-amodiaquine) and AS-SP (artesunate- sulfadoxine-pyrimethamine) we have explored; it of repeated administration of three ACTs on natural anti-malarial immune responses.

METHODS: Cytokines IL-2, IL-4, IL-5, IL-10, TNF-α, INF-γ and immunoglobulins: IgG directed against AMA-1, GLURP, MSP-3 and IgM, IgG1, IgG2, IgG3 directed against AMA-1 Ag, GLURP, MSP-3, GBP-130, MSP-6, SERP and LSA-5 Ag were analyzed by flow cytometry and ELISA in 212 symptomatic subjects aged 6 months or older, divided between the three treatment arms and the number of malaria episode.

RESULTS: Specific titles of anti-GLURP were significantly higher in those who have an episode compared to those who have 3 or more than 5 episodes of uncomplicated malaria (p <0.001). Specific titles of IgG anti-AMA-1 and anti-GLURP were significantly higher in the AR-L and AS-AQ than in the AS-SP arm (p <0.05 and p <0.001). The median levels of IL-2 and IL-4 were significantly higher in the AR-L and AS-AQ than in the AS-SP
arm (p <0.001). Conversely the rate of INF-γ was significantly higher in AS-SP and AS-AQ than in the arms AR-L (p <0.05).

CONCLUSIONS: Antibodies anti-Glurp was associated protection against malaria episode. Combinations AS-AQ and AR-L were associated with higher titers of anti-plasmodial IgG that the combination AS-SP. AS-SP and AS-AQ were associated with INF-γ response while AR-L was associated with IL-4.

P187: Quality control of the microscopic diagnosis of malaria in Senegal

Ibrahima Diallo, Julie Thwing, Michael Aidoo, Moustapha Cissé, Alioune Badara Gueye, Médéoune Ndiop, Fatou Ba Fall, Babacar Faye, Mady Ba

Blood film microscopy is considered the gold standard for diagnosis of malaria, but is highly dependent on the technical ability of the slide reader. High quality malaria microscopy has been difficult to implement outside reference laboratories. In order to improve quality of malaria microscopy in hospitals and health centers, the Senegal National Malaria Control Program adopted a strategy of giving laboratory technicians a week of intensive training followed by supervision visits, and quality control of a sample of slides taken from each laboratory. In 2009, 42 technicians were trained, with average pre-test and post-test scores of 55% and 64% respectively. Of 596 slides selected for quality control, there was a sensitivity of 95% and specificity of 73%, with 6.5% of slides unreadable. In 2011, 53 technicians were trained, with average pre-test and post test scores of 75% and 87% respectively. Among 866 slides selected for quality control, there was a sensitivity of 95% and a specificity of 77%, with 1.8% of slides unreadable. While laboratory technicians at the district level read slides with a high degree of sensitivity and reasonable specificity, the quality of slide preparation and coloration was noted to be mediocre, and very few technicians performed speciation or parasite density. The microscopy quality control of the Senegal NMCP follows the recommendations of WHO in terms of parasite detection, speciation, and quantification of parasitemia, as well as evaluation of quality of slide preparation and coloration. To further improve quality of microscopy at hospitals and health centers, in 2013, the regional biologists will be trained to help with supervision, technicians from all 76 district health centers will be retrained, all hospital and district health center labs will receive an onsite supervision visit including proficiency testing from a standardized panel, and slide quality control will continue to assess quality of microscopy.

P188: Sensitivity of Plasmodium falciparum to antimalarial drugs: relationship between molecular markers and treatment failures with Artemeter-lumefantrine and Artesunate- Amodiaquine

Diallo Salou1, Guiraud Issa1, Sorgho Hermann1,2, Tinto Halidou1,2,3, Guiguemde T. Robert2
1Clinical Research Unit of Nanoro (CRUN); 2Institut de Recherche en Sciences de la Santé (IRSS); 3Centre Murat Boub-Dioulasso

BACKGROUND: It is estimated that malaria affects annually about 350 million people causing more than one million deaths every year. Lack of treatment and treatment failure due to the resistance of the pathogenic agent to antimalarials is recognized as one of the major cause of this mortality. Therefore it has become imperative to identify the markers of resistance to the drugs commonly used. Our study focused on the relationship between markers of resistance such as Pfmdr-1 and Pfdrt genes and treatment failure when artemesunate amodiaquin (ASAQ) or artesarterium lumefantrin (AL) are used to treat uncomplicated malaria in Nanoro health district

METHODS: Between September 2007 and December 2008 uncomplicated malaria patients were recruited, treated with either AS-AQ or AL and followed up for 42 days according to the WHO drug efficacy assessment guidelines. Polymerase chain reaction (PCR) method was used for molecular analysis of the target genes.

RESULTS: Our study shows that at day 42, the treatment failure rates were 17.46% and 30.30% for ASAQ and AL respectively. Also the prevalence of mutations was 25.76%, 47.66%, 5.1% and 53.93% for Pfmdr-1 B6Y, Pfmdr-1 184F, Pfmdr1 1246Y and Pfcr 76T respectively. The analysis also showed a significant decrease of the wild alleles Pfmdr1B6Y, Pfcr 76T among patient treated with the ASAQ combination while the mutant alleles Pfmdr11246Y, Pfcr76T increased in the same group. By contrast the treatment with AL seemed to select only the wild allele Pfmdr1-N86 among the alleles analyzed. Finally your results showed an association between the treatment with ASAQ and an increase of the following molecular markers: 76T, 184F and haplotype B6Y/76T.

CONCLUSIONS: It will be important to monitor the efficacy of ASAQ and AL closely, especially in Burkina Faso where this drug selection pressure may be the greatest according policy health had adopted there in first line malaria treatment.

P189: Assessment of resistance markers level for artesunate+amodiaquine combination in the treatment of the uncomplicated malaria in Maferynay, Guinea

Diawara Ey1,2, Sylla M1,2, Diallo S1, Yattara M1,2, Camara D1, Sow A1,2, Camara G1, Dembele D1, Beavogui Ah1, Dumbou Ok1, Djimde Aa1
1Malaria Research and Training Center; 2National Center for Rural Health Research and Training of Maferynay

BACKGROUND: The use of the Amodiaquine in monotherapy, is associated with the selectionof resistance markers (Pfcr 76T and pfmdr1B6Y). Although there is not documented resistance, no resistance markers to artemisin derivatives it is important to assess the impact of artemisin based combination therapy (ACT) on the selection of markers associated with partner molecules.

OBJECTIVES: To evaluate the efficacy of Artesunate+Amodiaquine combinationin the treatment of uncomplicated malaria in Maferynay, Guinea Conakry;

To search for punctual mutations on Amodiaquine resistance genes (Pfcr 76T and Pfmdr1-B6Y); To assess for polymorphisms (MSP1, MSP2 & CA1) in order to discriminate new infection versus recrudescence.

METHODS: We assessed invivo efficacy of Artesunate+Amodiaquine (AS+AQ) on subjects aged from 3 months to 45 years living in Maferynay, near Conakry in Guinea. The efficacy of AS+AQ has been evaluated by WHO 28 days standard invivo test. Polymorphisms (MSP1, MSP2 & CA1) and punctual mutations on Amodiaquine resistance genes (Pfcr76T and Pfmdr1-B6Y) have been determined by PCR.

RESULTS: A total of 93 samples have been randomly selected before treatment and 11 samples with parasitemia after treatment have been analyzed. Baseline frequencies of Pfcr76T and Pfmdr1 mutations were respectively 67.7% (63/93) and 31.1% (28/93). These frequencies after treatment were respectively 50% for Pfcr76T and 54% for Pfmdr1 B6Y.

CONCLUSION: These data show an increased baseline level of Pfcr76T gene and a significant selection of AQ molecular maker trough AS+AQ.

L190: Determination of the proportion of naturally induced anti-AMA1 antibodies that bind to the 4G2 functional invasion-inhibition region of AMA1

E Dickson

The Plasmodium merozoite expresses many surface antigens among which are those that induce production of antibodies that have invasion-inhibitory activity and have been targeted as subunit vaccine candidates. Apical membrane antigen 1 (AMA1) is one such antigen, but anti-AMA1 antibodies are usually strain-specific since they bind to polymorphic
epitopes of the antigen.

To date only a single rat monoclonal antibody (mAb), 4G2, has been shown to bind to a conserved epitope on AMA1, making it a strain-transcending mAb.

To assess the importance of the region of AMA1 with this conserved epitope to malaria immunity in humans, the proportion of anti-AMA1 antibodies that are blocked by 4G2 binding to AMA1 was assessed using plasma from naturally-exposed individuals by ELISA techniques.

Data from ELISA was compared with data on the biological activity of affinity-purified anti-malarial antibodies measured by an in vitro growth inhibition assay (GIA). Measurable levels of anti-AMA1 antibodies blocked by 4G2 binding was generally less than 20% but correlated positively with GIA data (r = 0.047, p = 0.76). The proportions of naturally induced anti-AMA1 antibodies blocked by 4G2 binding was generally less than 20% but correlated positively with GIA data (r = 0.047, p = 0.76).

The region on AMA1 with the 4G2 binding epitope may thus be an immunologically important region in humans though only low proportions (up to 16%) of antibodies are made to this region of AMA1.

### P192: Evaluation of highresolutionmelting (HRM) to analyzegenetic mutations associated with resistanceto sulfadoxine-pyrimethamine.

Cyrille K. Diédhiou1*, Amy K. Bei2,3, AmbroseAhoudj1, OusmaneSarr1, Daou da Ndiaye2, Zul Premji3, Dyann F. Wirth4, Manoj Daruindingh5, Souleymane Mboup6

1 Laboratory of Bacteriology and Virology, Le Dantec Hospital, Dakar BP 7325, Senegal
2 Harvard School of Public Health, 665 Huntington Ave Blvd 1 Rm 706, Boston, MA 02115, * Department of Parasitology and Medical Entomology, Muhimbili University College of Health Sciences, Dar-es-Salaam, Tanzania

**BACKGROUND:** Malariaremainds a major public health challenge espeially in sub-Saharan Africa. Antimalarial drug resistance is alarming. In Tanzania, the first-line treatment (chloroquine) was replaced in 2001 by sulfadoxine-pyrimethamine (SP). Resistance to this drug, used in intermittent presumptive treatment(s) for malaria cases diagnosed in the laboratory for Pfdhfr and Pfmdgs genes. Our study compares the results of HRM and PCR-RFLP in the characterization of malaria infection in an endemic area with a high prevalence of mixed genotypes, but also assesses the use of HRM in the context of our countries’ development.

**METHODS:** Parasite DNA was extracted from infected blood spotted in filter paper by QiAmp Blood Mini kit. PCR-RFLP and HRM have been applied to analyze mutational associations with resistance to P. falciparum to SP. The NS151 and NS108 mutations in the dhfr genes were analyzed using both methods in this study.

**RESULTS:** A total of 47 malarial patients were recruited between 2003 and 2004 in Dakar, in an area of intense malaria transmission and multiple clone infections. PCR-RFLP and HRM respectively showed 66% and 61% of mutant isolates, 21% and 21% of wild, 11% and 18% of mixed parasites. PCR-RFLP and HRM respectively showed 70% and 76% of mutant isolates, 14% and 14% of wild, 16% and 10% of mixed.

The results of PCR-RFLP and HRM showed no statistically significant difference. **CONCLUSIONS:** In short, the high prevalence of mutations were observed. Time and relatively low cost experiments by HRM was more sensitive and attractive enough for to be considered an alternative to PCR-RFLP.
malaria diagnosed in the Fann CHNU declined significantly more than 80%, result of the various interventions of malaria carried out in Senegal.

P194: Assessment of pfcr7 76-76 haplotypes and mutants 76T after the introduction of ACT in Kinshasa, Democratic Republic of Congo

M Dieudonn

BACKGROUND: In 2001, WHO recommended the use of Artemisinin-based Combination Therapies (ACT) for first-line treatments of uncomplicated malaria cases, as monotherapies became ineffective in many parts of the world. As a result, many African countries changed their malaria treatment policies, including the Democratic Republic of Congo (DRC), which withdrew chloroquine (CQ) in 2002, a drug widely used until then. Nevertheless, data collected from 105 Plasmodium falciparum isolates in 2008, reported a frequency of 83.8% chloroquine resistance (CQR) in Kinshasa, DRC. Artesunate (AS)-amodiaquine (AQ) combination became the ACT of choice in DRC in 2005. AQ-resistance (AQr) has been reported in several parts of the world and mutations in codons 72-76 of pfcr (P. falciparum chloroquine-resistance transporter gene) have been strongly correlated with resistance, especially mutations encoding the SVMNT haplotype. This haplotype was first identified in South-East Asia and in South America but recently two studies have found it in two African countries neighboring the DRC. This fact raised the question of the presence of this haplotype in DRC, which would compromise the use of AS in ACT as partner drug.

METHODS: Parasite DNA was extracted from 213 thick blood films randomly collected from a pediatric clinic in Kinshasa, DRC in 2010. Plasmodium species were identified by real-time PCR and haplotypes of the pfcr gene were determined by sequencing in all P. falciparum parasites.

RESULTS: P. falciparum was correctly identified in 94.8% and all mutants found (73.2%) had the CVIET haplotype. No SVMNT haplotype was found in this study nor was the SVIET haplotype, which was previously described in DRC.

CONCLUSION: This study is the first to assess molecular markers of resistance to CQ and AQ after the introduction of ACT in DRC. Our results suggest that CQR is decreasing as wild type pfcr haplotypes were found in 26.8% of the samples. It also shows that SVMNT haplotype is not yet present in Kinshasa suggesting that AQ remains valid as partner drug in ACT in Kinshasa.

P195: Effects of malaria and viral hepatitis co-morbidities on immuno-haematologic status of HIV-1 infected patients from the Southwest region of Cameroon

M Dilonga

BACKGROUND: Malaria, HIV and viral hepatitis (B and C), though disparate diseases share an overlapping geographic distributions and are major public health concern worldwide. HIV co-infection with either malaria or viral hepatitis adversely affects the outcome of each disease and data on such studies in Cameroon are scarce and contradictory. We therefore evaluated the impact of malaria and viral hepatitis co-infections on the immuno-haematological status of HIV infected patients.

METHODS: This was a cross-sectional study undertaken between October 2010 and December 2011; involving HIV-1 infected adults (≥ 18 years), attending the HIV treatment centres of Limbe and Buea Regional Hospitals. Demographic and clinical data were obtained with a structured questionnaire. Full blood (Mindray BC-2800) and CD4 count (BD FACScount) were performed with standard automated techniques. Malaria parasitaemia was determined with conventional microscopy. HBV (Genetic System HbsAg, Bio-Rad Lab) and HCV (Chiron RIBA, Ortho Diagnostic) were done with enzyme immunoassays.

RESULTS: A total of 1202 participants were enrolled constituting: 884 females [mean age 37.1 years] and 324 males [mean age 41.8 years]. Malaria parasite and viral hepatitis prevalence were 18.7% and 15.3% (9.1% HBV and 5.5% HCV) respectively. Mean immuno-haematological parameters did not differ between hepatitis positive and negative patients. On the other hand, mean Hgb and RBC were lower in malaria parasitized patients (P < 0.01) and parasitaemia correlated significantly (P < 0.01) with Hgb (r = -0.587), RBC (r = -0.472) and platelets (r = 0.314) but showed no significance with CD4 count (r = -0.142, p = 0.292).

CONCLUSIONS: Malaria was associated with anaemia while viral hepatitis had no significant impact on immuno-haematologic parameters. However, the high prevalence of viral hepatitis warrants that adequate management is giving precedence in HIV/hepatitis co-infected patients.

P196: Ex vivo resistance profile of Plasmodium falciparum isolates recently collected in Abidjan, Côte d’Ivoire

Kigbafori D. Silué1,2, Sarah E. Mara3,–5, Xavier C. Ding1

1UF R Biosciences, Université Felix Houphouet-Boigny, 01 BP V34, Abidjan 01, Côte d’Ivoire, 2Département Environnement et Santé, Centre Suisse de Recherches Scientifiques en Côte d’Ivoire, 01 BP 1203, Abidjan 01, Côte d’Ivoire, 3Medicines for Malaria Venture, 20 rte de Pré Bois, Geneva CH 1215, Switzerland

BACKGROUND: Antimalarial drug therapies, similar to all anti-infective agents, are constantly at risk of losing efficacy due to resistance development in Plasmodium parasites. To avoid the potentially disastrous effects of using partially or totally ineffective therapies, it is necessary to monitor antimalarial drug efficacy as frequently and locally as possible. We report here an ongoing effort to characterize the resistance profile of Plasmodium falciparum isolates collected in Abidjan, Côte d’Ivoire.

METHODS: Intravenous blood samples were collected in two medical centers from informed and consenting adult patients suffering from uncomplicated malaria and with mono-species P. falciparum infections confirmed by RDT and microscopy. Parasitemia was determined by microscopy and reduced to 0.3% with uninfected blood if superior to this value. Drug susceptibility assays were performed within 24 hours of blood collection and parasite growth was measured after 72 hours using a SYBR Green II-based method and analyzed using the IVART tool provided by WWARN.

RESULTS: No ex vivo resistance was observed to amodiaquine (IC50 geomean: 14.62 nM, 95% CI: 8.8-24.4 nM, n=11), artesunate (4.08 nM, CI: 2.2-7.5, n=11), piperaquine (9.80 nM, CI: 5.58-17.23 nM, n=9), quinine (14.66 nM, CI: 5.2-41.0 nM, n=8), and lumefantrine (10.65 nM, CI: 4.8-23.8 nM, n=5). Resistance to chloroquine (IC50 > 70 nM) was observed in 5 out of 12 isolates (42%) and to pyrimethamine (IC50 > 150 nM) in 11 out of 12 isolates (92%).

CONCLUSIONS: The ex vivo activity of compounds currently used in Côte d’Ivoire as first line treatments (artesunate, amodiaquine, and lumefantrine for uncomplicated malaria, and quinine for severe malaria) appears to be fully preserved. Resistance to the formerly recommended chloroquine is still present and the high grade of resistance to pyrimethamine is a potential concern for the efficacy of the sulfadoxine-pyrimethamine combination, currently used for intermittent preventive treatment during pregnancy.
P197: Genetic diversity of Plasmodium falciparum among school-aged children living in a rural area of Man, western Côte d’Ivoire

Sarah E. Mara1,2, Kigbafori D. Silué1,2, Giovanna Raso1,3, Simon P. N’Guetta1, Eliezer K. N’Goran1, Jürg Utzinger1, Xavier C. Ding1
1UFR Biosciences, Université Felix Houphouët-Boigny, 01 BP V34, Abidjan 01, Côte d’Ivoire; 2Département Environnement et Santé, Centre Suisse de Recherches Scientifiques en Côte d’Ivoire, 01 BP 1303, Abidjan 01, Côte d’Ivoire; 3Department of Epidemiology and Public Health, Swiss Tropical and Public Health Institute, P.O. Box, CH-4002 Basel, Switzerland; 4University of Basel, P.O. Box, CH-4003 Basel, Switzerland

BACKGROUND: Plasmodium falciparum genetic diversity allows the molecular discrimination of otherwise microscopically identical parasites and the identification of individual clones in complex infections. We report here the P. falciparum prevalence, complexity of infections, and genetic diversity in the rural area of Man, western Côte d’Ivoire. We specifically investigated the relationship between these epidemiological factors and the local topography.

METHODS: Close to 300 blood samples were collected from school-aged children in four nearby villages located at varying altitudes (ranging from 265 to 784 meters above sea level). P. falciparum prevalence was determined by PCR (msp2) and parasitemia was established by microscopy. The multiplicity of infection (MOI) and the genetic diversity of the parasite populations were investigated by restriction fragment length polymorphisms on the msp2 PCR products (PCR-RFLP).

RESULTS: P. falciparum prevalences across the four villages ranged from 57.5% to 89.7% and decreased significantly with altitude. Genotyping of the isolates revealed 25 potentially new msp2 alleles and an overall MOI of 2.9. MOIs varied significantly across villages (ranging from 2.32 to 3.41) but did not correlate with altitude nor age, and only to a limited extent with parasitemia. An analysis of molecular variance (AMOVA) indicated that a small, but close to statistical significance (p=0.07), fraction of variance occurs specifically between villages of low and high altitude.

CONCLUSIONS: We provide a local characterization of the P. falciparum genetic diversity and extend the characterization of msp2 alleles present in Côte d’Ivoire. Higher altitude was associated with lower prevalence but not with reduced MOI, suggesting that, in this setting, MOI is not a good proxy for transmission. The evidence for partially parted parasite populations suggests the existence of geographical barriers that should be taken into account when deploying antimalarial interventions as they might contribute to their success.

P198: Ghanaian school children harbour antibody responses to antigens on the surface of P. falciparum gametocyte-infected erythrocytes

Bismarck Dinkó1, Teun Bousena2 and Colin J. Sutherland2
1Dept of Immunology, Noguchi Memorial Institute for Medical Research, Legon, Accra, Ghana; 2Dept of Immunology and Infection, London School of Hygiene and Tropical Medicine, London, UK

BACKGROUND: Little is known about the immune responses directed at circulating P. falciparum gametocytes in humans, knowledge of which would be useful in the development of intervention strategies to reduce and block malaria transmission.

METHODS: Consequently, antibody responses to surface antigens of P. falciparum gametocyte-infected RBCs (GSA) were determined in plasma samples from malaria asymptomatic Ghanaian school children between the ages of 5-17 years. These children were screened for malaria parasites and treated with dihydro-artemisinin piperaquine if positive, one week after first sample collection, and followed up weekly for one month.

Gametocytes were produced from a laboratory adapted parasite line, 3D7a and a recent patient isolate from Kenya (HL1204).

RESULTS: From a cohort of 113 children, all the children harboured plasma antibody responses that recognized GSA on a proportion of mature gametocyte-infected RBCs of 3D7 by flow cytometry. However, 56% of the children exhibited strong antibody responses to GSA (immune response above the median within the cohort per sampling time) by both the proportion of mature gametocytes bound to antibodies and the intensity of the antibody binding to GSA. Longitudinal data provided an additional 10% developing strong GSA responses during the 1 month follow-up. There were some children with antibody responses fluctuating around the median immune response within the cohort. Children with GSA antibodies present at enrolment, were less likely to develop new gametocyteemia at subsequent visits (odds ratio = 0.29, 95% CI 0.06 - 1.05; P = 0.034). 3D7a is a laboratory adapted parasite line so a selection of positive plasma samples was tested against mature gametocyte preparations from HL1204 and strong plasma antibody binding was again shown. No binding to the surface of RBCs infected with immature gametocytes of HL1204 was detected.

CONCLUSION: A proportion of malaria infected asymptomatic children harbour plasma antibodies which were strongly recognized by antigens on the surface of mature gametocyte-infected RBCs. Strong plasma antibody responses may contribute to the control of gametocyteemia in vivo. Ghanaian GSA responses recognized antigens on both 3D7 and an east African parasite line, suggesting these are relatively conserved.

P199: Malaria Immunological responses in children less than 10 years in Toubacouta district, Sokone, Senegal 2010, EDCTP/ WANETAM/Clinical Sites Project

Fode Diop

BACKGROUND: In order to establish the feasibility of a clinical and vaccine trial, studies have been conducted to gather baseline epidemiological, entomological and immunological data within the EDCTP program in the rural community of Toubacouta in Senegal. This study focused on the analysis of immune responses of children under ten to a number of P. falciparum antigens vaccine candidates merozoites crude extracts from different P. falciparum laboratory and field strains.

METHODS: In total, 1317 children (2 months to 10 years old) were recruited between October and December 2010 from 8 villages in the district of Toubacouta. During inclusion, blood samples were collected for laboratory investigations and thick and thin smears were prepared for parasitemia determination. Children were followed-up for clinical episodes including malaria for one year-period. On a random sample of 300 children, ELISA-based IgG antibodies responses were determined against antigens vaccine candidates MSP1, MSP3, AMA1 and GLURP. Antibodies responses were also determined against crude merozoites extracts from P. falciparum laboratory line (Palo Alto, PA) and field isolates adapted to in vitro culture (FP15 and 0703).

RESULTS: Prevalences of antibodies against merozoites crude extracts of P. falciparum strains were 56.7%, 54.3% and 81% respectively for PA, F15 and 0703. These prevalences were higher than those obtained with recombinant AMA1 (44.7%), MSP3 (17.3%) and GLURP (20%) antigens. Seroprevalence was significantly lower in children under five compared to older children (p<0.05). Reactivity against P. falciparum varied from 9% to 90% between villages.

CONCLUSIONS: Our study revealed heterogeneity of seroprevalence and high antibodies titers were also observed in some children. These findings are consistent with other results observed in the study: prevalence of parasiteemia (<13%), occurrence of clinical malaria (1.5 to 19% episodes) and entomological inoculation rate (4 to 30 infected bites per person).This result should help to establish vaccine clinical trial in villages where transmission is high.
P200: The diagnostic values of rapid diagnosis test based on Histidine rich protein-II (CareStart™) malaria in detecting malaria infection in pregnant women in San and Kita

Moussa Djimde, Etienne Guirou, Nouhoum Guindo, Binta Barry, Hamadoun Diakite, Moussa Niangaly, Siddiki Konate, Mohamed Keita, Kassoum Kayentao, Ogobara K. Doumbo
Malaria Research and Training Center, University of Sciences, Technics and Technology of Bamako

SUMMARY: We conducted from July 2009 to February 2010 in San and Kita (Mali), a study on the diagnostic values of the Histidine Rich Protein II (HRP-II) CareStartTM. Malaria compared to the thick smear for the detection of malaria infection during pregnancy as part of a pilot study for monitoring the therapeutic efficacy of Sulfadoxine-Pyrimethamine during in vivo testing of 42 days.

RESULTS SHOWED: Both the sensitivity and specificity of 95%. Positive and negative predictive values were 86% and 98% respectively, and the kappa value was 0.87. There was 5% of false positive and negative. During the in vivo day 42 follow-up, the sensitivity of HRP-II CareStart™ was higher than that of thick smear CareStart™. Malaria positivity was proportional to parasite density detected by the thick film.

CONCLUSIONS: The HRP-II CareStartTM Malaria has a good sensitivity, good specificity, easy to use and fast. It can be used in the detection of malaria infection in pregnant women.

P201: Effects of T helper cells immune response on the pathogenesis of malaria in women at delivery in Yaoundé

Megnekou Rosette1, 3, Djontu jean claude1, 3, Metenou Simon2, Fogako Josephine3, Leke FG Rose3
1Faculty of Sciences, University of Yaoundé I, Cameroon. 2National Institute of Allergy and Infectious Disease, NIH, USA. 3Biotechnology Center, University of Yaoundé I, Cameroon. 4Faculty of Medicine and Biomedical Sciences, University of Yaoundé I, Cameroon.

BACKGROUND: Although several studies associated malaria to the immunological dysregulations due to Plasmodium, the immunological mechanisms underlying the pathogenesis of pregnancy malaria are poorly understood. We therefore studied the effects of Th1, Th2, Th17 and Treg cells immune response on the pathogenesis of this disease.

METHODS: Peripheral and placental blood were collected in pairs, at delivery in 140 volunteers women, recruited in a Health Centre in Yaoundé-Cameroun. Parasitaemia was determined microscopically and blood hemoglobin level using the Hemocue. Peripheral and placental blood levels of different mediators of Th1 (INF-γ, TNF-α); Th2 and Treg cells were measured using ELISA method.

RESULTS: The malaria prevalence at delivery was significantly higher in primigravidae and secondigravidae than in multigravidae (p< 0.05 and p< 0.05 respectively) and the level of parasitaemia negatively correlated with parity (p = 0.02). Peripheral blood hemoglobin level of infected women was significantly lower than those of non infected women (p< 0.05). In the other hand, peripheral and placental plasma level of IL-10 positively correlated with parasitaemia (p< 0.001 in both cases) and negatively with peripheral hemoglobin level (p=0.07 and p< 0.001 respectively). In contrast, peripheral plasma levels of IL-17A negatively correlated with peripheral blood parasitaemia and positively with peripheral hemoglobin level (p<0.05 in both cases). Parity positively correlated with peripheral plasma level of IL-17A and negatively with peripheral plasma level of IL-10 (p< 0.01 in both cases).

CONCLUSION: These results suggest that IL-10 cytokine would favor pathogenesis of pregnancy malaria while IL-17A would protect against the pathogenesis of this disease.

P202: Is Anopheles funestus still susceptible to insecticides in Benin?: Exploring Mechanisms of Insecticide Resistance in a population of Anopheles funestus in Benin

Diouaka R1, Irving H1, Tukur Z1, Wondji CS2
1International Institute of Tropical Agriculture, Cotonou, Benin. 2Liverpool School of Tropical Medicine, Liverpool, United Kingdom. 3Bayero University, Kano, Nigeria.

INTRODUCTION/BACKGROUND: The insecticide resistance status of the malaria vector Anopheles funestus and the underlying resistance mechanisms remain un-characterized in many parts of Africa, notably in Benin, West Africa. To fill this gap in our knowledge, we assessed the susceptibility status of a population of this species in Pahou, Southern Benin and investigated the potential resistance mechanisms.

METHODOLOGY/PRINCIPAL FINDINGS: WHO bioassays revealed a multiple resistance profile for An. funestus in Pahou. This population is highly resistant to DDT with no mortality in females after 1h exposure to 4%DDT. Resistance was observed against the Type I pyrethroid permethrin and the carbamate bendiocarb. A moderate resistance was detected against deltamethrin (Type II pyrethroids). A total susceptibility was observed against malathion, an organophosphate. Pre-exposure to PBO did not change the mortality rates for DDT indicating that cytochrome P450s play no role in DDT resistance in Pahou. No L1014F kdr mutation was detected but a correlation between haplotypes of two fragments of the Voltage-Gated Sodium Channel gene and resistance was observed suggesting that mutations in other exons may confer the knockdown resistance in this species. Biochemical assays revealed elevated levels of GSTs and cytochrome mono-oxygenases in Pahou. No G119S mutation and no altered acetylcholinesterase gene were detected in the Pahou population. qPCR analysis of five detoxification genes revealed that the GSTe2 is associated to the DDT resistance in this population with a significantly higher expression in DDT resistant samples. A significant over-expression of CYP6P9a and CYP6P9b previously associated with pyrethroid resistance was also seen but at a lower fold change than in southern Africa.

DISCUSSION AND CONCLUSION: The multiple insecticide resistance profile of this An. funestus population in Benin shows that more attention should be paid to this important malaria vector for the implementation and management of current and future malaria vector control programs in this country.

P203: First record of kdr-e mutation in Anopheles arabiensis and probable involvement of oxidases enzymes in mosquitoes resistance to pyrethroids and organochlorine insecticides in Ndiop, Sénégal.

S. Doucoure, O. Ndiiath, S. Sougoufara, N. Diagne, C. Sokhna and J.F Trape
Institut de Recherche pour le Développement, URMITE, Sénégal.

BACKGROUND: Long lasting insecticide nets (LLINs) and indoor residual spraying (IRS), have been decisive tools in reducing the malaria worldwide burden. However, the spread of Anopheles resistance to insecticides may impair the recent progress. Thus, the monitoring of mosquito resistance is crucial for improving vector control program. In this study, Anopheles susceptibility to four groups of insecticides was investigated after implementation of LLINs at community level in Ndiop, Senegal.

METHODS: Two years after implementation of LLINs, Anopheles larvae were collected in the field and breed in insectary into adults. The World Health Organisation standard test was used to evaluate: i) Anopheles susceptibility to carbamates, organophosphate, pyrethroids and...
organochlorine insecticides and ii) to assess the effect of pre-exposure to piperonyl butoxide (PBO) synergist on insecticides susceptibility. Additional tests were conducted to indentify Anopheles at species and molecular level and to evaluate the frequencies of knock down resistance (kdr) and acetylcholinesterase gene (Ace-1) mutations.

**RESULTS:** Among the 300 specimens sampled, An. Arabiensis, An. coluzzii (formerly An. gambiæ s.s M form) and An. gambiæ s.s.S form represent 65.6%, 26.3% and 8% individuals, respectively. A significantly higher susceptibility to organophosphate and carbamate was observed with a mortality rate of 97% and 100% by fenitrothion and bendiocarb, respectively. In contrast, 60%, 57%, 51% and 75% of mortality were recorded by Lambdacyhalothrin, Deltamethrin, Permethrin and DDT, respectively. The pre-exposure to PBO synergist re-store the fully mosquito susceptibility to pyrethroids and organochlorines. The Ace-1 and L1014F kdr target site mutation were not found while a low frequency of L1014S kdr resistant allele is recorded only in An. arabiensis.

**CONCLUSION:** This study suggests that detoxifying enzymes may be involved in mosquito resistance to pyrethroids and organochlorine. For the first time the kdr-e is recorded in Senegal. Further studies are needed to understand the impact of LLINs implementation on mosquito susceptibility to insecticides.

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**P204: Analyse of antibody responses against three blood stage antigens (AMA-1, MSP-142, GLURP-R2) of *P. falciparum* in naturally exposed children aged from 1 to 5 years old in Bandiagara, Mali**

**Carlota Dobaño**

**INTRODUCTION:** Development of effective antimalarial vaccine is still a challenge for researcher. Several malaria candidate vaccines have been tested with limited success. One of the most important issues is lack of correlare of protection. Antimalarial immunity is age dependent and require many mosquitos bites during several years. The results of the vaccine trials show that a single antigen could not be sufficient to induce protection.

**OBJECTIVES:** Measure the association between antibody response against several vaccine candidate antigens and protection against malaria

**METHODOLOGIE:** We have conducted cohort study in Bandiagara (Mali) in 211 children. We measured IgM, IgG and IgG subclasses specific to AMA-1, MSP-1 et GLURP-R2, 3 blood stage antigens by ELISA. A six month follow up of the cohort were performed to evaluate the malaria incidence. by thick smears.

**RESULTS:** Malaria incidence was 0.69 per children per year. Anti-malaria IgG seropositivity was high in children having parasites at the start of transmission season. Levels of d'IgG, d'IgG1 and d'IgM to AMA-1 were higher in children aged from 3 to 5 years compared to younger children. IgG levels to MSP-1 and and GLURP-R2 in children from 3 to 5 years were comparable to those less than 3 years. There was no correlation between antibody levels and protection against malaria episodes.

**DISCUSSION:** As usualy described the natural acquisition of antibodies against these 3 antigens was age dependent. No association between the presence of any isotype of malaria antigen specific antibody and protection against the occurrence of clinical episode has been observed as described in studies of Dodoo and Kusi in Ghana. This result needs more investigation.

**CONCLUSION:** The antibodies against three antigens is not associated with protection against malaria in this population of children 1-5 years old in Bandiagara a setting of seasonal transmission area.

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**P205: Assessment of Guibourtia coleosperma and Diospyros chamaethamnus extracts for in vitro antiplasmodial activity in Namibia**

**Iwanette du Preez, Davis Mumbengegwi**

**University of Namibia, Multidisciplinary Research Centre, Science, Technology & Innovation Division, Windhoek, Namibia**

**BACKGROUND:** Malaria is one of three major causes of morbidity and mortality around the world. However, in Namibia the malaria incidence and deaths have reduced dramatically. The number of confirmed cases has decreased from 477,786 in 2000 to 133,464 in 2008; and 199 deaths were reported in 2008 compared to 679 in 2000. Hence, the country is seeking to eliminate malaria by 2020. A barrier in achieving this target is that not all communities use the WHO recommended treatment for malaria, opting to use traditional medicines. Traditional plant-based medicines are used by many communities deeming them safe for human consumption. Even so, they need to be scientifically validated to allow for their safe and effective use as antimalarials. The use of traditional medicine alongside Western medicines will provide treatment options, thus ensuring the use of effective antimalartials in all communities helping Namibia achieving its aim to remove all Plasmodium reservoirs. Guibourtia coleosperma and Diospyros chamaethamnus are used to treat malaria-like symptoms in an ethnomedicinal setting in the Caprivi region of Namibia. These uses were validated using chemical profiling and in vitro assays using Plasmodium falciparum 3D7A.

**METHODS:** Briefly, aqueous and organic extracts of Guibourtia coleosperma and Diospyros chamaethamnus were prepared using distilled water and dichloromethane-methanol (1:1v/v). Phytochemical analysis was conducted for known antimalarial classes of compounds using thin-layer chromatography. Extracts were diluted to 5, 10 and 50 µg/ml using water and DMSO and were incubated with synchronized Pf infected RBCs for 48 hours. Growth inhibition was determined using parasitaemia.

**RESULTS:** Thin layer chromatography revealed the presence of anthraquinones, coumarins and tannins. Antimalarial activity was observed for both plant extracts at 5 µg/ml. G. coleosperma organic extract exhibited a higher percentage parasitaemia reduction (58.75 %) than the aqueous extracts (47.5 %). Whereas for *D. chamaethamnus*, the aqueous extract had a higher reduction in parasitaemia (41.25 %) compared to the organic extracts (33.75 %).

**CONCLUSION:** These results support the ethnomedicinal use of the plants and how these herbal remedies are prepared in the traditional setting. The safety profiling of the extracts will be determined using Vero cell lines.

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**P206: Public versus private health sector services on access to malaria healthcare.**

**Dwight Atanga**

**Catholic University of Cameroon Bamenda.**

**BACKGROUND:** Malaria is one of the illnesses with the highest prevalence in Sub Saharan Africa. As a result many individuals end to spend a lot on trying to cure themselves f the illness. The fact is, many of them cannot afford for treatment for malaria because the majority of the population fall below the poverty line. As a result millions of individuals die each year because of the inability to have access to drugs due to high prices. Most of the drugs available are provide through private health sector services such as private hospitals or clinics, mission hospitals or clinics also known as confessionals, private pharmacies etc. most patients cannot afford this drugs and tend to go to the public health service providers who usually don’t have the drug available in stock. A plan to either subsidize drug prices in private health care provider or make provisions for the availability of drugs in the public health care provider sector which are more affordable, would seem feasible.
**P207: Evaluation of the anti-plasmodial activity of the methanolic root extracts of Anthocleista nobilis G. Don, Nauclea latifolia Smith and Napoleona imperialis P.Beauv.**

Ijeoma Ogbuieh, Omotayo Ebong; Eme Asuquo, Chijioke Nwauche, Lucy Yagwo-Ide
Centre for Malaria Research and Phytomedicine (CMRAP), University of Port Harcourt, Port Harcourt, Nigeria.

**BACKGROUND:** Malaria is one of the major health problems in Nigeria. The emergence of multi-drug resistant strains of the malaria parasite has necessitated the continued search for other effective, safe and cheap plant-based antimalarial agents. This study was carried out to evaluate in vivo the antiplasmodial effect of the extract of a combination of three plants as used in traditional medicine in South-east, Nigeria.

**METHODS:** Dried and ground roots of the three plants: Anthocleista nobilis, Nauclea latifolia and Napoleona imperialis were combined in equal weight and extracted in 70% methanol. The compound extract (125, 250 and 500mg/Kg), was given orally to chloroquine-sensitive Plasmodium berghei (NK 65) infected Swiss albino mice (15 – 25 mg/Kg) to investigate its repository, suppressive and curative antimalarial effects compared with the effects of each plant extract and with that of Chloroquine 25mg/kg and phosphate-buffered saline. The basal temperature of mice was measured by introducing 1.5 cm of digital thermometer into the rectum.

**RESULTS:** Dose-dependent evaluation of extract activity showed that there was no significant difference in parasite inhibitory activity between doses 250mg/kg and 500mg/kg for all extracts. After 7 days of treatment with extracts, the Nauclea latifolia extract exhibited the highest potency, in the curative test, with 78.7 ± 0.7 mean parasite inhibition. This was followed by the compound extract, with mean of 70.4 ± 0.4 inhibition. The Chloroquine group caused 91.6± 0.2 mean inhibition (p < 0.05). For antipyretic activity, Napoleona imperialis extract showed the highest anti-pyretic activity, similar to that of the control–Chloroquine. (P≤0.05).

**CONCLUSIONS:** The results show that the antiplasmodial action of the three plant extracts is mainly due to the activity of N. latifolia in the mixture. It is most probable that the compound extract as used by traditional healers, gives improved symptomatic relief for malaria because of the added antipyretic effect of Napoleona imperialis.

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**P208: Molecular profile of resistance to sulfadoxine-pyrimethamine two years after its implementation as preventive treatment in Manoka archipelago**

M Ebumbou

**BACKGROUND:** Despite efforts in many countries to control and eliminate malaria, this endemic remains the most feared of tropical parasitic diseases in Africa. In 2010, malaria accounted for 36% (vs. 38% in 2009 and 41% in 2008) of all hospital admissions and mortality from malaria was 24% (vs. 29% in 2009) in Cameroon. Over the past two years, malaria prevention intervention increased in Cameroon through free distribution of more than 8 million LLINs and IPT based on SP for pregnant women. In the Manoka archipelago located around 60 km from Douala, there is no data on treatment failure of SP adopted for IPTp since 2011. Resistance to SP is highly variable and has been reported in some areas of Cameroon, with incidence ranging from 8% to 40%. The aim of this study is to evaluate the SP resistance level in Manoka archipelago.

**METHODS:** SP resistance molecular markers will be investigated in 300 parasite samples collected from pregnant women from three islands of Manoka archipelago. Using RFLP, 7 SNPs of SP resistance molecular markers (dhfr and dhps) will be assayed.

**EXPECTED RESULTS:** The prevalence of SP resistance markers will be discussed. Linkage disequilibrium blocks and resistance haplotypes will be assessed to characterize parasite genetic factors that are involved in the SP resistance.

**CONCLUSION:** These findings would be benefit for clinical decision-making on therapeutic approaches.

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**P209: Target site and metabolic mechanisms cause extreme insecticide resistance and cross-resistance in Anopheles gambiae s.s. from West Africa**

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Malaria control depends on mosquito susceptibility to insecticides. With resistance to pyrethroids and DDT now widespread, carbamates are an increasingly important alternative for indoor residual spraying (IRS). Yet mechanisms of resistance to carbamates are poorly understood and critical knowledge of potential cross resistance with other insecticide classes is lacking. We assayed insecticide resistance in Anopheles gambiae mosquitoes from southern Côte d’Ivoire and applied synergist, target site assays and whole genome microarrays to investigate resistance mechanisms. Mosquitoes were resistant to insecticides from all four approved classes. Such complete resistance, which includes exceptionally strong phenotypes, presents a major threat to malaria control. The G119S target site resistance mutation was strongly associated with bendiocarb survivorship, but bioassays with PBO, which synergises P450 enzymes, restored significant insecticide efficacy, suggesting a previously unappreciated role of metabolic resistance in carbamate resistance. This observation was confirmed by microarray analyses, which implicated the involvement of multiple P450s in carbamate resistance. The role of the strongest candidate P450, Cyp6M2, which is also linked with pyrethroid and DDT resistance in An. gambiae was validated via production of a bendiocarb resistant phenotype in transgenic Drosophila melanogaster.

Our results demonstrate strong roles for target site and metabolic mechanisms in producing extreme levels of carbamate resistance in Anopheles gambiae, in addition to a concerning potential for cross resistance via overexpression of a specific P450 gene.

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**P210: Antimicrobial properties of taraxasteryl acetate: a Triterpenoid Isolated from Lagerra pterodonta(DC.) Sch. Bip.**

3Egharevba, O. Henry; 2Emeje, O. Martins; 3Okwute, K. Simon; 4Okogun, I. Joseph
1Department of Medicinal Plant Research and Traditional Medicine (MPR&TM), National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria; 2Centre for Nanomedicine and Biophysical Drug Delivery, Advanced Biology and Chemistry unit, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria; 3Department of Chemistry, University of Abuja, Nigeria.

This study investigates the bioassay-guided fractionation of Lagerra pterodonta extract in order to isolate and identify active antimicrobial compound(s). The aerial part of the plant was extracted successively with hexane and ethyl acetate and thereafter, the more active ethyl acetate extract was subjected to standard chromatographic separation, isolation and purification procedures. The compound 3β,18α,19α-Urs-20(30)-en-3-ol,3-acetate also known as taraxasteryl acetate: a Triterpenoid Isolated from Lagerra pterodonta exhibit a MIC of 25 µg/mL for all the clinical isolates. It is suggested that the compound taraxasteryl acetate: a Triterpenoid Isolated from Lagerra pterodonta possesses strong anti-infective properties and may serve as cheaper alternative to the current expensive and multi-drug resistant antibiotics.

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**6th MIM Conference**
P211: Computer Aided Antimalarial Drug Design from Phytochemicals with in-vitro antiplasmodial activities: Chemoinformatics and Docking analysis.

Samuel Ejieye1, James Syce1, Alan Christofell1, Sarel Malan1

1School of Pharmacy, University of the Western Cape, Cape Town, South Africa.

INTRODUCTION: Identification of compounds with different mechanism of actions (MOA) is highly desirable to circumvent resistance of Plasmodium falciparum to current antimalarial drugs. A large number of phytochemicals have shown varying in-vitro antiplasmodial activities. The highly diversified chemical space occupied by these phytochemicals gives them the potential to interact with a variety of drug targets in P.falciparum. Substructural and pharmacophore features can characterize the interaction of these phytochemicals with drug targets in P.falciparum with a potential to identify those with possible unique MOA and drug-likeness, which is essential for in-vivo/clinical evaluation.

RESEARCH QUESTIONS: How similar/diverse are these phytochemicals from current antimalarial drugs? Do these phytochemicals have the potential to be orally bioavailable? What are their binding affinities and mode to drug targets in P.falciparum?

METHOD: The substructural similarities (Tanimoto coefficient) between these phytochemicals and current antimalarial drugs were measured using PubChem structural fingerprints. Scaffold analysis was used to identify unique scaffolds. Molecular Operating Environment software was used to compute pharmacophore features and features that defines drug-likeness (Lipinski’s model). Similarity matrices were constructed and data visualized as cluster trees and heat maps.

RESULTS: The results show that the majority of these phytochemicals (93%) have substructural features that are diverse from current antimalarial drugs but similar pharmacophore features. Unique scaffolds were identified and up to 64% possess desirable drug-like/oral bioavailability properties.

CONCLUSIONS: These phytochemicals possess what is required to interact with novel drug targets. They contain pharmacophore groups with unique substructural features that could interact with different drug targets than the existing drugs or to the same drug targets but with a different interaction pattern, possibly providing a unique MOA. Drug-likeness/potential for oral availability calculations were favourable for in-vivo and/or clinical evaluation. To assess binding affinity and mode, docking of these phytochemicals to drug targets are now under way using MOE software.

P212: Long-lasting insecticidal bednets catching on fire: a threat to bednet users and to successful malaria control?

Marc Egrot1, Roch Houngnihin1, Carine Baxerres1

1 Institut de Recherche pour le Développement, France; 2 Université d’Abomey Calavi, Bénin

BACKGROUND: In 2011, a research in Benin showed that distributions of bednets did not imply their correct use. The prevention’s efficacy depends on how individuals perceive and use the object. For this reason, we developed an anthropological program to better understand the cultural factors that might explain the gaps between LN use and coverage rates. And, one of the many reasons mentioned by some people to explain a possible refusal to use bednets is that LNs can catch on fire.

METHODS: Our study combine direct observation (23), focus group (3), individual interviews (91), with many catches of videographic and photographic images. Survey data were processed by qualitative content analysis.

RESULTS: Cultural factors that influence the use of bednets to Tori-Bossito and surrounding villages are varied. We can classify them into three categories: Cultural representations of illness and bednets: The popular nosological wecivozon (in fonble language) does not exactly cover the disease “malaria”. Etiological interpretations of illness does not necessarily mobilize mosquito. The bednets itself refers to other objects whose use is subject to cultural norms; Difficulties encountered with the daily management of bednets in domestic spaces: Overload domestic work related to the (un)installation, reorganization of domestic space and sleeping places in the house, or also wash and dry bednets, etc.; Sometimes a very good acceptability of bednets, but for a multitude of uses other than sleeping below: football goal, fishing net, safety net against pests in agriculture, etc.

CONCLUSIONS: The results produced by this research provide a better understanding of the low use of bednet in the study area. Moreover, the reasons which determine people’s choices regarding their uses are extremely diverse. Sometimes, for the same person, the decision to use or not the bednet based on multiple factors and a tangle of different logics. In addition, the acceptability of bednets can’t be understand only with reference to the function initially assigned to the object by manufacturer and Public Health. This is important to consider because these misuse may provide ideas for recycling 294 million bednets distributed over the last years.

P213: Use bednets or not? Yes but why? An anthropological study in Benin

Marc Egrot1, Roch Houngnihin1, Carine Baxerres1

1 Institut de Recherche pour le Développement, France; 2 Université d’Abomey Calavi, Bénin

BACKGROUND: Several studies in Africa, especially Benin in 2011, show that utilization of bednets are well below the coverage rates. However the effectiveness of malaria control depends on the quality of reception of bednets by people. An anthropological study conducted in Benin in 2011-13 analyzes the social life of bednets and cultural factors determining their uses.

METHODS: Our study combines direct observation (23), focus group (3), individual interviews (91), with many catches of videographic and photographic images. Survey data were processed by qualitative content analysis.

RESULTS: Cultural factors that influence the use of bednets to Tori-Bossito and surrounding villages are varied. We can classify them into three categories: Cultural representations of illness and bednets: The popular nosological wecivozon (in fonble language) does not exactly cover the disease “malaria”. Etiological interpretations of illness does not necessarily mobilize mosquito. The bednets itself refers to other objects whose use is subject to cultural norms; Difficulties encountered with the daily management of bednets in domestic spaces: Overload domestic work related to the (un)installation, reorganization of domestic space and sleeping places in the house, or also wash and dry bednets, etc.; Sometimes a very good acceptability of bednets, but for a multitude of uses other than sleeping below: football goal, fishing net, safety net against pests in agriculture, etc.

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P214: Emphasizing Environmental Sanitation in a Malaria Vector Control Programme: A Study in Ota, Southwest Nigeria
Ehi-Eromosele C.O.
Department of Chemistry, Covenant University, P.M.B. 1023, Ota, Nigeria.

BACKGROUND: One effective way to control malaria is through vector control. The use of insecticide treated nets (ITNs), long-lasting ITNs (LLINs) and indoor residual spraying (IRS) have dominated most Integrated Vector Management (IVM) programmes for malaria control in Africa even with the prohibitive costs of these materials, the cases of insecticide resistance and adverse health and environmental impacts. Environmental Sanitation presents a more sustainable option in IVM for malaria control for most African neighbourhoods which usually have poor environmental sanitation characterised by increased slums, poor drainage and waste disposal. This study aims to assess the impact of Environmental sanitation on malaria transmission in Ota, Southwest Nigeria.

METHODS: The study will be conducted in the area of Ota, Southwestern Nigeria which has a high prevalence of malaria as a result of the many breeding grounds for mosquitoes in its neighbourhoods. Data which include the background malaria case data, the type of vector control being used, the knowledge of malaria and its causes will be obtained from a study of literature, in-depth interviews and questionnaires to residents and experts. Environmental sanitation of this area will be mobilized and sustained through community participation twice a week for a year (May 2013 to May 2014) with the monitoring of the malaria cases in selected respondents and from the health centers located there.

RESULTS: Over the study period, malaria cases are expected to decline greatly amongst the respondents and in the health centers in the area. The knowledge of respondents about malaria transmission and control is expected to be high but may have been incapacitated by the costs of procuring ITNs, LLINs, IRS and the poor environmental sanitation of their neighbourhoods.

CONCLUSION: Environmental sanitation presents a sustainable option for malaria control, results in a multitude of socio-economic benefits and can contribute positively to all the Millennium Development Goals. Hence, it must be emphasized in the malaria vector control programmes in Africa.

P215: Perception and management practices of malaria in Ojoto, south east Nigeria
Chinyelu A Ekwunife¹, Bernice Ogolo¹, Chinyere Ukaa³
¹Department of Parasitology and Entomology, Nnamdi Azikiwe University, Awka, Nigeria; ²Department of Nursing Services, Nnamdi Azikiwe University Teaching Hospital Trauma Cente, Oba, Nigeria; ³Department of Animal and Environmental Biology, Imo State University, Owerri, Nigeria

BACKGROUND: Perception, awareness, and beliefs of communities contribute immensely to sustainable control of endemic diseases such as malaria. Strategic malaria control involves primary prevention which focuses on vector elimination and personal behavior change specifically through the consistent use of insecticide-treated nets (ITNs) and prompt and effective case management. This study aimed to assess the local community understanding of malaria transmission, recognition of signs and symptoms, treatment seeking behavior and preventive measures in south east Nigeria.

METHODS: A structured questionnaire covering information on respondent demographics, knowledge, and management practices was administered upon receipt of an informed consent for participation. This study investigated the local communities’ understanding of malaria transmission, recognition of signs and symptoms, perceptions of cause, treatment-seeking patterns, preventive measures and practices.

RESULTS: A substantial number of research participants showed reasonable knowledge of malaria, including correct association between malaria and mosquito bites, its potential fatal consequences and correct treatment practices. Almost 70% (n = 300) of the respondents stated that they would seek treatment within 24 hours of onset of malaria symptoms with health facilities as their first treatment option. Most people (66.6%) perceived clinics and vector control practices as central to treating and preventing malaria disease. Few respondent (8.3%) use local herbs, (7%) use the prayer houses while (18%) procure drugs from patent medicine stores. Most (86%) of the respondents posses treated bed nets but 62% of them did not like sleeping under the bednets because they feel uncomfortable in the net.

CONCLUSIONS: This assessment of perception and management practices showed a broad awareness of malaria and its consequences among residents of malarial areas in Ojoto, Idemili South, local Government Area Anambra State, South East Nigeria. However enlightenment programmes in churches, schools and health centers should be mounted so that people who do not like sleeping under treated bednets should learn to do so. Knowledge and awareness can only be fruitful if the correct management ways is put into practice.

P216: Potential antimalarial activity of methyl jasmonate and its effect on lipid profiles in Plasmodium berghei infected mice
Oladapo E Oyinloye , ¹ Ayokulehin M Kosoko , ² Obukwoh B Emikpe , ³ Catherine O Falade, ¹, ₄ George O A O Adegowo, ¹, ₄
¹Department of Pharmacology and Therapeutics, college of Medicine, University of Ibadan, Ibadan, Nigeria; ²Department of Biochemistry, college of Medicine, University of Ibadan, Ibadan, Nigeria; ³Department of Veterinary Pathology, University of Ibadan, Ibadan, Nigeria; ⁴Institute for Advanced Medical Research and Training, College of Medicine, University of Ibadan, Ibadan, Nigeria

BACKGROUND: Malaria is a major threat to public health and economic development in Africa. Effort at controlling malaria has been hampered by parasite resistance to commonly used and affordable antimalarial drugs. There is an urgent need for search and discovery of new antimalarial agents. This study aimed to evaluate the in vivo antimalarial activity of methyl jasmonate (MJ) which is a plant hormone, known since as a fragrant component in the essential oil from flowers of Jasminum grandiflorum.

METHODS: The antimalarial activity of MJ against Plasmodium berghei (P. berghei) was evaluated using the Rane test procedure. Albino mice weighing between 18g and 30g were distributed into10 groups of seven animals each. Four groups were uninfect, a group received ethanol, while others received 10mg, 25mg and 50mg MJ per kg body weight respectively. Six groups were infected with P berghei , a group was not treated while the remaining five groups were treated with 3mg arteether (AE), 10mg chloroquine (CQ), 10mg, 25mg and 50mg MJ respectively. Parasitaemia was monitored daily and screened under X 100 magnification using a light microscope. The PCV and lipid profiles were also investigated. Data were analyzed computed and processed using SPSS version 15.0 at P<0.05.

RESULTS: The suppressive effect of 50mg MJ, CQ and AE on parasite growth were demonstrable from day 1 (post treatment) and with suppression reaching 81.9%, 96.9% and 97.3% respectively by day 3.There was recrudescence from day 5 in AE treated group. 70.3% and 70.4% suppression of parasitaemia were observed in 10mg and 25mg MJ treated groups respectively on day 3. A dose dependent increase in PCV growth were demonstrable from day 1 (post treatment) and with at 100 days of treatment. An important finding of this study is that MJ and CQ had a significant effect on the lipid profiles in infected mice. The cholesterol levels increased in a dose dependent manner in MJ treated groups while a reduction in triglyceride levels was observed as the dose increase.

Conclusions
P217: The effects of climate changes on malaria epidemiology in Sudan Region

Rasha A. Aal1 and Ayman A. ElShayeb1,2
1Department of Environmental Studies – School of Life Sciences- Faculty of Science and Technology-Alneelain University-Khartoum-Sudan; 2University of Khartoum, Faculty of Science, Department of Microbiology. Khartoum - Sudan

INTRODUCTION: Many diseases in Africa such as malaria are known to be sensitive to climate factors there is a positive correlation between malaria, cholera, meningitis, and climatic elements. This study was conducted in Sudan region to evaluate the direct and indirect effects of climate changes on malaria distribution and spread in the period 1997 – 2007.

MATERIALS AND METHODS: Data collected for three categories; climate’s factors records, vector distribution and malaria records to evaluate the effects of climate changes on malaria and mosquito spreading. The selected time frame for historical data is 1997-2007. Climatic change scenarios required for endemic zones are temperature, rainfall and precipitation and their data was collected from the Sudan Meteorological Authority. The malaria records were used by the assist of the Ministry Health National Centre for Health Information. We also search the United Nations humanitarain website data for Sudan.

RESULTS AND DISCUSSION: Regular limits of malaria allocation indicate that malaria could develop for several months in most areas of Sudan due to climate variables, we found that the malaria cases related to temperature increasing was clearly recognized in the Northern State with 1.59% out of the state population, the most endemic state was Blue Nile with 2.45% in the Rich Wet Savannah, and the lowest state of malaria infections was Western Darfur with 0.06% cases, the total of malaria infections in northern Sudan is 15.19% of 29 million population, the prevalence of malaria in southern Sudan was 24.5% of 9.5 million population (including 2004 and 2005 returns) of those examined were infected. Whilst in Khartoum State the mean number of cases increased to reach 60869 in the study period 5.5 million of population as the largest inhibited state.

P218: Nanoencapsulation increases Pavetta crassipes antimalarial efficacy against Chloroquine Resistant Plasmodium falciparum in vitro

Martins Emje

BACKGROUND: Malaria is the most important endemic parasitic infection in humans, accounting for more than 1 million deaths per year, especially in Africa. Delivery systems such as liposomes and nanoparticles have been studied for intracellular infections because they are able to deliver the drug to the specific target in the human body where the parasite is located. Nanocapsules are a specific type of nanoparticle which has been used for the protection of drugs against inactivation in the stomach and is improved for bioavailability; protection of the mucosa from drug toxicity; control of drug release; and reduction of drugs side effects. The aim of this work was to develop pavetto (PV)-loaded microcapsules and evaluate its antimalarial efficacy in vitro.

METHODS: Microcapsule dosage form with sodium alginate containing Pavetta crassipes alkaloidal extract was prepared using ionotropic gelation technology. The efficacy of the pavetto-loaded microcapsules was evaluated in chloroquine-sensitive (HB3) and chloroquine-resistant (FCM29) P. falciparum strains. Three standard drugs; artemisinin, chloroquine and quinine were used as reference standards for comparism.

RESULTS: Pavetto-loaded microcapsules presented an adequate particle size (173 nm), narrow particle distribution (0.20), positive zeta potential (14mV) and high drug content (98.88 %) and encapsulation efficiency (67.01 %). The extract exhibited significant (P < 0.05) antimalarial effect against both Pf HB3 and Pf FCM29. The efficacy of pavetta-loaded microcapsules as measured by in vitro assay doubled (11.20) when the extract was microencapsulated compared with the free extract (22.08). IC 50 of 11.20 and 10.60 µg/ml for Pf HB3 and Pf FCM29 respectively, representing an almost 50 % increased activity were recorded for Pf HB3 and Pf FCM29 respectively, with the IC 50 of 22.08 and 21.91 µg/ml respectively for the free extract.

CONCLUSIONS: Therefore, microencapsulation increased the interaction between the extract and the erythrocyte and this mechanism may be responsible for the extract’s increased efficacy when microencapsulated. Our current findings show that PV and its microcapsule formulation may be a useful preparation in the treatment of acute chloroquine-sensitive and chloroquine resistant malaria.

P219: Elimination of Incessant Attacks of Malaria Through the Use of Fumigation System

Anaebonam Emeka1, Paul Nwosu1, Okey Edozie1 2Udegboka Nkechi 3Emeka Anaebonam health research foundation, Onitsha, Anambra state, Nigeria.

BACKGROUND: Fegge in Onitsha is a highly densely populated area with a population of about 500, 000 out of which 60 - 70% are illiterates living in poorly kept environments with a very poor drainage system and lakes, which makes the environments conducive for high breeding of mosquitoes. With the high cases of malaria recurrent and deaths among the people, millions of dollars are spent to import and distribute mosquito treated net (MTN), however the people rarely use MTN to sleep, the compliance is very low. This study aimed to elimination of mosquitoes and permanent solution to incessant and frequent attack of malaria using the fumigation system of malaria control.

METHODOLOGY: The environment was fumigated especially the ponds, stagnant gutters and refuse dumb sites. This was done on two weekly basis, then monthly, fumigating the environment and sustained for two months. The fumigation was done in the areas where mosquitoes breed and so when mosquitoes came to breed, they die.

RESULTS: Over a period of 1-2months, 90% of all the mosquitoes in the fumigated environment died. Within a period of 3months, there were no longer mosquito bites experience among the people. Patients that come for malaria treatment drastically reduced from 60 to 13 per week in a small hospital. The fumigation gave a swift result in cutting down drastically the number of mosquitoes by 90 - 95%. The incidence of malaria highly dropped in the fumigated community, the people feel healthier for longer periods of time.

CONCLUSION: Though i am still studying and applying the system in other regions, so far fumigation system of malaria control is a cost-effective way to reduce the incidence of malaria attack. If replicated in other states, it would save more lives of African women and children. A law/bill should be passed for government to take over the fumigation of the environment to control malaria.

P220: Toxicological evaluation of combination therapy of artemisinin derivative (arthemeter and lumefantrine) with ivermectin in male albino rats (rattus norvegicus)

Emmanuel T IDOWNU1, Olubunmi A OTUBANO1, Abdurrahmon B. TALABI2, Gideon C. ALIMBA2
1Department of Zoology, University of Lagos, Akoka, Lagos State, Nigeria.; 2Department of Zoology, University of Ibadan, Oyo State, Nigeria.

BACKGROUND: Combined therapies in the co-endemic treatment of parasitic infections are ongoing in sub-Saharan Africa. Artemisinin based Combination therapy (ACTs) is the drug of choice recommended by World Health Organization in the treatment of malaria while annual rounds of ivermectin (IVR) administration is ongoing in areas of Africa where onchoceriasis is endemic. Combined therapies pose potential drug toxicity, rare adverse events and complications

METHODS: Behavioural biochemical and histopathological effects of artemisinin derivative ACT and IVR administered using normal and double human therapeutic doses (HTDs) in male albino rats were investigated. Rats
were exposed daily to IVR for 15 days while, ACT exposure was undertaken for 3 days prior to rats sacrifice. The doses of these drugs were calculated based on the mean average weight of animals assigned to the group and administered orally. The estimations of L-asparate amino transferase (AST), L-alanine amino transferase (ALT), alanine phosphatase (ALP), total albumin, total protein and cholesterol were determined using the automated chemistry analyzer. Histopathological alterations of liver and kidney were also assessed. RESULTS: The result showed no significant loss or gain of body weight (p>0.05) of the animals in all the treated groups. Biochemical assessment also showed no significant alteration (p>0.05) in values of biochemical parameters including ALT, AST, ALP, Total protein, cholesterol, creatinine, total bilirubin and urea analyzed compared to the control. Histopathological damage of the kidney was minimal, however mild to moderate congestion of the liver were recorded in rats exposed to HTD of IVR and HTDs of IVR+ACT. CONCLUSION: The results of this study validate previous findings that co-administration of IVR+ACT is safe and the two drugs can be co-administered where the diseases are co-endemic.

P221: Ethnobotanical Study of Antimalarial Plants in Denbia District, North Gondar, Amhara Region, Northwest Ethiopia

Abyot Endale1, Zewedu Berhanu1, Alemayehu Berhane1, Bayew Tseg1
1School of Pharmacy, College of Medicine and Health Sciences, University of Gondar, Ethiopia

BACKGROUND: Medicinal plants play an important role in the treatment of malaria especially in developing countries where resources are limited. Thus it is crucial to document medicinal plants used for treatment of malaria and other diseases. This study documented medicinal plants that are traditionally used for treatment of malaria in Denbia District, Northwest Ethiopia.

MATERIALS AND METHODS: The study was conducted in four malarious village of Denbia District, Amhara Region, Northwest Ethiopia in March 2013. Information was collected by interviewing traditional healers using semi-structured questioner. Specimens of the reported antimalarial plants were collected and stored at the Department of Pharmacognosy, University of Gondar, following identification.

RESULTS: A total of 30 traditional healers were interviewed of which 96.7% were males and 3.3% females. Twenty four plants species used in the treatment of malaria were identified. Detailing information such as common and vernacular names, parts used, methods of preparation, frequency and duration of use were compiled. Of the plants identified during the survey, Allium sativum (32.2%), Aethadoto schimperiana (22.6%), Croton macrostachys (6.4%) and Brassica nigra (6.4%) showed the highest incidence of encounter. The traditional usage of fresh bife from domestic goat, Capra aegagrus, (6.4%) and white fish (3.2%) in the treatment of malaria is also reported by the healers.

CONCLUSIONS: The results provide data for further pharmacological and toxicological studies and development of commercial antimalarial phytotherapy products.

P222: Knowledge, attitude and perception of malaria in a Nigerian semi-urban setting: A hospital-based study

Obiora Enemuo1,2, Adaora Obiaguwu3, Chinenyenwa Mbegbu1
1Department of Parasitology and Entomology; 2Department of Community Medicine

INTRODUCTION: Malaria remains a major public health problem in Nigeria. Health education has been identified as a major means of correcting traditional beliefs and perceptions of malaria among pregnant women, a group hardest hit by the disease.

RESEARCH STATEMENT: This study was conducted in order to understand how pregnant women in a semi-rural Nigerian setting recognize malaria. Their general knowledge and perception of malaria, its causes, preventive measures taken and treatment sources were measured.

P223: Health workers and community members perceived sources, role of information and communication on malaria prevention and control.

Jane C Enemuoh1,2, Uzochukwu BSC1,2,3, Nkoli Ezumah1, Onuwjwike Obinna1,2, Lindsay Mangham-Jefferies1

BACKGROUND: Malaria co-exist with other prevailing health problems [bcc strategy 2008] and affects children under five years of age, pregnant women and migrants/visitors from non-malarious regions. It is also responsible for the high rate of absenteeism among school children, reduction in work productivity and capacity. Most often, malaria symptoms are perceived wrongly, not recognized and go untreated. Up to 70% of malaria reported cases treated at home were mal administered with ineffective treatment. Provision of adequate information and communication among community members and health workers can assist recognize malaria symptoms, proper treatment seeking behaviour, total compliance to treatment and improve the usefulness of resources and services in place. The study inquires on what the perception of health workers are on sources and role of information on malaria prevention and control.

METHODS: Qualitative data from 6 selected communities; 3 in urban, 3 in rural is collected to serve as a data set of socio economic variables and health indices. 18 FGDs among 179 community members and 26 IDI among health workers in public and private health providers were used to reveal sources and role of information and communication on malaria.

RESULTS: The major finding was that sources of information on malaria from health workers and community members were through adverts, workshops and seminars organized by donor agencies, facility supervision, posters, other health care providers television and radio adverts. Health workers indicated the usefulness of information on malaria prevention and control in updating knowledge on treatment using new treatment guidelines and current rapid diagnosis for malaria. Information from sources encouraged appropriate malaria treatment seeking among community members.

CONCLUSION: The result that use of different sources of information and communication for malaria prevention and control can extensively improve the effectiveness of the current resources. Resourcing for malaria prevention are scare therefore, expanding information and communication sources will extend the possibilities for health providers’/communities’ extended knowledge using local medium, participatory development and knowledge sharing.
P224: Patients' satisfaction and quality of care in a tertiary institution in Southeast Nigeria

Jane Enemuoh

OBJECTIVE: To determine the factors enhancing and deterring patients' satisfaction in a tertiary institution and the quality of care in southeast Nigeria.

Methods: The study was a cross sectional study in which 360 systematically selected participants completed 5 point likert scale self-administered questionnaire to rate their satisfaction level and quality of services provided, as well as factors of importance where best service was provided.

Results: Overall, participants were quite satisfied (Mean score = 3.75) with the services provided by the different service providers. Respondents also indicated that overall the quality of care of the health facility was good (mean score = 3.45). Pharmacy received the highest satisfaction level with a mean rating of 4.1. Over a third (38%) rated the services provided by the doctors as best despite giving the highest quality ratings with a mean of 3.9 to pharmacy compared to mean ratings of 3.4 for the doctors. Respondent's greatest displeasure was with the time spent at the facility as 63.9% of them were displeased. More than a third (36.9%) was most pleased with information given to them as a factor of importance.

Conclusion: Participants were quite satisfied with the services provided as well as the quality of care by the different service providers of the health facility. There is a need for interventions in terms of time spent at the facility which would promote good customer focused service delivery

P225: Perceived Roles of Traditional Birth Attendants for Malaria Prevention during Pregnancy and Benefits of Utilizing Traditional and Skilled Births Attendants: A Qualitative Approach

Enobong Ndekhe

BACKGROUND: Traditional Birth Attendants (TBAs) are still popular though discouraged by the Safe Motherhood (SMH) initiative that stresses skilled birth attendants (SBAs). In Nigeria only 58% of pregnant women made at least one antenatal care visit, while only 35% delivered at clinics (NDHS, 2008). This study investigated birth place choice (TBAs, SBAs) by women and perceived roles of the TBAs on prevention and care in a tertiary institution in Southeast Nigeria. This study investigated birth place choice (TBAs, SBAs) by women and perceived roles of the TBAs on prevention and care in a tertiary institution in Southeast Nigeria.

METHODS: The study was a cross sectional study in which 360 systematically selected participants completed 5 point likert scale self-administered questionnaire to rate their satisfaction level and quality of services provided, as well as factors of importance where best service was provided. This study investigated birth place choice (TBAs, SBAs) by women and perceived roles of the TBAs on prevention and care in a tertiary institution in Southeast Nigeria.

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Conclusion: Participants were quite satisfied with the services provided as well as the quality of care by the different service providers of the health facility. There is a need for interventions in terms of time spent at the facility which would promote good customer focused service delivery

P226: Identification of HERPES SIMPLEX VIRUS TYPE I in febrile children suspected having malaria

ENDO Anna A, Louise Stéphanie MAKEMGUÉ

Département of Animal Biology and Physiology, Faculty of Sciences, University of Yaoundé I, Cameroon.

BACKGROUND: Plasmodium malaria remains one of the major parasitic infections in the tropics and in Cameroon in particular. The similarity of clinical symptoms of malaria to that of some infectious diseases such as herpes labialis, caused by the Herpes Simplex Virus Type 1, complicates malaria diagnosis in children. This situation results in misdiagnosis and consequently increased morbidity, especially when they occur in mixed infections. That is why we sort to determine any relationship between the two pathogens in febrile patients suspected having malaria.

METHODS: Blood samples were bled from 90 febrile children (49 boys, 41 girls) aged between 2-15 years with the consent of their parent or legal guardian, for the identification of Plasmodium spp. by microscopy and two rapid detection tests (SD Bioline Malaria Antigen Pf/Pan and ICT MALARIA Pf). The antibody titre of Herpes Simplex Virus Type 1 was also determined by Enzyme Immunoassay test.

RESULTS: Microscopic examinations revealed 20(22.22%) positive cases; only Plasmodium was detected. Rapid detection tests showed 45(50%) positive cases with 23(51.11%) cases of Plasmodium falciparum in single species infections and 22(48.89%) in co-infections with Plasmodium malariae and Plasmodium ovale. The serological assay revealed 47(52.22%) of the Herpes Simplex Virus Type 1; children in the 10-15 age group were the most affected (61,29%). Plasmodium spp. was in coinfection with Herpes in 23(25.56%) cases.

CONCLUSIONS: Rapid Detection Tests were obviously more sensitive than microscopy for the malaria diagnosis. The high prevalence of Herpes Simplex Virus Type 1 emphasizes the importance of a thorough laboratory diagnosis of malaria in febrile patients, following clinical observations.

P227: The prevalence of Plasmodium falciparum compared to that of routinely investigated pathogens in transfusion blood in the Essos hospital center, Yaoundé, Cameroon.

Anna A Eno, Christelle Laure G Yongo

University of Yaoundé 1, Faculty of Science, Yaoundé, Cameroon.

BACKGROUND: Plasmodium falciparum should be systematically investigated in transfusion blood in malaria endemic countries because the development of malaria following transfusions further endangers the health of recipients. The aim of this study was to highlight the relative importance of Plasmodium falciparum, the most prevalent Plasmodium specie in Cameroon, to that of systematically investigated pathogens in transfusion blood, using two diagnostic methods.

METHODS: Two (2ml) of peripheral blood was randomly collected from 224 donors of both sexes between 18 and 62 years, recruited in the Essos hospital center in Yaoundé from March to June 2010. These samples were screened for Plasmodium falciparum by the rapid diagnostic test (RDT), « ICT Malaria Pf. » and microscopy. The serological identification of the human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), and Treponema pallidum were done with HIV Vidas Duo Quick, HBV Vidas, SERODIA-HCV, and SERODIA-TP PA, respectively.

RESULTS: Plasmodium falciparum was detected in 26 (11.61 %) samples, with an average parasitaemia of 75.92 parasites per µl of blood, 14 (6.25 %) of which were detected by « ICT Malaria Pf. » test only, 7 (3.13 %) by microscopy, and 5 (2.23 %) by both identification methods. Using microscopy as the reference test, the « ICT Malaria Pf. » showed a sensitivity of 61.67 % and specificity of 93.40 %. The prevalence of the other pathogens in descending order were 27 (12.05 %), 15 (6.70 %), 6 (2.68 %) and 5 (2.23 %) for HBV, HCV, T. pallidum and the HIV respectively.

CONCLUSION: With a prevalence of 26 (11.61 %), Plasmodium
P228: An analysis of alternative technologies for the management of malaria vectors
Julian C Entwistle
Xenex Associates Ltd, West Sussex, United Kingdom

BACKGROUND: Conventional chemical insecticide approaches to the control of malaria vectors are threatened by the development of insecticide resistance. Exophagic vectors are not effectively controlled by the conventional methods of insecticide treated mosquito nets and indoor residual insecticide sprays. Therefore there is a need to consider alternative technologies for the management of malaria vectors and this study involves a literature search and analysis of information on a set of alternatives to establish the current status and future prospects for deployment.

METHODOLOGY: A literature search was conducted on alternative technologies for mosquito management, including methods to reduce mosquito populations, reduce mosquito biting rates and reduce vectorial capacity. Methods already commonly deployed in control programmes were excluded. Personal contact was made with the organisations involved in their development where necessary to gain further information. The technologies were categorised in terms of their stage of development, target application, performance level, ease of use, supply chain viability, regulatory considerations and commercial interest.

SUMMARY OF RESULTS AND CONCLUSION: A large number of alternative approaches for mosquito management are in development, several have been tested in open trials and some have been deployed in a limited way in control programmes. Some are being developed primarily for control of vectors of other diseases, particularly dengue, but also have significant potential for malaria in particular situations, for example the Release of Insects with Dominant Lethality and Wolbachia. Some other genetic approaches may prove highly effective but are at an early developmental stage and will require careful safety checks and risk assessment. Additional use of insecticide treated fabrics and more widespread application of spatial decision support systems appear particularly promising and the selective deployment of ivermectin as a human drug that kills mosquitoes and inhibits sporogenesis offers potential in some situations. Further development and testing are required for sugar baits, repellents and trap-based approaches. Alternative methods will need to be incorporated with established approaches and new insecticides in integrated vector and disease management plans appropriate for local circumstances.

P229: Using management approaches to improve malaria interventions at district level – early experiences of the Community and District Empowerment for Scale-up (CODES) project in Uganda

Sara Eriksson,  Eric Sseguga,  Peter Waiswa,  Anne Katahoire,  Danstan Bagenda and Stefan Peterson
1Department of Public Health Sciences, Karolinska Institutet, Sweden; 2School of Public Health, Makerere University, Kampala, Uganda

BACKGROUND: Malaria remains one of the major causes of mortality in children under five years. Most of these deaths occur in sub Saharan Africa. Uganda has the third largest burden of malaria in Africa, causing 100,000 deaths every year. Various interventions targeting management of malaria are running. However, poor health care performance at district level and lack of prioritizing optimal interventions affect child survival in Uganda. We describe early experiences of using management interventions to improve malaria management and control at district level.

METHODS: The Community and District Empowerment for Scale-up project (CODES) is a five year project aimed at building capacity for district management to design targeted interventions to match malaria, pneumonia and diarrhea burden among children under five. It empowers district management teams to use evidence based tools for performance improvement and increase community oversight to strengthen demand and accountability. CODES is implemented in 5 high mortality districts in Uganda. Data was collected through 38 interviews, document reviews and field observations. Atlas.ti software was used for data management. Content analysis was applied for reviews and manual analysis for interviews, using codes and sub-codes developed based on pre-determined themes.

RESULTS: using the lot quality assurance survey (LQAS), districts were able to identify district performance regarding malaria services. The bottleneck analysis (BNA) helped the districts to identify obstacles for service delivery addressing malaria interventions. This information acted as a basis to design specific interventions such as (Integrated management of childhood illness (IMCI), integrated community case management (ICCM), trainings, motivation of Village Health Teams). Using the management analysis district worked identified bottlenecks hindering actions targeting malaria.

CONCLUSIONS: By providing district teams with evidence based tools and mentorship, districts are able to prioritize malaria interventions and improve service delivery. However, additional resources and continuous facilitation is needed to address bottlenecks.

P230: Dynamics between genetic polymorphism and phylogenetic in Plasmodium vivax isolates from malaria endemic provinces in People’s Republic of China and Myanmar

Ernest Tambo1, Xia Zhou1,*, Ming-Bo Yin1,*, Jing-Su1, Metoh Theresia Njabe1, Xiao-Dong Sun1, Jun-Hu Chen1*, Xiao-Nong Zhou1
1National Institute of Parasitic Diseases, Chinese Center for Diseases Control and Prevention, WHO Collaborating centre on Malaria, Schistosomiasis & Filariasis; 2Medical college of Soochow University, PR China; 3Fudan University, People’s Republic of China; 4UP Centre for Sustainable Malaria Control, Department of Biochemistry, FNAS, University of Pretoria; South Africa; 5Department of Biochemistry, University of Bamenda, Republic of Cameroon; 6Yunnan Institute of Parasitic Diseases, Dali-Puer, PR China

We analyzed the genetic polymorphism and natural selection of the merozoite surface protein 1 (PvMSP1) gene among 77 P. vivax isolates collected from China-Myanmar border areas in Yunnan province and the inland cases in P.R. China during 2010-2012. Sequence analyses revealed 41 haplotypes, 30 were new haplotypes whereas 35 of 59 individuals in Myanmar, characterizing of multiple clonality. surprisingly a common single haplotype was documented in each of the 6 isolates from Anhui, and in contrast all 6 isolates from Yunnan have different haplotypes, and 2 haplotypes from 6 patients in Zhejiang. The difference between the rates of non-synonymous and synonymous mutations suggested that the region has evolved under natural selection. A BLAST search on Plasmodb genebank database of P. vivax to compare these successfully sequenced isolates with those previously identified from China and the Asia-pacific sub-region showed the network system of identified haplotypes originated from Myanmar followed by Yunnan and Zhejiang provinces of PR China. The phylogenetic analysis revealed that newly identified haplotypes from China were clustered differently due to selective pressure on these regions identified of PvMSP12 of Myanmar-China border areas exhibiting dynamics and diverse genetic polymorphisms. These results have significant implications for understanding the dynamic of the P. vivax population circulating in China-Myanmar border areas and provide useful information towards China malaria elimination campaign strategies.
P231: Seasonal variation on a 175kda Plasmodium falciparum polymorphic vaccine candidate antigen (erythrocyte binding antigen-175) in Nite, South Cameroon

Esemu L.F.1, Bigoga J.D.1, Fodjo Y.B.1, Fossi K.G.1, Otla V.S.1, Moyou S.R.2, Leke R.G.F.1
1 The University of Yaounde I, Biotechnology Center, Molecular Parasitology and Disease Vector Research Laboratory, National Reference Unit for Vector Control, P.O. Box 3851-Messa, Yaounde, Cameroon; 2 Institut de Recherches Médicales et d’études des Plantes Médicinales, Centre de Recherches Médicales, Laboratoire de Parasitologie (IMPM).

BACKGROUND: The erythrocyte binding antigen 175 kDa (EBA-175) of Plasmodium falciparum is one of the major ligands implicated in red blood cell invasion by merozoites and its gene has been shown to be polymorphic within the parasite population (FCR3 and CAMP alleles), with the degree of polymorphism varying between eco-geographic locations. However, whether this polymorphism is influenced by season is yet to be verified in Cameroon. This study thus aimed at assessing the genetic polymorphism of EBA-175 during the dry and wet seasons in Nité, South Cameroon.

METHODOLOGY: Peripheral finger prick blood was collected from 914 participants (488 during the dry season and 426 during the rainy season). The blood was used to prepare thin and thick blood smears for microscopy. A portion of the blood specimen collected on Whatmann 3MM filter paper was used to extract DNA for Plasmodium species identification by PCR. P. falciparum mono-infections were genotyped for of EBA-175 polymorphism using nested PCR in 100 individuals.

RESULTS: Alongside the already known FCR3 allele and CAMP allele, two new allelic forms with band sizes of ~400bp and ~350bp were also observed in both seasons. The prevalence of the FCR3 and CAMP alleles were 66% (66/100) and 34% (34/100) respectively while the new alleles were found in 11% (11/100; ~400bp) and 12% (12/100; ~350bp) of isolates.

CONCLUSION: These findings thus reveal that EBA-175 is more polymorphic in Nité than it was reported by literature. This is shown by the identification of two new alleles (~400bp and ~350bp). The observed surge in the polymorphism of the gene of this protein may compromise the efficacy of EBA-175 based vaccines in this locality.

P232: Submicroscopic infections of Plasmodium falciparum in symptomatic individuals of Franceville, southeastern Gabon

Jean Claude Bitéghé Bi Essone, Samoline Mvé Mbeze, Benjamin Ollomo, Fousseny S Touré, Nduo and Richard Onanga
International Center for Medical Research of Franceville (CIRMF), Franceville, BP 769, Gabon.

INTRODUCTION: Malaria still remains a life threatening disease. Watered throughout of year by two rainy seasons, malaria transmission is perennial in Gabon. P. falciparum represents more than 95% of all species from the country. Patients with P. falciparum submicroscopic infection (infection without circulating plasmodies by standard microscopy examination) are not detected and therefore remain untreated. PCR provides a powerful tool to diagnose this kind of infection allowing an accurate management of disease with regard to its eradication policy.

OBJECTIVE: To determine the prevalence of both patent and submicroscopic infections of P. falciparum in symptomatic patients.

MATERIALS AND METHODS: A cross-sectional study was carried out in Franceville, during two periods from May to July 2011 and from February to May 2012. A total of 595 symptomatic patients were enrolled, 250 in the first and 345 in the second period. A clinical examination and parasitological diagnosis by microscopy and PCR was carried out in all patients.

RESULTS: Of the 250 patients enrolled in the first study period, the prevalence was 24% (60/250) and 9.6% (24/250) for patents and submicroscopic infections respectively, a true prevalence of P. falciparum infection was 33.6% (84 / 250). In the second period of the study, a prevalence of 21.45% (74/345)for patent infections and 9.57% (33/345) for submicroscopic infections was obtained. The true prevalence of malaria due to P. falciparum was 31.01% (107/345). The prevalence of submicroscopic infection significantly decreases from 18.18% to 8.20% in February until May, while that of patent infection increases from 15. 91% to 26.23% in the same period. Sixty-nine percent (69%) of the samples assessed by microscopy and PCR were negative for malaria.

CONCLUSION: The submicroscopic infections occur in symptomatic patients in an average of 9% of cases with a relative high prevalence at the beginning and the end of the rainy season.

PERSPECTIVES: The results of this work allow us to consider extending the study over a period of at least one year in urban as well as in rural areas. It would be also of interest to investigate the etiologic agents associated with symptoms from individuals without P. falciparum infection.

P233: Plasmodium falciparum var gene expression homogeneity as a marker of the host-parasite relationship during acquisition of natural immunity to malaria

K Esther

BACKGROUND: Acquired immunity to Plasmodium falciparum infection causes a change from frequent, sometimes life-threatening, malaria in young children to asymptomatic, chronic infections in older children and adults. Little is known about how this transition occurs but antibodies to the extremely diverse PEM1 parasite antigens are thought to play a role. PEM1 is encoded by a family of 60 var genes that undergo clonal antigenic variation, potentially creating an antigenically heterogeneous infecting population of parasites within the host.

METHOD: Previous theoretical work suggests that antibodies to PEM1 may play a role in “orchestrating” their expression within infections leading to sequential, homogeneous expression of var genes, and prolonged infection chronicle. Here, using a cloning and sequencing approach we compare the var expression homogeneity (VEH) between isolates from children with asymptomatic and clinical infections.

RESULTS AND CONCLUSION: We show that asymptomatic infections have higher VEH than clinical infections and a broader host antibody response. We discuss this in relation to the potential role of host antibodies in promoting chronicity of infection and parasite survival through the low transmission season.

P234: Evaluation of new tools for malaria vector control in Cameroon: focus on long lasting insecticidal nets

Josiane Etang1,2, Philippe Nwane1, Michael Plameu1, Blaise Manga1, Daniel Soupo1, Parfait Awono-Ambene1
1 Laboratory of Medical Entomology, Organisation de Coordination pour la lutte contre les Épidémies en Afrique Centrale, Yaoundé, Cameroon; 2Faculty of Medicine and Pharmaceutical Sciences, The University of Douala, Cameroon; 3Faculty of Science, The University of Yaoundé I, Cameroon; 4Centre Supérieur des Sciences de la Santé, Université catholique d'Afrique Centrale, Cameroon; 5Division of Health Promotion, Ministry of Public Health, Cameroon; 6Service of Pestce regulation, Ministry of Agriculture and rural development, Cameroon

BACKGROUND: From 2006 to 2011, biological activity of insecticides for Indoor Residual Spraying (IRS), conventional treatment of nets (CTNs) or Long lasting insecticidal nets (LLINs) was evaluated before their approval in Cameroon. The objective of the study was to select the best tools for universal malaria vector control coverage.

METHODS: Bioassays were performed using WHO cones and the Kisumu bioassay.
P235: Perception and acceptability of malaria vaccines among Maternal and Child Health clinic attendees at the University of Calabar Teaching Hospital, Calabar, Nigeria.

INTRODUCTION: Ninety-percent of the world’s malaria cases occur in sub-Saharan Africa. Pregnant women and children aged under-five are the most vulnerable. It is estimated that about half of Nigeria’s 160 million population suffer from malaria every year. There are challenges in implementing ongoing malaria control strategies such as widespread resistance of the mosquito to insecticides and development of multi-drug resistance by the malaria parasite. As a result, there is need for newer vaccines among the respondents was high, there is need for more education toward universal coverage of malaria vector control in Cameroon.

RESULTS: All the 14 tested products were found effective (95-100% knockdown and mortality rates), although a slight decrease of effectiveness was seen with lambda-cyhalothrin WP IRS, alpha-cypermethrin CTNs and LLINs (p< 0.05). However, the efficacy of Interceptor nets did not decrease during the 5 months evaluation, even after 25 washes. Meanwhile Fendona SC nets displayed a drastic decrease of activity after 5 washes. No significant difference was seen in bio efficacy of nets washed at community level compared with those washed in laboratory conditions, either with Interceptor or Fendona nets (P>0.05), suggesting that washing procedures at community level was well done.

CONCLUSION: This study provided useful data for decision making and community education toward universal coverage of malaria vector control in Cameroon.

P236: Perceptions and utilisation of the antimalarials artemether-lumefantrine and DHA-PPQ in the Chikhwawa district of Malawi: a mixed methods study

INTRODUCTION: Adherence to antimalarial dosing schedules is essential to ensure effective treatment. Measuring adherence is challenging due to recall issues and the participants’ awareness of the desired behaviour influencing their actions or responses. This study used qualitative methods, which allow for rapport building, to explore issues around antimalarial utilisation, and used the results to guide the development of a context specific questionnaire on perceptions and adherence to artemether-lumefantrine and DHA-PPQ.

METHODS: Qualitative data collection was conducted between September 2010 and February 2011: 12 focus group discussions explored community perceptions of antimalarials, and experiences of administering medications to children. Critical incidence interviews were conducted with 22 caregivers to explore experiences of administering Coartem or Coartem dispersible to children during recent febrile episodes. Quantitative data was collected between November 2011 and May 2013. A structured questionnaire was used to gather data on experience of recent treatment and adherence to antimalarials during follow-up visits with 218 caregivers whose child was recently treated with either Coartem dispersible or DHA-PPQ.

RESULTS: We will present the findings of this mixed-methods investigation, including the overall level of treatment adherence; perceived determinants of drug efficacy; and other factors influencing antimalarial utilisation, such as ease of administration to young children. The results will be used to identify potential intervention opportunities to reduce barriers to appropriate, effective antimalarial treatment.

P237: Malaria parasitaemia among settled Fulani pastoralists in Rivers State, Nigeria

BACKGROUND: Malaria infection is a leading cause of morbidity, mortality and a serious impediment to economy and social development in Nigeria. The Fulani Pastoralists play an important role in the economy and nutrition of many African Countries, yet they have the least access to health care services and basic amenities since the settlements are often located far from government facilities. This study was carried out to provide some baseline data on Malaria parasitaemia in this group.

METHODS: Venous blood was collected by veno-puncture from Fulani herdsmen in Rivers State, after obtaining ethical clearance. Standard thick and thin blood smears were used to examine the blood specimens. Number of asexual parasites per 200 white blood cells was counted and parasite densities computed assuming a mean WBC count of 8,000/µl. Packed cell volume was determined by centrifugation of blood in hermepenized capillary tube and was read using microhaematocrit reader. Oral questionnaires were administered with the aid of an interpreter and data analysed using Anova.

RESULTS: Of the 593 Fulani pastoralists examined 464(78.2%) were infected. Male herdsmen were more infected (79.9%) than females (75.4%). There was no statistically significant difference in the occurrence of malaria infection by sex of subjects (p>0.05).The age group 20-30 years had significantly higher prevalence of malaria 99(81.8%) than the
age group 51 years and above. Most of the infections were of moderate parasitaemia with parasite density of 11-100 parasites per 100 high power fields and was higher among females. Anaemia was higher among the age group 21-30 years 28(23.1%) and in females. Forty- three (10.5%) of the Fulani Pastoralists owned insecticide net (ITN) while majority used coil 160(38.9%) as a preventive measure against mosquito bites. 

CONCLUSION: The result of the study indicated high parasitemia among the Fulani pastoralists in their various bush encampments and the parasitemia and their vector is advocated.

P238: Survey of Antimalarial Drugs Use Pattern in Retail Outlets in Enugu Urban, South East Nigeria; Implication for malaria treatment policy

Charles C. Ezendu1, Brian O. Ogbonna2
Department of Clinical Pharmacy & Pharmacy Management, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University Awka, Agulu campus, Nigeria

BACKGROUND: As a key source of malaria treatment in developing countries, regular and accurate information on the private retail sector is essential for enhancing strategies for improving the use of Artemisinin-based Combination Therapy (ACT), for effective malaria treatment. The study analyzed the demand and sales pattern of antimalarial drugs in private retail outlets to assess the current state of compliance to policy.

METHODS: A prospective cross-sectional survey of randomly selected retail drugs outlets, by type and location in Enugu urban south east Nigeria, was conducted between April and June 2013, to determine the types, range, prices and sales pattern of antimalarial drugs demanded and dispensed as well as concomitant medications, from pharmacies and patent medicine outlets. Data was collected for antimalarial drugs demanded for and sold by self-treatment/medication, recommendation/treatment by retail outlets and prescription from hospitals, and analysed based on descriptive statistics.

RESULTS: ACTs accounted for 78% (1,841/2,360) of dispensed drugs, while monotherapy (SP and other products) was 22%. AMFm drugs contributed 28.2% (520/1,841) of ACTs. The AL brand was the most used ACT, at 67% (1,233/1,841). 51.7% (1,220/2,360) of the drugs were dispensed from self-treatment by patients. Treatment by the retail outlets accounted for 39.8% (939/2,360) while about 8.5% of the dispensed drugs were prescriptions from hospitals. (100%) of prescriptions from hospitals were ACTs. Prescriptions from retail outlets were approximately 99% ACTs while self-medication by patients contained 57.4% of ACTs. Men (64%) are more likely to use the retail outlets than women (36%) while prescriptions from hospitals were mostly children, 65%. The median cost of the ACTs, ($3.23) is about three times the median cost of monotherapy ($0.97). Total cost of treatment, including concomitant medications averaged $3.34 ($±1.90).

CONCLUSION: ACTs have become the predominant antimalarial drugs of choice in the retail outlets, with significant contribution of the AMFm drugs. This portends positive implications on the goals of malaria case management. However, due to significant incidence of presumptive malaria treatment in the sector, interventions to enhance accuracy of diagnosis are needed for more effective and cost-effective treatment of malaria fevers in the retail sector.

P239: Peripheral antinociceptive and heme biominerization inhibitory effects of *Entada africana* leaf extract with activity against drug resistant *Plasmodium falciparum*

Ifeoma Ezennyi, Lasaloaminrina Ranarivelo1, Oluwakanyinsola Salawu1, Martins Emeye1
1National Institute for Pharmaceutical Research and Development, Idu Abuja, Nigeria; *Centre National d’Application de Recherches Pharmaceutiques, Antananarivo, Madagascar*

BACKGROUND: *Entada africana* is a medicinal plant widely used in West Africa for the treatment of different diseases including malaria. Its roots are mostly used in traditional preparations against malaria and this has raised concerns regarding the sustainable use of the plant in combating malaria. Our study therefore explores the antimalarial potential of the ethanol leaf extract of *Entada africana* (EA)

METHODS: The activity of EA against chloroquine sensitive (HB3) and chloroquine resistant (FCM29) *Plasmodium falciparum* was determined as well as its peripheral antinociceptive and anti-inflammatory properties. The effect of the extract on human monocytes (THP-1) cells was recorded as a measure of cytotoxicity, while the inhibitory effects of the extract on heme detoxification was evaluated as a possible mechanism of antiparasomal activity.

RESULTS: At a concentration of 100 µg/mL, EA was non-cytotoxic and displayed moderate antiparasomal activity with IC50 values of 26.36 and 28.86 µg/mL against P. falciparum HB3 and FCM29 respectively. It also exhibited concentration-dependent inhibition of synthetic heme, with an IC50 value of 16 mg/mL. The extract (200 mg/kg body weight) showed significant (P < 0.05) inhibition (35 %) of egg albumin-induced edema at 150 min and this effect was not dose-dependent as similar effect was not observed at higher doses of EA used. Conversely, EA significantly (P < 0.01, 0.05) reduced the number of abdominal writhes induced by acetic acid (58.62 – 65.51 %) and its effect was higher compared to diclofenac (50 %, P < 0.05).

CONCLUSIONS: These findings suggest that peripheral antinociceptive effects, inhibition of parasitic heme detoxification and parasitidal activity contribute to the antimalarial properties displayed by EA. These properties can be employed in the identification and isolation of antimalarial compounds for further development from *Entada africana* leaves, a sustainable alternative to *E. africana* roots, as effective therapy against malaria infection.

P240: *In vivo* animal model antimalarial activity of trichilia megalantha harms (meliaceae) extracts and fractions

Fadare Dorcas A., Abiodun Oyindamola O., and Ajaiyeoba Edith O.
1Department of Pharmacognosy, University of Ibadan, Nigeria.  
2Department of Pharmacology and Therapeutics, University of Ibadan, Nigeria.

BACKGROUND: Malaria is the most important parasitic disease in tropical areas. The cost of effective treatment is prohibitive for the large majority of the populations due to resistance to several antimalarials by the parasites. This necessitates urgent need for discovery and development of new chemotherapeutic compounds of low cost which can be achieved through the use of medicinal plants.

METHOD: The crude methanol extracts of leaf, stem bark and stem bark fractions of *T. megalantha* were screened for *in vivo* animal antimalarial activities in mice against a chloroquine resistance *Plasmodium berghei* ANKA clone was assessed using the Peters 4-day suppressive test procedure.

RESULTS: Of all the seven extracts studied *T. megalantha* stem bark gave the highest activity. At 200 mg/kg of mouse, it had complete suppression of parasite growth (100%). Least activity was observed for the leaf extracts while the root bark had a parasite suppression of 98.4% at 800 mg/kg comparable to that of Chloroquine. The most active fraction, *T. megalantha* stem chloroform had 95.0% parasite growth suppression at 800 mg/kg. Percentage suppression of parasite growth on day 4 post infection ranged from 3.3% to 96.1% in mice infected with *P. berghei* and treated with extracts and fractions of *T. megalantha* when compared with chloroquine disphosphate, the standard reference drug which had a chemosuppression of 96.2%

CONCLUSION: A promising antimalarial potential has been demonstrated by *T. megalantha* plants parts. Efforts are been geared towards isolation of active constituents.
P241: Burden of malaria in HIV +ve persons in Ibadan southwest Nigeria

Catherine Falade, Bukola Adesina-Adewole, Olusegun Ademowo, Isaac Adewole
College of Medicine, University of Ibadan, Ibadan, Nigeria, I

BACKGROUND: In endemic areas, malaria is usually diagnosed presumptively. HIV increases susceptibility to malaria with the result that HIV +ve persons are treated for presumed malaria very frequently.

METHODS: In a cross-sectional study, 2,082 people living with HIV (PLWHIV) were evaluated for presence of malaria parasite by expert malaria microscopy of Giemsa stained thick blood film. A self/investigator-administered questionnaire was used to collect information on demographic details, symptoms of malaria and antimalarial drug use. Study population was drawn from the HARVARD partnered President’s Emergency Plan for AIDS Relief (PEPFAR) funded APIN adult ARV outpatient clinic, University College Hospital, Ibadan, Nigeria.

RESULTS: The mean age of enrollees was 36.7 ± 9.1 years (range 16-70). 81.5% (1696/2082) were female. Prevalence of malaria parasitemia was 15.8% (329/2082); geometric mean parasite density of 503/μL (range 38 – 229,440/μL). Parasite density was <200/μL in 49.4% of enrollees and >500/μL in 41.2%. Prevalence of malaria parasitemia was significantly higher among female PLWHIV compared to male (17.1% versus 10.1%; p <0.0001). Significantly, prevalence of patent parasitemia decreased as monthly income increased (p=0.044). Males had higher income, better educated and were engaged in occupations that exposed them less than females (p <0.0001, 0.003 and <0.0001 respectively). 49.2% (1024/2082) of the enrollees had one symptom or another at enrolment. Fever (70.1%), headache (63.2%), loss of appetite (44.3%), abdominal pains (32.1%), chills & rigors (22.3%) and vomiting (22.3%) were the five most common. Only vomiting was significantly associated with patent parasitemia. Temperature >37.4°C was not significantly associated with malaria parasitemia. 43.9% had received antimalarial drugs in the preceding three months. 251 (12.1%) reported three or more attacks of presumptively diagnosed malaria in the same time frame. 13.9% (35/251) reported 6 to 10 episodes each. Antimalarial drugs used within two weeks of enrolment include sulfadoxine-pyrimethamine (310; 14.9%), chloroquine (205; 10%) and artemisinin-based-combination therapy (46; 2.2%). 316 (15.2%) of the PLWHIV perceived that they had malaria more frequently and each attack was more severe than before their HIV status changed.

IN CONCLUSION: Malaria is less frequent than believed among PLWHIV. Parasite-based diagnosis will reduce overtreatment with antimalarial drugs.

P242: Does prior exposure to the malaria parasite enhance the efficacy of Lophira alata in combination with artemisinin?

Mofolusho O. Falade, Favor A. Komoni and Roseangela I. Nwuba
Cellular Parasitology Programme, Cell Biology and Genetics Unit, Department of Zoology, University of Ibadan, Ibadan, Nigeria.

BACKGROUND: In malaria endemic areas, acquired immunity gained by residents through repeated exposure to the malaria parasite may lead to enhanced antimalarial activity of some plant extracts used locally for the treatment of malaria. This hypothesis suggests a synergy between plant extract and acquired immunity in treating infections. To test this hypothesis, an immune rodent malaria model was used to test the efficacy of Lophira alata (LA) leaf extracts in treating malaria infections.

METHODS: Two rounds of blood infection (with Plasmodium berghei) and drug-cure (with pyrimethamine) were used to establish immunity in swiss albino mice (Immune mice). Immune and naive mice challenged with P. berghei were then used to determine the influence of acquired antimalarial immunity on the efficacy of Lophira alata (LA) leaf extracts, administered alone or in combination with Artesunate (ART) in the treatment of malaria.

RESULTS: There was significant reduction in parasitemia in immune mice when compared to naive mice (P <0.001) irrespective of treatment regimen; although LA leaf extracts administered alone did not significantly reduce parasitemia in immune mice when compared with ART treated animals. When LA was administered in combination with ART, there was a significant reduction (LA 32.9% ≤ 8.4%, LA+ART 3.13% ≤ 2.1, P < 0.0032) of patent parasitemia in the immune group of animals and furthermore, survival was prolonged (P = 0.0109) in the immune treated animals irrespective of treatment protocol, with the combination LA+ART animals surviving longer.

CONCLUSIONS: These findings suggest that the action of LA in treating malaria infections in a murine model is enhanced by prior exposure to the malaria parasite. Thus the requirements of using plants in treating malaria in endemic populations may differ for those used in western systems, where trials are carried out with naive malaria patients. Combining an artemisinin derivative and a medicinal plant in immune populations may provide an alternative control measure in malaria endemic regions and may justify the continued use of these plants by indigenous populations in treating malaria.

P243: The Larvicidal Activities of the Leaves and Stem barks of Two Jatropha Species Grown in Nigeria.

Funmilayo G. Famuyiwa¹, Samson O. Famuyiwa¹, Adedunni A. Ajiboye¹
¹Department of Pharmacognosy, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria; ²Department of Chemistry, Faculty of Science, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria.

BACKGROUND: Malaria infects about 300 to 500 million people annually killing more than a million and a half majority of which are African children. The high cost of treatment and the development of resistance to available drugs remains a great barrier to the eradication of the disease. Preventing the breeding and maturation of mosquitoes is an important and cost effective method for controlling malaria. The non selective and non-biodegradable nature of the available synthetic larvicides (methoprene and temephos) dictates continued investigation of plants for natural bioactive compounds. This study aimed to assess the leaves and stem barks of Jatropha gossypifolia and Jatropha multijuga for larvicidal activity against the vector of malaria.

METHODS: The plant parts were collected, authenticated, dried and extracted into petroleum ether 60-80°C, ethylacetate and methanol. The extracts were concentrated in vacuo and each was tested against the larvae of Anopheles gambiae according to WHO (2005) with slight modifications. The number of surviving larvae was counted after 24h and 48h. Endosulfan and distilled water were used as the positive and negative controls respectively. Number of replicates was six. The LC₅₀ and LC₉₀ values were subjected to statistical analysis.

RESULTS: The stem bark extracts of the two plants were more active than the leaf extracts. The petroleum ether extract of the stem bark of J. gossypifolia was the most active and had LC₅₀ and LC₉₀ values of 1.58 ± 0.02, 3.02 ± 0.03 mg/ml that were comparable to those of Endosulfan (0.9 ± 0.09, 1.44 ± 0.11 mg/ml). The order of activities of the extracts of the two plants was petroleum ether > ethylacetate > methanol.

CONCLUSIONS: Further purification of the most active petroleum ether extract of the stem bark of Jatropha gossypifolia could afford compounds that could be used in the control of the vector of malaria.
P244: Antimalarial activities in crude extracts and chemicals from some central African medicinal plants.

Guy Raymond Feyua Tchouya1, Jean Bikili2, Fabrice Fekam Boyom2, Bruno Lenta Djakou2, Jean Claude Tchouankeu1

1Département de Chemistry-Biochemistry, Scientific and Technical University of Masuku (U.S.T.M.), Franceville, Gabon; 2Faculty of Science, University of Yaoundé I, Yaoundé, Cameroon; 2Department of Chemistry, Teacher’s training school, Yaoundé, Cameroon

BACKGROUND: In Cameroon and Gabon, many plants are traditionally used by a wide part of the poor population as antimalarials or antipyretics. Entandrophragma angolense, Picralima nitida, Schumanniopython magnificum and Thomandria hensii, are among the most important. In the present study, the in vitro antimalarial activity of these plants extracts was assessed against *P. falciparum* W2 chloroquine-resistant strain. This study also includes the isolation and the antimalarial activities of chemicals from the dichloromethane-methanol extract of the stem bark of *Entandrophragma angolense*. The structure-activity relationship study of limonoids from *Entandrophragma angolense* is also reported.

METHODS: Dried and ground plant materials were successively extracted with hexane and dichloromethane-methanol (1:1) at room temperature for 96h. The extracts were filtered and concentrated. For the isolation of biomolecules, the extract was subjected to column chromatography over Si gel (70-230 mesh) eluting with n-hexane-ethyl acetate gradient of increasing polarity. The molecules were characterized through common spectroscopic methods. To assess antimalarial activities, different dilutions of the extracts and compounds were incubated at 37°C with cultured W2-strain of *P. falciparum* parasites for 48 hours. Parasites were thereafter fixed and stained, and parasitemias of treated and control cultures were determined. Results were means, compared to untreated controls, from 3 experiments.

RESULTS: The plant extracts demonstrated that they possess antimalarial activity. Except *S. magnificum*, the dichloromethane-methanol extracts were more active than the hexane extracts. The dichloromethane-methanol (1:1) extract of *E. angolense* was the most active. The phytochemical investigation of the stem bark of *E. angolense* led to the isolation of ten compounds among which 7α-obacunyl acetate and a cycloartane derivative exhibited a good activity, with IC50 of 2 and 5.4 μg/ml respectively.

CONCLUSIONS: The results of this study can be correlated to the traditional use of the cited species in the treatment of malaria, that should be standardized. The plants also possess antimalarial compounds. From the structure-activity point of view, although the presence of α, β-unsaturated ketone is an important feature for enhanced antimalarial activities of limonoids, the localisation of α, β-unsaturated carbonyl moiety in ring A seems to be critical for the enhancement of that activity.

P245: Antimalarial activities in crude extracts and chemicals from some central African medicinal plants.

Deepika Fernando1, Renu Wickremasinghe1 Pandu Wijeyaratne1

1Faculty of Medicine, University of Colombo, Sri Lanka; 2Faculty of Medical Sciences, University of Sri Jayawardeneppura; 3Tropical and Environmental Diseases and Health Associates (Pvt) Ltd, Colombo, Sri Lanka.

INTRODUCTION: Following the end of the civil conflict the ability of the Anti Malaria Campaign to carry out malaria diagnostic services in the Eastern and Northern Provinces of Sri Lanka was hampered due to a breakdown in health infrastructure and a large number of vacancies in the preventive and curative cadres. Tropical and Environmental Diseases and Health Associates (TEDHA), a private sector organization was called upon to assist in intensified surveillance in these provinces to achieve the goal of malaria elimination in Sri Lanka by 2014.

METHODS: TEDHA set about training two categories of laboratory personnel who would be involved in the laboratory component of surveillance. Fever Surveillance Assistants were trained towards receptivity of patients suspected to have malaria and preparation of blood smears by finger prick method. Parasitology Surveillance Assistants were trained to stain and examine blood smears for malaria parasites. Hospitals to establish laboratories were identified in areas where government services were lacking, and refurbishments carried out where needed.

RESULTS: Following the above process, 50 fully functional laboratories have been established and are actively carrying out malaria diagnosis by microscopy by a staff of 112 persons. Quality assurance procedures are in place with field monitoring taking place regularly. From January 2010 till 31st December 2012, TEDHA has screened 584,081 blood smears for malaria. It is noteworthy that hospital based laboratories have now been extended to outreach through mobile malaria clinics and hospital village clinics.

CONCLUSIONS: The adequacy of TEDHAs training of laboratory personnel and their performance in the current level of malaria diagnosis is a positive solid indicator of the processes adopted. TEDHA has contributed in a number of ways to supplement the national malaria elimination efforts in the Northern (Mannar district) and Eastern Provinces (Amara, Batticaloa and Trincomalie districts), based on the fact that the private sector has the flexibility and capacity to reach populations and provide services which would require longer time commitments to be accomplished by government or civil society. The success of this public-private partnership is an example that can be used in other countries attempting malaria elimination.

P246: Anopheles gambiae s.l. does not avoid mature aquatic habitats colonised by predatory invertebrates

Ulrike Fillinger1, Andrew Githeko2, Bryson Ndenga3

1International Centre of Insect Physiology and Ecology (icipe), Mbita, Kenya; 2London School of Hygiene and Tropical Medicine, London, UK; 3Kenya Medical Research Institute, Kisumu, Kenya

Malaria vector control measures in Africa do not protect against outdoor biting vectors, a problem that grows as vectors develop behavioural resistance to insecticides used indoors. Consequently, there has been an increased interest in attacking the aquatic vector stages. To implement effective larval source management it is necessary to better understand the vector’s habitat choice and larval ecology. In this study we set out to investigate the following questions: Is *An. gambiae* a pioneer species that colonizes aquatic habitats as soon as they occur and for a short period of time? Does *An. gambiae* avoid to oviposit in older habitats? Does the larval density decrease with the increase of predatory invertebrates in the habitat? To study the succession in colonisation of a habitat 20 similar burrow pits (1m2) were created in a valley bottom of the western Kenya highlands (altitude 1,400m). The habitats were sampled daily for four months (Feb–May) with a sweep net and presence and abundance of early and late instar culicine and anopheline larvae, Odonata, Coleoptera and Heteroptera monitored. Abiotic factors, vegetation cover and presence of algae were recorded. All pupae were collected and emergence observed to estimate the vector productivity over time. *Anopheles* colonised all the habitats from the second week of their creation. Peak densities of early instar anophelines were reached from week three and were constant throughout the following three month. Late instar anopheline density increased over time reaching maximum numbers when the habitats were mature indicating a better survival at that time. In contrast culicine larvae occurred in large numbers in the first week but as the habitats aged the proportion of habitats with culicine larvae consistently declined. This decline coincided with an increase in Odonata, Heteroptera and Coleoptera. Highest malaria vector productivity was recorded from habitats older than two months. The presence of grass, algae mats, Odonata and Heteroptera in the habitat was strongly associated with presence and density of anopheline larvae. Consistent colonisation indicates that anophelines were not expelled from laying eggs in mature habitats and in habitats where predators were present in high abundance.
P247: Population pharmacokinetic modeling and simulation of pyronaridine in pediatric malaria patients.

Stephan Duparc1, Janthima Methaneethorn2, Isabelle Borghini-Fuhrer3, Jangsik Shin4, Lawrence Fleckenstein5
1Medicines for Malaria Venture, Geneva, Switzerland; 2College of Pharmacy, The University of Iowa, Iowa City, Iowa, USA; 3Shin Poong Pharmaceuticals, Seoul, Republic of Korea

BACKGROUND: In 2011, there were about 3.3 billion people at risk for malaria worldwide. Approximately 85% of malaria deaths globally were in children under 5 years of age. Pyronaridine/Artesunate (PA) 3:1 fixed dose combination is a novel artemisinin-based combination therapy for an acute uncomplicated P. falciparum or P. vivax malaria. Adult tablet formulations recently received a positive Article 58 Opinion from the European Medicines Agency and a pediatric granule formulation is under development for clinical use. The objectives of this study were to determine pharmacokinetic properties of pyronaridine in pediatric malaria patients by means of population pharmacokinetic modeling and to use Monte Carlo simulation to explore pyronaridine exposures among current PA dosing regimens in pediatric malaria patients.

METHODS: Population pharmacokinetics of pyronaridine was conducted using a non-linear mixed effect modeling approach. A total of 332 pediatric malaria patients participating in five Phase II-III clinical trials were included in the analysis. Age and weight of this population ranged from 0.6 to 15 years and 5 to 56.2 kg, respectively. Influence of weight was treated as a fixed covariate using an allometric scaling approach. The effects of seven covariates including age, formulation, creatinine clearance, alanine aminotransferase, aspartate aminotransferase, gender, and baseline hemoglobin were evaluated. Monte Carlo simulations were performed to reflect pyronaridine exposure in pediatric malaria patients with weights between 5-20 kg using covariate-parameter relationship of the final model and the current dosage recommendations.

RESULTS: Pyronaridine pharmacokinetics in pediatric malaria patients was best described by an allometric twocompartment model with first order absorption and elimination from the central compartment. The only significant covariate-parameter relationship in the final model was the effect of age on apparent clearance (CL/F). The exposures of pyronaridine, estimated by AUCs, were consistent across the current weight-based dosing recommendations of PA granule labeling for pediatric malaria patients.

CONCLUSIONS: The current PA weight-based dosing for pediatric malaria patients of weight between 5 and 20 kg produces consistent pyronaridine exposures across dosing groups. These results suggest that the use of pharmacokinetic modeling and simulation can be used to support appropriate dosing recommendations for children with malaria.


M Florence

BACKGROUND: Insecticide treated bednets (ITNs) are the most widely adopted intervention for the prevention of malaria in African children. Studies suggest that improved house structure may lower the density of mosquito vectors offering an additional means of reducing the risk of malaria.

METHODS: This study explored the relationship between materials used in house construction and the incidence of malaria in a cohort of children followed prospectively as part of a randomized clinical trial of antimalarial chemoprevention. A total of 600 HIV unexposed and exposed children aged 4-5 months living in different houses were enrolled using convenience sampling in Tororo, Uganda a rural area with perennial high transmission intensity. Children received an ITN at enrollment and were followed for all their health care needs 7 d/wk. Children were randomized to one of four chemoprevention arms: HIV unexposed infants were randomized at 6 months of age and HIV exposed children were randomized approximately 6 weeks after cessation of breast feeding (median age 10 months). Approximately 1 year after the start of the study a survey was performed at each child’s house detailing the materials used in house construction. Associations between house structure and the incidence of laboratory confirmed malaria between 6-24 months of age by passive surveillance were estimated using a negative binomial regression model with measures of association expressed as the protective efficacy (PE=1-incidence rate ratio)

RESULTS: The final analyses include 515 children. The prevalence of houses with non-earth floors, non-thatched roofs, and non-mud walls was 14.6%, 43.1%, and 17.5%, respectively. After controlling for chemoprevention, having a non-earth floor was associated with a 64% lower incidence of malaria (95% CI, 53-72%, p<0.001), having a non-thatched roof was associated with a 27% lower incidence of malaria (95% CI, 14-39%, p<0.001), and having non-mud walls was associated with a 53% lower incidence of malaria (95% CI, 42-63%, p<0.001). Houses constructed with all non-natural materials were associated with a 70% lower incidence of malaria (95% CI, 60-77%, p<0.001).

CONCLUSION: Most houses in this rural area of Uganda were constructed with basic natural materials. Better-constructed houses using non-natural materials were associated with a much lower incidence of malaria.

P249: Vector diversity and malaria transmission in Ndop, North West Region Cameroon

Barrière Fodjo1, Raymond Tabue1, Thomas Ném1, Jean Atangana3, Salomon Patchôle1, Frédéric Thouné1, Etienne Fondjo2, Jude Bigoga1, Rose Leke1
1National Reference Unit for Vector Control, the Biotechnology Center, National Reference Unit for Vector Control, the Biotechnology Center, University of Yaoundé I. 2Department of Biochemistry, University of Yaoundé I. 3National Malaria Control Programme.

BACKGROUND: Malaria is endemic in Ndop. There is the need to simultaneously use different control measures to fight this disease. Knowledge of vectors involved in the transmission of malaria using evidence collected in the field are required to make decisions about the combination of control measures and improved malaria control strategies. This work aims to study the vector fauna and the role of existing Anopheles species involved in malaria transmission in Ndop.

METHODS: In three neighborhoods within the Ndop health area (Backyt, Mbafuh and Mbadishi), a series of longitudinal entomological surveys were conducted during one year (2011). Anopheline mosquitoes were collected through human landing night catches and identified morphologically. Four consecutive indoor and outdoor night catches were carried out in each zone per month. Molecular identification (PCR) was carried out for An. gambiae s.s. Infectivity with Plasmodium falciparum was detected by Enzyme-linked immunosorbent assay (ELISA).

RESULTS: A total of 3,972 anophelines was collected, belonging to 09 species: Anopheles gambiae, An. ziemanni, An. christyi, An. implexus, An. maculipalpis, An. nili, An. funestus, An. tenebrosus and An. pharaohensis. An. ziemanni was the predominant species in this area (93.19%) and had the highest infection rates, followed by An. gambiae (4.7%). Overall the entomological Inoculation Rate (EIR) for the malaria vectors was 0.13 ib/m/n. The Average overall EIR for An. gambiae s.l. and An. ziemanni were 0.01 ib/m/n and 0.12 ib/m/n respectively. Only the M form of An. gambiae s.s was found.

CONCLUSION: This study shows that in the absence of the major vector (An. gambiae), other species may play an important role (An. ziemanni) in malaria transmission. However, studies on the behavior and the susceptibility of vectors to insecticides are needed in order to assist the planning and implementation of improved malaria control strategies in this zone.
**P250: Hepatitis C Virus infection may lead to slower emergence of P. falciparum in blood**


**BACKGROUND:** Areas endemic for *Plasmodium falciparum*, hepatitis B virus (HBV) and hepatitis C virus (HCV) overlap in many parts of Sub-Saharan Africa. HBV and HCV infections develop in the liver, where takes place the first development stage of *P. falciparum* before its further spread in blood. The complex mechanisms involved in the development of hepatitis may potentially influence the development of the liver stage of malaria parasites. Understanding the molecular mechanisms of these interactions could provide new pathophysiological insights for treatment strategies in Malaria.

**METHODOLOGY:** We studied a cohort of 319 individuals living in a village where the three infections are prevalent. The patients were initially given a curative antimalarial treatment and were then monitored for the emergence of asexual *P. falciparum* forms in blood, fortnightly for one year, by microscopy and polymerase chain reaction.

**Principal FINDINGS:** At inclusion, 65 (20.4%) subjects had detectable malaria parasites in blood, 36 (11.3%) were HBV chronic carriers and 61 (18.9%) were HCV chronic carriers. During follow-up, asexual *P. falciparum* forms were detected in the blood of 203 patients. The median time to *P. falciparum* emergence in blood was respectively 140 and 120 days in HBV- and HBV+ individuals, and 135 and 224 days in HCV- and HCV+ individuals. HCV carriage was associated with delayed emergence of asexual *P. falciparum* forms in blood relative to patients without HCV infection.

**CONCLUSIONS:** This pilot study represents first tentative evidence of a potential epidemiological interaction between HBV, HCV and *P. falciparum* infections. Age is an important confounding factor in this setting however multivariate analysis points to an interaction between *P. falciparum* and HCV at the hepatic level with a slower emergence of *P. falciparum* in HCV chronic carriers. More in depth analysis are necessary to unravel the basis of hepatic interactions between these two pathogens, which could help in identifying new therapeutic approaches against malaria.

**P251: How do health workers perceive and practice monitoring and evaluation of malaria control interventions in south-east, Nigeria?**

Freya Fowkes

**BACKGROUND/PROBLEM STATEMENT:** The Anambra state Malaria Control Booster Project (ANMCBP) depends on an effective monitoring and evaluation (M&E) system to continuously improve the implementation of the malaria control interventions. However, it is not clear how the health workers that are expected to be the fulcrum of the malaria M&E perceive and practise M&E. The study was carried out to determine the knowledge, perception, and practice of Malaria M&E among selected health staff, and to identify related socio-demographic factors.

**METHODS:** Semi-structured questionnaire and observation checklist were used to collect information from selected health workers in public primary health centres in all 21 local government areas of the State. Multistage sampling technique was used in selection of respondents. The questionnaire explored knowledge, perception and practice of malaria M&E from 213 health workers. The observation checklist was used to record the actual practice of malaria M&E as observed by trained supervisors.

**RESULTS:** While 81.5% of health workers were able to correctly identify the malaria M&E forms and 97.6% of them felt there was need to keep proper records, less than half of them had correct understanding of malaria data management in terms of what malaria data is (37.6%), and the malaria data generation (48.8%), analysis (48.8%) and transmission (40.8%) processes for M&E. Observation of filled M&E forms showed that 61% of the respondents had at least one wrongly filled form, and half of them had no properly filled form. Across age groups and cadres, no significant difference was found in the health workers’ knowledge, perception and practice of malaria M&E.

**CONCLUSION:** Gaps still exist in health workers’ understanding of malaria data management, perception of efficient data transmission and observed practice of malaria M&E. Socio-demographic variables such as age and cadre do not significantly affect health workers’ perception and practice of malaria M&E.

**P252: How long do antibodies to malaria last?: Evidence from immuno-epidemiology studies**


1Macfarlane Burnet Institute of Medical Research, Melbourne, Victoria, Australia; 2Shkolia Malaria Research Unit, Mae Sot, Tak, Thailand; 3Centre for Molecular, Environmental, Genetic and Analytic Epidemiology, University of Melbourne, Australia; 4Division of Chemical Biology, the Walter and Eliza Hall Institute of Medical Research, Bundoora, Victoria, Australia; 5Malaria Vaccine Development Branch, National Institute of Allergy and Infectious Diseases/ National Institutes of Health, Rockville, Maryland, USA; 6Department of Biochemistry, La Trobe University, Victoria, Australia; 7Malaria Research Unit, Cell-Free Science and Technology Research Centre, Ehime University, Ehime, Japan; 8Kenya Medical Research Institute, Kilifi, Kenya; 9Department of Tropical Medicine, Medical Microbiology and Pharmacology, University of Hawaii at Manoa, Honolulu, HI

It is widely said that anti-malarial antibodies are very short-lived, but there are little published data that address this question. This is due to the lack of epidemiological studies with repeated consecutive antibody sampling at close intervals. How antibodies to malaria antigens, including vaccine candidates, are acquired, maintained and persist in field settings is therefore unclear. We determined antibody levels to a panel of *P. falciparum* and *P. vivax* merozoite antigens, and to variant surface antigens expressed on the surface of the infected erythrocyte (both pregnancy [PfVAR2CSA] and non-pregnancy-specific antigens) in multiple longitudinal epidemiological studies. These studies included immunological studies in children and pregnant women living in Asia and Africa and experiencing different levels of malaria exposure. Antibodies to *P. falciparum* and *P. vivax* were highly variable over time, and maintenance of high levels of anti-malarial antibodies involved highly dynamic responses resulting from intermittent exposure to infection. There was evidence of boosting with each successive infection suggesting the presence of immunological memory. Interestingly, the response half-life of anti-merozoite antibodies to *P. falciparum* conserved and variant merozoite antigens as well as responses to *P. vivax* merozoite, was similar regardless of antigenic or species diversity (1-5 years). Conversely, antibody response half-life to variant surface antigen (PfVAR2CSA) was considerably longer (decades). However, the half-lives of *Plasmodium* antibody responses were relatively short compared to measles (457 years). These data in pregnant women and children are valuable to quantify the impact of malaria interventions and control programs on the maintenance of protective immunity to malaria as well as the development of sero-surveillance tools to assess malaria elimination.
P253: Antimalarial and toxicological activities of Clerodendron polycephalum

Adewoyin F. B., Odalbo A. B., Adewunmi, C. A. Omisore, N. O and Iwalewa
G. E.

Clerodendron polycephalum is a medicinal plant used traditionally to treat malaria. The leaves were collected and investigated for effectiveness against Plasmodium berghei in vivo using mice. Using appropriate standard methods, prophylaxis, curative and suppressive activities were carried out. The methanolic extranolic extract showed significant antimalarial activity compared to standard drug used. Toxicity studies of methanolic extract was also done using haematological parameters. There are slight changes in body weights compared to the control. The control animals generally showed increase in body weights while the test animals had slight decrease and then increase. The incidence of the later increase could be inferred as possible recovery from any toxic activity of the extract. The use of C. polycephalum could be encouraged as a good herbal material against malaria.

P254: Role of alpha-3.7 deletional thalassemia and glucose-6-phosphate dehydrogenase deficiency (G6PDA-) on Plasmodium falciparum transmission

Yao Franck

INTRODUCTION/BACKGROUND: Human genetic factors play a key role in determining the resistance/susceptibility to infectious diseases. It is unknown whether the genetic makeup may also influence the host efficiency to transmit pathogens. As to malaria, a major selective force in recent human evolution, field studies performed in Burkina Faso demonstrated that genetic variation in human HBB is associated with Plasmodium falciparum transmission. The objective of this study was to evaluate the role of alpha-3.7 deletional thalassemia and glucose-6-phosphate dehydrogenase deficiency (G6PDA-) on the efficiency of Plasmodium falciparum transmission from the human host to the mosquito vector.

METHODOLOGY: A total of 70 asymptomatic children aged 3-15 years from the village of Soumouso, South-West of Burkina Faso, with known alpha-thal and G6PD genotypes, were recruited in the study. Ex vivo malaria transmission experiments based on artificial membrane feeding of naive insectary-reared mosquitoes collected at the larval stage from the same Soumouso study area were performed during the high malaria transmission season. Transmission experiments were performed in blind, that is without checking for the presence of gametocytes in peripheral blood before blood collections. A total of 4129 Anopheles were dissected on day seven after membrane feeding and oocysts number counted.

RESULTS: The results showed that alpha-3.7 deletional thalassemia and glucose-6-phosphate dehydrogenase deficiency (G6PDA-) were associated with a higher parasite transmission from the human host to the mosquito vector.

DISCUSSION AND CONCLUSION: These preliminary results confirmed the influence of human genetic variation on Plasmodium falciparum transmission from humans to mosquitoes. More investigations are needed to explain the mechanisms underlying these differences.
P256: The Larvicidal Activities of the Leaves and Stem barks of Two Jatropha Species Grown in Nigeria.

Funmilayo G. Famuyiwa1, Samson O. Famuyiwa1, Adedunni A. Ajiboye1
1Department of Pharmacognosy, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria.
2Department of Chemistry, Faculty of Science, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria.

BACKGROUND: Malaria infects about 300 to 500 million people annually killing more than a million and a half majority of which are African children. The high cost of treatment and the development of resistance to available drugs remains a great barrier to the eradication of the disease. Preventing the breeding and maturation of mosquitoes is an important and cost effective method for controlling malaria. The non selective and non-biodegradable nature of the available synthetic larvicides (methoprene and temephos) dictates continued investigation of plants for natural bioactive compounds. This study aimed to assess the leaves and stem barks of Jatropha gossypifolia and Jatropha multijida for larvicidal activity against the vector of malaria.

METHODS: The plant parts were collected, authenticated, dried and extracted into petroleum ether, ethylacetate and methanol. The extracts were concentrated in vacuo and each was tested against the larvae of Anopheles gambiae according to WHO (2005) with slight modifications. The number of surviving larvae was counted after 24h according to WHO (2005) with slight modifications. The number of surviving larvae was counted after 24h and 48h. Endosulphan and distilled water were used as the positive and negative controls respectively. Number of replicates was six. The LC50 and LC90 values were subjected to statistical analysis.

RESULTS: The steam bark extracts of the two plants were more active than the leaf extracts. The petroleum ether extract of the stem bark of J. gossypifolia was the most active and had LC50 and LC90 values of 1.58 ± 0.02, 3.02 ± 0.03 mg/ml that were comparable to those of Endosulphan (0.9 ± 0.09, 1.44 ± 0.11 mg/ml). The order of activities of the extracts of the two plants was petroleum ether > ethylacetate > methanol.

CONCLUSIONS: Further purification of the most active petroleum ether extract of the stem bark of Jatropha gossypifolia could afford compounds that could be used in the control of the vector of malaria.

P257: Larvicidal activity, qualitative and quantitative components of Parquestina nigrescens leaf extracts on different larval stages of an African malaria vector.

O Funmilola

BACKGROUND: Hemosporidians are parasites of significant health importance considering the morbidity and mortality rates recorded globally as a result of the diseases they caused. Malaria is one of such diseases which affect both humans and other animals. Plasmodium species responsible for human malaria are vectored by female Anopheles mosquitoes. Drug resistance displayed by Plasmodium contributed to the difficulty in combating malaria which has necessitated the need to search for alternative control measures. Traditionally, plants and herbs play crucial roles in controlling diseases. Parquestina nigrescens is one of these plants. This study the larvicidal activity of different extracts of Parquestina nigrescens were investigated on Anopheles mosquito larvae.

METHODS: The leaf part of Parquestina nigrescens was collected, air dried and blended in preparation for extraction processes using different solvents namely methanol, ethanol and aqueous solution. Qualitative and quantitative components of the leaf extracts were assessed. The bioactivity of these extracts were conducted on laboratory reared second and fourth instars of Anopheles mosquito larvae for 48 hour and the mortality recorded at 12 hours intervals. Each treatment was carried out in triplicates. The LC50, resistance ratio and significant differences of each treatment was calculated using log dosage-Probit mortality regression analysis software and ANOVA.

RESULTS: In ethanol extract treatment, the highest percentage mortality of 95% was recorded in the fourth instars while 84% mortality was recorded in the methanol extract. The highest impact of the plant bioactivity was recorded on the second instars where 100% mortality was recorded in the ethanol treatments. The treatments were significantly different when P<0.05. The qualitative screening of the plant revealed the presence of compounds such as Saponin, Alkaloid and Flavonoid while quantitative assessment showed that alkaloid has the highest value of 1.8.

CONCLUSION: This study revealed that the leaf extract of Parquestina nigrescens has larvicidal property on Anopheles. Hence it might be a potential biological control agent for malaria vector.

P258: Gametocytes and multiple-clone carriage among long lasting asymptomatic Plasmodium falciparum carriers in absence of therapy

Amal Godalla1,2, Petra Schneider1, Elkhansaa Nassir2, Abdel-Muhsin A Abdel-Muhsin1, Sarah Reece3 and Hamza Babiker2
1 Tropical Medicine Research Institute, National Center for Research, Kartoum, Sudan; 2 College of Medicine, Sudan Qaboos University, Alkhoud 123, Muscat, Oman; 3 School of Biological Sciences, University of Edinburgh, EH9 3JT, UK

Asymptotic malaria represents a major challenge to control efforts and the prospect of malaria elimination. In areas of seasonal malaria, asymptomatic parasitaemia can persist during the transmission-free period, and raise new cases following the start of annual rains and resurgence of Anopheles mosquitoes. However, little is known on how asymptomatic parasitaemia persist for several months/years and sustain ability to produce infective gametocytes. Here we monitored P. falciparum persistence, multiplicity of infection and gametocyte production among asymptomatic carriers, over several months during the dry season in an area of seasonal transmission. Thirty eight participants, with persisting P. falciparum infection between Jan 2001 (start of the dry season) and Sep 2002 (end of the dry season), were examined. Monthly samples from each participant were examined for gametocytes using RT-PCR and parasite density using qPCR. Multiplicity of infection was identified by typing P. falciparum pfg377 and msp2 polymorphic genes. Asymptomatic P. falciparum parasitaemia that persisted throughout the dry season, were characterized by (a) low parasite density ranging between 5-474 parasites/µl. (b) high probability of multiple genotypes (20-65%) and (c) high probability of gametocyte carriage (20-40%). Interestingly, gametocyte carriage and multiplicity of infection increased at the end of the dry season, coinciding with the start of the ensuing transmission season.

P259: Variation of the haematological parameters in children less than five years with Plasmodium asymptomatic infection: Implication for malaria vaccine trials.

Adama Gansane1,2, Ouedarogo N. Issa1, Noelle Bere Henry1, Issiaka Soulima1, Esperance Ouedraogo1, Jean-Baptiste Yaro1, Amidou Diarra1, Seydou Sombie1, Amadou T. Konate1, Alfred Tione1, Sodilomon Sirima1,2
1Centre National de Recherche et de Formation sur le Paludisme (CNRP), Ouagadougou, Burkina Faso; 2Groupe de Recherche Action en Santé GRAS), Ouagadougou, Burkina Faso

BACKGROUND: During the season of high malaria season, most of children are infected by Plasmodium which targets red blood cells affecting hematological parameters. Vaccine immunogenicity is evaluated using WBCs and subsets and the methods used require a high level of viable peripheral blood mononuclear cells (PBMCs) after blood collection. Thus, the impact of malaria parasites on WBCs could affect the validity of in vitro proliferative assays, such as the [3H]thymidine uptake assay, which is widely used in areas of endemicity to detect specific cellular responses to Plasmodium falciparum antigens. The aim of this study was to determine
the potential of hematological variations as confounding factors in vaccine trials in a malaria-endemic area.

**METHODS:** Cross-sectional survey was conducted at the peak of malaria transmission season in rural area of Burkina Faso. After informed consent and clinical examination, blood samples were obtained from participants for malaria diagnostic and full blood count. The subjects with no detected malaria parasites were assigned to the control group and the subjects with malaria parasites to the infected group. To test for any association between malaria parasitaemia and a specific haematological parameter, we stratified *P. falciparum* parasitaemia into four categories (null, 1-1,000, 1,000-10,000 and > 10,000 parasites/µL). ANOVA and t tests were used to compare the means of the haematological parameters for the two groups.

**RESULTS:** From 414 children included in analysis, 192 were Plasmodium uninfected and 222 were asymptomatic carriers of plasmodium infection. The mean age of infected children was 41.8 months (range 26.4-57.2) vs. 38.8 months (range 22.4-55.2) for control group (p=0.06). Asymptomatic infected children tend to have significant lower mean hemoglobin level (10.8g/dl vs. 10.4g/dl; p<0.001), mean lymphocyte count (1403/µL vs. 1192/µL; p<0.001), mean red blood cell count (4.388x10^6 vs. 4.158x10^6/µL; p=0.004), mean platelet count (266x10^3 vs. 385x10^3/µL; p<0.001), mean red blood cell count (4.388x10^6/µL vs. 4.158x10^6/µL; p<0.001) and higher mean monocyte count (1403/µL vs.1192/µL; p=0.001) as compared to control group.

**CONCLUSION:** Malaria parasites may affect the hematopoiesis of children living in malaria-endemic areas. Special attention should be applied when interpreting hematological parameters and also when evaluating immune responses in children living in malaria-endemic areas and enrolled in vaccine trials.

**P261: Repellency**

**R Gayaram**

**BACKGROUND:** This study was initiated to establish whether any South African ethnomedicinal plants (indigenous or exotic), that have been reported to be used traditionally to repel or kill mosquitoes exhibit effective mosquito repellent properties.

**METHOD:** Extracts of a selection of such taxa were tested for repellency properties in an applicable mosquito feeding-probing assay using unfed female *Anopheles arabiensis*.

**RESULTS:** Although a water extract of the roots of *Chenopodium opulifolium* was found to be 97% as effective as DEET after 2 mins, time lag studies revealed a substantial reduction in efficacy (to 30%) within two hours.

**CONCLUSIONS:** None of the plant extracts investigated exhibited residual repellencies >60% after three hours.

**P262: Cytokine responses to the VAR2CSA vaccine candidate in pregnant Beninese**

**Gbedané Komí1, Samad Ibitokou2, Bertin Vianou3, Carine Agbowai3, Marita Troye-Blomberg4, Thor Theander5, Ali Salanti6, Achille Massougbodji7, Nicaise Tuikue Ndam8, Philippe Deloron3,4, Adrian J F Luty4, Nadine Fievet1,4**

1Centre d’Étude et de Recherche sur le Paludisme Associé à la Grossesse et à l’Enfant (CERPAGE), Faculté des Sciences de la Santé, Université d’Abomey-Calavi, Benin; 5PRES Sorbonne Paris Cité, Université Paris Descartes, Faculté de Pharmacie, France; 6Department of International Health, Institute of International Health, Immunology and Microbiology, University of Copenhagen, Denmark; 7Department of Immunology, Wenner-Gren Institute, Stockholm University, Stockholm, Sweden; 8Department of Medical Microbiology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; 2Institut de Recherche pour le Développement, UMR D216 Mère et enfant face aux infections tropicales, Paris, France.

**INTRODUCTION:** The STOPPAM consortium conducted 2 longitudinal cohort studies in pregnant women in Benin and Tanzania in order to evaluate the immunopathological consequences of infections with *Plasmodium falciparum* during pregnancy. In this context, parasite antigen-specific cellular responses, in particular to the vaccine candidate antigen VAR2CSA, have received little attention. Here we evaluated the cytokine (IL10, IL13, IL17, IFN—) profiles that reflect the acquisition of a specific cellular memory response to the vaccine candidate according to gravidity or previous infection. We also evaluated the cytokine concentrations that are reflective of infection earlier in the pregnancy. The amounts of IL10, IL13, IL17, IFN—DBL5 were quantified in supernatants of stimulated PBMC.

**METHODS:** In Come, southwestern Benin, we conducted a longitudinal prospective study of ~1000 pregnant women. Women at ≥ 24 weeks of pregnancy were enrolled and followed at each antenatal visit until delivery. Peripheral blood mononuclear cellular (PBMC) responses to VAR2CSA-DBL5 in vitro were determined in subgroups of 150 women at inclusion and 100 at delivery. In each subgroup those harbouring *P. falciparum* infections were matched by gravidity and gestational age with mothers with no infection at inclusion and those with no history of infection earlier in the pregnancy. The amounts of IL10, IL13, IL17, IFN—DBL5 were quantified in supernatants of stimulated PBMC.

**RESULTS:** At the time of writing, all data have been collected, cytokine concentrations were evaluated and analyses are under way.

**DISCUSSION:** Results will be discussed firstly in the context of cytokine profiles that reflect the acquisition of a specific cellular memory response to the vaccine candidate according to gravidity or previous *P. falciparum* infection. Secondly, we will discuss cytokine profiles as protection markers in the context of infection, anemia and birth weight.
INTRODUCTION: Most rural communities and inmates of crowded refugee camps in Africa rely on herbs for treatment of malaria; as access to efficacious antimalarials against the fast spreading multidrug-resistant *Plasmodium falciparum* is a challenge. However, very few of these folklore herbs have been scientifically investigated and authenticated.

METHOD: Ten alcoholic and nine alcoholic extracts from *Adenia cissampeloides* Planch. ex Hook., *Anthocleista nobilis* G. Don, *Calliandra calothyrsus*, *Eleaees guineensis* Jacq., *Entandrophragma angolense* (Welw.) CDC, *Mallotus oppositifolia* (Geisel.) Mull. Arg., *Sarcocephalus latifolius* (J.E.Sm) E. A Bruce, *P. longifolia* (K1 strain) by the parasite lactate dehydrogenase (pLDH) assay.

RESULTS: Thirteen extracts (7 alcoholic and 6 alcoholic extracts) out of a total of 19 showed antiplasmodial activities with IC\(_{50}\) < 50 µg/ml. *P. longifolia* extracts exhibited the most potent activity (IC\(_{50}\) < 23 µg/ml). Three clerodane diterpenes (16-hydroxycleroda-3,13-dien-16,15-olide, 16-oxocleroda-3,13E-dien-15-16-olide, 3,16-dihydroxycleroda-4(18),13(14)Z-dien-15,16-olide), two alkaloids (Darienine and Stepholidine) and a steroid (β-Stigmasterol) were isolated from the *P. longifolia* extracts. While the alkaloids and the steroid showed weak antiplasmodial activity (IC\(_{50}\): 22 -105 µg/ml) the clerodane diterpenes exhibited significantly potent activity (IC\(_{50}\): 3 - 6 µg/ml) against the multidrug resistant malaria parasite.

CONCLUSIONS: The results have shown that 68.4% of the studied extracts possessed antiplasmodial activity against the multidrug resistant *P. falciparum* and therefore appeared to support the traditional reputation placed on these herbs as phytotherapies for malaria in Ghana. This is the first report of antimalarial property of *P. longifolia* extracts, 16-oxocleroda-3,13E-dien-15-16-olide, 3,16-dihydroxycleroda-4(18),13(14)Z-dien-15,16-olide. These clerodane may therefore serve as leads for any antimalarial drug development agenda.

P265: Change of malaria risk in Africa: a spatio-temporal analysis of two rounds of Malaria Indicator Survey data assessing the effectiveness of vector control interventions

Federica Giardina\(^1\) and Penelope Vounatsou\(^1\)

\(^1\)Swiss Tropical and Public Health Institute, Basel, 4051, Switzerland;

University of Basel, Basel, 4051, Switzerland.

BACKGROUND: Declines in malaria disease during the past five years are mainly associated with the expansion in the implementation of vector control measures like insecticide-treated nets (ITN) and indoor residual spraying (IRS). In this study, we produced spatially explicit estimates of the change in malaria risk quantifying the relative contribution of intervention strategies after adjusting for environmental and socio-economic factors.

METHODS: We analysed data collected in two rounds of Malaria Indicator Surveys (MIS) in five countries in Africa: Senegal, Rwanda, Liberia, Tanzania and Angola. To estimate the current malaria risk and the change relative to the period between the last two national surveys we used geostatistical regression. We applied Bayesian variable selection procedures to select the most relevant ITN and IRS measures in reducing malaria risk and performed spatial kriging over the study area to produce intervention coverage maps. We estimated the contribution of (or the scale up of) ITN and IRS in reducing malaria risk after adjusting for climatic and socio-economic factors assuming spatially varying effect to take into account potential interaction with endemicity levels.

RESULTS: We observed an overall decline in malaria risk in most of the countries. The use of IRS (measured as the proportion of households in sprayed in the last 10 months) was not always associated with a significant change in malaria risk. ITN was an important factor in reducing the malaria risk under different definitions of coverage. For instance, the proportion of households in the cluster with at least one ITN was identified by the variable selection procedure as the measure mostly associated with the change in malaria risk in Tanzania while the mean people-to-net ratio had the most significant effect in Angola.

CONCLUSIONS: The described methodology is useful in identifying areas where declines or increases of malaria risk occurred as well as in detecting possible changes in the geographical pattern of the disease. The effectiveness of interventions varies geographically probably because of interactions with endemicity. These maps provide a powerful visual tool for the NMCPs to identify areas where targeted strategies and resources are most needed or likely to have the greatest impact.
P266: Proteomic Analysis Of Antibodies Isolated From Children With Cerebral Malaria

E.N. Gitau 1,2, A. Waithaka 1, J. Berkley 1, B. Urban 1,3, and K. Marsh 1,2
1 KEMRI-Wellcome Trust Collaborative Programme, PO Box 230, Kilifi, Kenya; 2 Liverpool School of Tropical Medicine, Pembroke Place, Liverpool L3 5QA, UK; 3 Centre for Tropical Medicine, CCMRM, Oxford University, Oxford OX3 7LJ, UK.

BACKGROUND: In sub-Saharan Africa, neurological impairment in children with fever is a common presentation to hospital, causing significant mortality and long-term disability. Easily identifiable causes include severe malaria and bacterial meningitis, but many children have neither. The interaction between pathogens and the immune system remains incompletely understood and identifying structural differences between antibodies isolated from patients could help identify better diagnostic and therapeutic targets that could be used in patient management.

METHODS: Proteins were extracted from plasma samples from patients who had confirmed S. pneumoniae meningitis (n=3), CM (n=7) and aseptic meningitis (n=9) and separated on 10% SDS-PAGE gels followed by western blot analysis with patient plasma with confirmed bacterial meningitis, confirmed CM, malaria hyper-immune adult sera and malaria non-immune sera from a European donor. Antibodies were then isolated from the patient plasma samples and characterized using mass spectrometry.

RESULTS: Western blot results indicate that plasma from children with bacterial or aseptic meningitis have similar patterns to those blotted using hyper-immune sera while blots from children with confirmed cerebral malaria are similar to those blotted using non-immune sera.

CONCLUSION: We are now characterizing the antibodies isolated from the patient samples using mass spectrometry to identify structural differences, which may help elucidate the differences seen in western blot analysis. Results of this study could be exploited in design of therapeutic and diagnostic monoclonal antibodies.

P268: Geographic disparities in long-lasting insecticide-treated nets survivorship and physical condition after universal distribution campaign in Benin

Virgile Gnanguenon1, Roseric Azondekon1,2, Frederic Oke-Agbo1 and Martin Akogbeto1
1 Centre de Recherche Entomologique de Cotonou, Cotonou, Benin. 2 University of Massachusetts Amherst, Amherst, USA.

BACKGROUND: Mass-distribution of long-lasting insecticide-treated nets (LLINs) was conducted in Benin in 2011 as part of the national malaria control strategy. To answer the question of the best time for replacement, a monitoring tool had been implemented. Two World Health Organization indicators (survivorship and fabric integrity) were used to estimate nets durability in the field. This study presents the results of the assessment of those nets one year after their distribution.

METHODS: We used the monitoring tool in four different geographic sites where access to water for washing was either full or less. Following distribution, 500 houses were randomly selected at each site. The monitoring team visited each selected house and, verified that a replacement, a monitoring tool had been implemented. Two World Health Organization indicators (survivorship and fabric integrity) were used to estimate nets durability in the field. This study presents the results of the assessment of those nets one year after their distribution.

RESULTS: At T12, LLINs survivorship declined to 75%. Survivorship was significantly higher in northern sites than in southern sites (p<0.05). 76.06% of LLINs assessed was found with holes. The mean pH was 600. LLINs found with holes were higher (p<0.05) in locations with full access to water for washing. 40.46% of LLINs was still in good condition, 34.37% was serviceable and 25.17% need to be replaced. LLINs found in good conditions were higher (p<0.05) in location with less access to water for washing. Washing frequency and low LLINs maintenance were significant (p<0.05) predictors for loss of integrity.

CONCLUSIONS: One year post distribution, approximately three LLINs out of ten had been removed from the houses where they were hung and the number of LLINs with poor physical conditions was higher in areas with full access to water.

P269: Entomological surveillance of behavioural resilience and resistance in residual malaria vector populations

Nicodem J. Govella, Prosper Chaki, Gerry Kililea

The most potent malaria vectors rely heavily upon human blood so they are vulnerable to attack with insecticidetreated nets (ITNs) and indoor residual spraying (IRS) within houses. Mosquito taxa that can avoid feeding or resting indoors, or by obtaining blood from animals, mediate a growing proportion of the dwindling transmission that persists as ITNs and IRS are scaled up. Increasing frequency of behavioural evasion traits within persisting residual vector systems usually reflect the successful suppression of the most potent and vulnerable vector taxa by IRS or ITNs, rather than their failure. Many of the commonly observed changes in mosquito behavioural patterns following intervention scale-up may well be explained by modified taxonomic composition and expression of phenotypically plastic behavioural preferences, rather than altered innate preferences of individuals or populations. Detailed review of the contemporary evidence base does not yet provide any clear-cut example of true behavioural resistance and is therefore consistent with the hypothesis presented. Caution should be exercised before over-interpreting most existing reports of increased frequency of behavioural traits which enable mosquitoes to evade fatal contact with insecticides: this may simply be the result of suppressing the most behaviourally vulnerable of the vector taxa that constituted the original transmission system. Mosquito taxa which have always exhibited such evasive traits may be more accurately described as behaviourally resilient, rather than resistant. Ongoing national or regional entomological monitoring surveys of physiological susceptibility to insecticides should be supplemented with biologically and epidemiologically meaningfully estimates of malaria vector population dynamics and the behavioural phenotypes that determine intervention impact, in order to design, select, evaluate and optimize the implementation of vector control measures.

P270: The impact of egg-retention in gravid females of Anopheles gambiae on the fecundity, eggs development, trophic and oviposition behaviours

Renaud Govoetchan*, Martin Akogbeto*
* Centre de Recherche Entomologique de Cotonou (CREC)

BACKGROUND: The scarcity of breeding sites that characterizes droughts force gravid mosquitoes to delay oviposition and retain eggs in their ovaries. In laboratory conditions, we explored the possible consequences of egg carriage on spawning capacity, egg viability, trophic behavior and preference of breeding sites in gravid females of An. gambiae waiting for eggs laying in a context of breeding site absence.

MATERIAL AND METHODS: The study was performed with An. gambiae ss. KISUMU reared at CREC. Newly emerged females aged 5-days old were twice blood-fed and submitted to egg-laying delay following the preset modalities as immediate egg-laying versus 10 to 40-days retention. The batches of eggs laid were counted under binocular microscope including the number of embryonated eggs and then introduced into no-chlorine water to determine hatchability and emergence rates of larvae. To assess whether gravid females waiting to lay eggs continue to take blood meals, gravid specimens were blood fed every 48 hours and the blood feeding rate was recorded. Moreover, batches of gravid anopheles with 3-weeks
oviposition delay were offered 3 kinds of nest box in order to explore the choice of oviposition site.

RESULTS: Overall, the mean anopheline fecundity per brood ranges from 75.64 eggs for immediate egg-laying to 79.50 eggs for 40-days retention (RR: 1.05; 95% CI (RR): 0.97-0.97; p = 0.2031). The embryo, hatchability and emergence rates significantly decreased gradually as the retention time is extended. 53.3% of gravid females deprived of nest were able to take up to 8 blood meals and 21.4% reached 13 blood meals before dying. Egg-retention has not been identified as a factor that can change the behavior of Anopheles in their choice of oviposition site.

CONCLUSION: Egg-retention in An. gambiae has no impact on female fertility and the choice of oviposition site but influences the egg development cycle. The data recorded in our simulations are very encouraging. It is important to conduct the same study under natural conditions.

P271: The potential public health impact and cost-effectiveness of the RTS,S vaccine in Africa

Jamie T Griffin and Azra C Ghani
MRC Centre for Outbreak Analysis and Modelling, Department of Infectious Disease Epidemiology, Faculty of Medicine, Imperial College London

BACKGROUND: The RTS,S vaccine has recently been shown in phase three trials to be partially effective in preventing infection and clinical malaria in infants, and decisions will soon need to be taken on whether to recommend the vaccine for routine use.

METHODS: We use a simulation model of malaria transmission to quantify the possible reduction in morbidity and mortality, and hence the cost-effectiveness of the vaccine when delivered via the Expanded Programme on Immunization (EPI). The model uses Africa-wide data on Plasmodium falciparum prevalence, insecticide-treated net coverage and treatment use to capture malaria transmission within each country. Estimates of potential country-specific dates of introduction and coverage of the vaccine were provided by the Global Alliance for Vaccines and Immunisation.

RESULTS: In sub-Saharan Africa as a whole, we estimate that vaccination at EPI (at 6 to 14 weeks of age) would avert 150 million cases of malaria and 570,000 deaths over the period from 2017 to 2030. The number of deaths averted per 100,000 vaccinated infants varies from below 100 if the parasite prevalence is below 5%, to above 200 deaths averted in areas with moderate to high transmission. Vaccination at age 8 to 10 months would avert around twice as many cases and deaths, due mainly to the greater efficacy of the vaccine at that age. If the vaccine cost US$5 per dose, then the cost per disability adjusted life-year (DALY) gained is US$125 or US$44 with vaccination at the younger and older ages respectively.

CONCLUSIONS: Routine use of the RTS,S vaccine at EPI could have an important public health impact, and in most countries in Africa would be considered cost-effective by global standards as long as the cost is not substantially more than US$5 per dose. However in order to maintain the reductions in transmission that have occurred to date, it is important that resources for vector control are not diverted.

P272: Behaviour patterns of malaria cases investigated in five provinces of South Africa.

Mary Anne Groepe1, Ntsiene Ramalwa1,2, Allison Tatarksy1, John Nawn1, Ishen Seocharan, Mbavahelo Shandukani1, Eunice Misiani1, Devanand Moonasar1

1World Health Organization, Pretoria, South Africa, 2School of Health Systems and Public Health, University of Pretoria, Pretoria, South Africa 3South Africa Field Epidemiology and Laboratory Training Program, National Institute for Communicable Diseases of the National Health Laboratory Services, Johannesburg, South Africa 4Clinton Health Access Initiative, Boston, Massachusetts, USA, 5National Department of Health South Africa, Pretoria, South Africa 6Malaria Research Programme, Medical Research Council, Durban, South Africa.

INTRODUCTION: Epidemiological analysis has revealed that 64% of all malaria cases reported in South Africa are imported from endemic areas outside the country. The WHO classification of risk of malaria transmission, occupation and other demographic information of malaria cases can affect the effectiveness and efficiency of the malaria programme. This study aims to gain a better understanding of the behavioural patterns of high risk populations in order to improve targeting of interventions as South Africa moves toward malaria elimination by 2018.

METHODS: In this study, data was collected prospectively from April to June 2013. Participants included all malaria cases over the age of 18 years reported during the study period in five provinces: KwaZulu-Natal, Mpumalanga, Limpopo, Gauteng and North West. Data was collected from information captured during routine case investigation, and a brief questionnaire was administered to obtain data on work history and migratory patterns. Data was captured and analyzed on an Access database.

RESULTS: Preliminary findings for the study sample of 648 cases indicate that the majority of cases (83%) were between 18 and 40 years of age, of which 268 (58%) were male and 272 (42%) were female. Common home and spoken languages were Tsonga and Tshivenda. Of total cases, 107 (16%) were employed, of which 8% worked in the agriculture industry. The survey revealed that 161 (25%) of the cases had travelled across the borders, with 67 (42%) of these crossing once a year to visit friends and relatives and 32 (20%) crossing quarterly. Of the cases that crossed the border between January 2012 and January 2013, 152 (86%) had travelled by road; 66% crossed through Lebombo and 16% through the Beitbridge border. Nineteen percent of the cases indicated that South Africa was their home country and 15% that Mozambique was their home country.

CONCLUSION: While further analysis is required, it is clear from the preliminary findings that evidence on the behaviour and demographic information of malaria cases will enable the malaria programme to improve targeting of interventions at high-risk populations, in the relevant languages, at the appropriate location, and towards high-risk occupations. This intelligence will be valuable as South Africa moves toward elimination.

P273: Mapping Artemisinin Resistance: Smart Surveillance

Philippe J Guerin1,2
1WorldWide Antimalarial Resistance Network (WWARN), Oxford, UK; 2Centre for Tropical Medicine, Nuffield Department of Clinical Medicine, University of Oxford, Oxford OX3 7LE, UK

BACKGROUND: Artemisinin resistance has been detected in several loci in South East Asia. Current studies are ongoing to measure the extent of the spread. However, due to limited resources and the complexity of the methods used to characterize resistance, the number of sites remains limited. A methodology is proposed that will allow candidate sites to be chosen in an informed way.

METHODS: Using malaria endemicity and human population density estimates, along with maps of uncertainty in resistance, we aim to assess the effect of adding additional candidate sites to the dataset. The objective is to find the candidate sites that most reduce the uncertainty of resistance mapping and maximize the useful information. Using parasite clearance half-life (HL) estimates from studies conducted in the Mekong to characterize artemisinin resistance, the data are dichotomized to define the number of ‘positive’ responses as those with a HL above a cutoff. The cutoff is defined on a distribution of HLs in populations expected to be as sensitive to artemisinin as those observed in settings in Africa.

RESULTS: We develop a Bayesian geostatistical model of the proportion of individuals with a HL more than the cutoff and a predictive map is generated on a regular grid of the Mekong region. For each prediction location, probabilities of the HL being greater than the cutoff are drawn and the distribution summarized as the median of this set. The associated uncertainty maps that accompany the median maps are created by calculating the coefficient of variation.
CONCLUSIONS: The methods outlined here could play an important role in identifying where future efficacy studies should be done and inform decisions on surveillance of artemisinin resistance and elimination in the Mekong region or in region where it could emerge.

P274: Out of Pocket Treatment for Malaria-a major Cost constrain in the Fight Against malaria in less developed countries

Nazah Gwat

Malaria inflicts significant costs on households and on the economy of malaria endemic countries. There is evidence that the economic burden is higher among the poorest in a population, and that cost burdens differ significantly between wet and dry seasons. What is not clear is whether, and how, the economic burden of malaria differs by disease endemicity. The need to account for geographical and epidemiological differences in the estimation of the social and economic burden of malaria is well recognized, but there is limited data, if any, to support this argument. Malaria is considered as one of the poverty related diseases and is the number one killer disease in sub-Saharan Africa. This is due to the fact that the environments in which they live in are enabling environments which enable the breeding grounds of malaria. The greater part of money spent on health care often comes from the private pockets of those who are directly or in directly suffering from the burden of the illness concern. When planning on healthcare expenditure, it is important for governments to strive as much as possible to put in place strategies that will cut down on the out of pocket payment of the cost of health care by the population. In a country where close to 45 % of the population leave on less than a dollar per day, and where the population has to battle with this ancient Scotch –malaria on daily bases, it becomes imperative for a cost effective intervention model to be put in place that will provide acceptable and affordable standard of care as far as malaria prevention and treatment is concern. All the indirect cost involves in the treatment of those suffering from malaria has to be well analyzed in order to effectively quantify the financial burden posed by this disease on the population. The aim of this study is to reduce or if possible eliminate out of pocket payments for malaria treatment and promoting a cost-effective malaria case management.

P275: Monitoring of long-lasting insecticide treated bednets (LLINs) in Rwanda: Preliminary results of a 3 year longitudinal LLIN durability study.

Emmanuel Hakizimana1, Beatus Cyubahiro1, Alphonse Rukundo1, Allan Kalayiza2, Eric Tongren2, Mike Green2, Ray Beach2, Corine Karema2

1Malaria and other Parasitic Diseases Division, Kigali, Rwanda. 2 Presidential Malaria Initiative, Kigali Rwanda.

BACKGROUND: The proper use of long lasting Insecticide-treated nets (LLINs) is a proven effective malaria control intervention. While LLINs are thought to last for 3-5 years or more than 20 washes, the reality of net effective life in Rwanda, was found different. The LLINs have been introduced in Rwanda since 2005 with an achievement of universal coverage (1 net per 2 people) in February 2011, and 6.1 million LLINs were distributed. Meanwhile, in December 2010, a 3 year longitudinal study was initiated to monitor the net durability at 6 sites under operational conditions.

METHODS: A prospective longitudinal monitoring was conducted in 3,000 LLINs (1500 polyethylene/permethrin; 1500 polyester/deltamethrin) distributed through national campaigns. At every 6-monthly interval, 30 LLINs are sampled from each site and tested for bio-efficacy using WHO cone bioassay. A standard questionnaire was used for collection of data on the status of each coded LLIN. The fabric integrity was estimated using a Proportionate Hole Index (PHI).

RESULTS: The preliminary results showed that at the interval of 6,12,18 and 24 months following the LLIN distribution, 12.9%, 17.8%, 28.4% and 32.1% of LLINs were missing cumulatively. LLIN cone bio-efficacy decreased to an average of 84.3% at 6 months, 83.8% at 12 months, 81.1% at 18 months and dropped down to 68.2% at 24 months. The assessment of LLIN fabric integrity indicated that for a threshold of PHI>768, the LLINs required replacement after the intervals above are respectively 10.6% (polyethylene: 6.7%; polyester: 14.4%), 32.2% (polyethylene: 36.4%; polyester: 28.1%), 31.3% (polyethylene: 36.7%; polyester: 25.8%), 58.3% (polyethylene: 68.9%; polyester: 47.8%).

CONCLUSIONS: The durability monitoring indicates ineffectiveness of LLINs after 24 months. These observations highlight the need to conduct LLIN efficacy and durability studies to guide LLINs replacement and maintain effective universal coverage.

P276: Acute Renal Failure (ARF) occurring during hospitalization of patients under severe malaria treatment at the CHU of Kamenge (Centre Hospitalo - Universitaire de Kamenge)

Desire H.1, Anatole N.1,2, Petronie N.1,4

1 University of Burundi, Faculty of Medicine, CHU of Kamenge; 2 Health Healing Network Burundi; 4 Hospital of Murure.

BACKGROUND: To investigate the prevalence of ARF in severe malaria hospitalizations; analyze the impact of ARF on the prognosis of severe malaria, indicate the major factors of poor prognosis in severe malaria

METHODS: A retrospective descriptive study on 12 months and prospective on 6 months. Were selected for the study all patients hospitalized with one or more criteria of severe malaria with impaired renal function: blood creatinine > 265 Mmol.l-1 and/or oligoanuria with positive malaria parasitaemia. We asked the Complete Blood Count (CBC), the serum electrolytes, lumbar puncture and blood sugar.

RESULTS: Over the period of 18 months, 73 cases of acute renal failure (ARF) on severe malaria have been observed at the University Hospital of Kamenge. The prevalence of ARF in severe malaria cases is of 13.12%. The average age of our patients was 30.7%. The patients whose trenched of age goes from 21 to 40 years are most affected by the IRA with 67.1% of cases. The sex ratio was 3.3 for men. 75.3% of the cases were soldiers, farmers, students and pupils. The majority of cases came from Bujumbura suburbs which are the less serviced and low-income settings. The study demonstrated a delay in hospitalization on average of 5.2 days after the severe malaria. The ARF was clinically associated with a fever (86%), had jaundice (72.6%), coma with Glasgow Score <9 (32.8%), digestive disorders (64.4%), oligoanuria (69.8%) and respiratory distress (35.6%). It was noted the death rate (33.9%) and the total healing (47.1%).

CONCLUSIONS: The rate of ARF increases with the late consultation of the patients; Adult-young are the most affected; Deaths are remarkable when the severe malaria is associated to the ARF than isolated severe malaria.
P277: Impact of intermittent screening and treatment for malaria among school children in Kenya: a cluster randomised trial

Katherine E. Halliday, George Okello, Elizabeth L. Turner, Kiambo Ngaji, Carlos Mcharo, Juddy Kengo, Margaret M. Dubeck, Matthew C.H. Jukes & Simon J. Brooker

BACKGROUND: Improving the health of school-aged children can yield substantial benefits for cognitive development and educational achievement. However, there is limited experimental evidence of the benefits of alternative school-based malaria interventions or how the impacts of interventions vary according to intensity of malaria transmission. We investigated the effect of intermittent screening and treatment (IST) of malaria on health and education of school children in an area of low to moderate malaria transmission.

METHODS: A cluster randomised trial was implemented in 101 government primary schools on the south coast of Kenya, 2010-2012. The intervention was delivered to children randomly selected from Classes 1 and 5 who were followed up for 24 months. Once a school term, children were screened by public health workers using malaria rapid diagnostic tests (RDTs), and children (with or without malaria symptoms) found to be RDT-positive were treated with a six dose regimen of arteether-lumefantrine (AL). The primary outcomes were anaemia and educational achievement. Secondary outcomes included malaria parasitaemia and classroom attendance. Data were analysed on an intention to treat basis.

RESULTS: During the intervention period, an average of 88.3% children in intervention schools were screened at each round, of whom 17.5% were RDT-positive. 80.3% of children in the control and 80.2% in the intervention group were followed-up at 24 months. No impact of the malaria IST intervention was observed for prevalence of anaemia or *P. falciparum* at either 12 or 24 months or on scores of classroom attention. No effect of IST was observed on educational achievement in the older class, but an apparent negative effect was seen on spelling scores in the younger class at 9 and 24 months and on arithmetic scores at 24 months.

CONCLUSION: In this setting in Kenya, IST as implemented in this study, is not effective in improving the health or education of school children. Possible reasons for the absence of an impact are the marked geographical heterogeneity in transmission, the rapid rate of reinfection following AL treatment, the variable reliability of RDTs and the relative contribution of malaria to the etiology of anaemia in this setting.


Hamid

BACKGROUND: The on-going rapid spread of resistance to dichlorodiphenyltrichloroethane (DDT) and pyrethroid insecticides in *Anopheles gambiae* complex is a major setback to malaria interventions targeting the vector with indoor residual sprays (IRS) and insecticide-treated bed nets (ITNs). Knockdown resistance (kdr) is a target-site resistance mechanism against DDT and pyrethroid insecticides resulting from point mutations at the domain II of the voltage-gated sodium channel gene of *Anopheles gambiae* complex. Indications of DDT and pyrethroid resistance among *Anopheles gambiae s.l* in The Gambia were reported in a recent bioassay study. The aim of the present study was to investigate the underlying target-site resistance mechanism and associated kdr haplotypes in the same mosquito population.

METHOD: A combination of allele-specific polymerase chain reaction (AS-PCR), a modified high resolution melting (HRM) assay and sequencing was employed to describe the kdr genotypes in Gambian *Anopheles gambiae* populations sampled in previous bioassays. The genotypic frequencies at the kdr locus and the agreement within the assays were determined.

RESULTS: Both L1014S, kdr east and L1014F, west mutations and five kdr haplotypes were identified with a total frequency of 22% mainly in *Anopheles arabiensis* from four sites. With the modified HRM assay, four wild and one mutant kdr haplotypes were detected involving V/C (Y), T/A (W), G/T (K), C/G (S) and A/T(W) substitutions. There was a strong agreement between the HRM assay and sequencing in the detection of kdr haplotypes.

CONCLUSION: This study showed for the first time the presence of five haplotypes of kdr in wild mosquito populations in The Gambia. HRM was useful in detection of kdr haplotypes and could be applied for wide scale scanning for mutant kdr haplotypes in mosquito populations as DDT and pyrethroid-based insecticidal interventions are rolled out in large scale.

P279: School performance after six years of intermittent preventive treatment using artemisinin-based combination therapy in Mali

Hamma Maiga, Breanna Barger-Kamate, Issaka Sagara, Oumar Bila Troare, Mamadou Tekete, Intimbeye Tembine, Antoine Dara, Zoumana Isaac Troare, Modibo Diarra, Samba Coumare, Aly Kodjo, Bouran Sidibe, Aboubacrine Haidara, Nouhoum Diallo, Ogobara K. Doumbo, and Abdoulaye A. Dijemde

BACKGROUND: Previous studies showed that in areas of seasonal malaria transmission, intermittent preventive treatment of school children (IPTs) targeting the transmission season, reduced the rates of clinical malaria. The efficacy of ACTs in the context of longitudinal IPTs is poorly investigated and school performance has not been thoroughly evaluated.

METHODS: This was an open randomized controlled trial of seasonal IPT among school children aged 6–13 years in Kolle, Mali. The study began in September 2007 and completed follow-up in June 2013. Students were randomized to one of three study arms: Sulphadoxine–pyrimethamine (SP), Artesunate/Pyronaridine (AQ) or Control (C). All students received two full treatment doses, given 2 months apart during the season of high transmission from September to December. Groups were compared with respect to school performance, incidence of clinical malaria, asymptomatic parasitaemia and anaemia.

RESULTS: A total of 296 students were randomized, and retention in the study was 99.3%. Yearly grade average and success rate in the SP/AS and AQ/AS arms were (5.37; 79.1%) and (4.87; 70.5%) respectively vs. control (4.81; 68.7%) (P < 0.05). Clinical malaria incidence in the SP/AS and AQ/AS arms was reduced by 50.9% and 26.6%, respectively, vs. control (P < 0.001). There were fewer all-cause clinic visits among the children receiving SP/AS or AQ/AS (P < 0.001). The prevalence of asymptomatic parasitaemia was higher in the control group than in the SP/AS or AQ/AS (P < 0.001) groups. At the end of the transmission period, children treated with IPT showed a trend towards lower rates of anaemia (SP/AS 4.2%; AQ/AS 7.8%; Control, 12.7%; P = 0.012).

CONCLUSION: IPTs with SP/AS reduced the rates of clinical malaria, all-cause acute clinic visits and asymptomatic parasitaemia and trended towards a reduction in anaemia among school-aged children while improving markers of school performance.
P280: Effects of changing interventions on malaria incidence in a highland Kenya area of low malaria transmission

Karen E.S. Hamre1, George Ayodo2, Jackson Abuya3, Chandy C. John1,2
1Division of Global Pediatrics, University of Minnesota, Minneapolis, Minnesota, United States of America
2KEMRI-University of Minnesota Malaria Research Program, Centre for Global Health Research, Kenya Medical Research Institute, Kisumu, Kenya

BACKGROUND: Interruption of local malaria transmission is the first step towards elimination. We reported evidence of local interruption in the highland areas of Kipsamoite and Kapsissiya from April 2007–March 2008 after the Kenyan Ministry of Health (MOH) implemented widespread indoor residual spraying (IRS) in both areas in 2007 and switched to artesiminin combination therapy for first line treatment of malaria. Subsequently, the MOH concentrated malaria control efforts (focused IRS and increased insecticide treated net (ITN) distribution) on surrounding areas with higher malaria transmission. We assessed the relationship of these changing interventions to malaria incidence in the study sites.

RESEARCH QUESTIONS: Is sustained interruption of transmission in areas of low malaria transmission possible without continued malaria-specific interventions in the area, if the interventions are conducted in surrounding areas of higher transmission?

RESEARCH METHODOLOGY EMPLOYED IN STUDY: Annual demography and MOH dispensary-based clinical malaria surveillance were performed from 2003-2011, with travel assessment, ITN use and IRS treatment assessed from 2005-2011.

SUMMARY OF RESULTS: The IRS campaign implemented in the study area started in 2005 and increased to widespread (>85%) coverage in 2007 contributed to local interruption reported from March 2007–April 2008. IRS was conducted in 71% of households in 2008, 89% in 2009 and 54% in 2010. In 2009, IRS did not commence until malaria cases were already being reported. In 2011, no households were sprayed. The standard policy of distributing ITN to pregnant mothers and their newborn children was continued, but ITN use remained low (<17% for all years). Incidence of clinical malaria was low, but malaria was noted in all subsequent years (annual incidence per 1000 person-years, 2008,1.7; 2009, 7.4; 2010, 3.1; 2011, 8.5).

CONCLUSION: Interruption of malaria in areas of low transmission will likely require continued high-level IRS or ITN coverage. However, it is unclear if even these measures will sustain local interruption, if surrounding areas still have higher-level malaria transmission. Further study is required to determine if the seasonal increases in malaria in this area were due to continued infection with parasites present at the site or to infection with new parasite genotypes.

P281: Assessing Progress and Revising Strategies for Malaria Control in Zambia: 2006-2011

Mulakwa Kamulivo1, Emmanuel Chanda1, Uby dul Haque2, Mercy Mwanza-Ingwe1, Chadwick Sikaala1, CeciliaKatebe-Sakala1, Victor M. Mukonka2, Douglas E. Norris3, David L. Smith3, Gregory E. Glass1, William J. Moss3
1Ministry of Health, National Malaria Control Centre, Lusaka, Zambia;
2Copperbelt University, School of Medicine, Department of Public Health, Ndola, Zambia;
3Johns Hopkins Malaria Research Institute, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, Maryland, USA

OBJECTIVE: Evaluate the impact of increased coverage with long-lasting insecticide-treated nets (LLINs) and indoor residual spraying (IRS) on the burden of malaria in Zambia from 2006 to 2011.

METHODS: National passive malaria surveillance data from 2006 to 2011 were analyzed. A district-level, random effects model with Poisson regression analysis was used to explore the association between malaria cases and coverage with LLINs and IRS. Malaria cases and LLIN and IRS coverage were mapped to visualize the spatio-temporal variation in malaria for each year.

FINDINGS: From 2006-2011, 24.6 million LLINs were distributed and 6.4 million houses were sprayed with insecticide. Both IRS and LLIN coverage were associated with a reduction in malaria cases in Zambia, although an increase in the number of malaria cases was reported in some districts over the study period. A high burden of malaria persisted in north-eastern Zambia, whereas a reduction in the number of reported malaria cases was observed in western and southern Zambia. Coverage with LLINs was not uniformly distributed over the study period and IRS was consistently targeted to central and southern districts where malaria transmission was low.

CONCLUSION: Enhanced and targeted interventions in north-eastern Zambia where the burden of malaria remains high, as well as sustaining low malaria transmission in the south-west, will be necessary for Zambia to achieve the national goal of being malaria free by 2030.

P282: The in vitro effect of ethylacetate extract of callandra portoricensis on malaria parasite

Bello Hassan onimisi, Kasim L S
Department of medicinal chemistry /pharmaceutical chemistry Olabisi Onabanjo University Sagamu, Ogun state Nigeria,

BACKGROUND: Malaria has been a ravaging disease in Nigeria and by extension Nigeria. Despite many effort by the government of African nations, the disease is still the deadliest in the continent. In Nigeria especially when the baseline of malaria treatment has been changed for some times in the hospitals from chloroquine to artesiminin. This has not however change the course of things. It has been reduced to the bearers minimum the resistance of the organism towards the drugs. The major obstacle before Africans now is the financial obligation to meet up with the new drug. Artesiminin is a challenge that must be surmounted if the vision of health free Africa must be attain. hence the use of alternative medicine has come to the rescue.

METHOD: Malaria parasite was collected from some patients in an enclosure or hood and kept in a refrigerator for some few hours before they are used. Different doses of ethylacetate extract was prepared and used in incubating the blood samples in blood agar plates. These plates were incubated for 24-48 hours at 27 degree centigrade. The plates were collected and counted after 48 hours.

RESULTS: The result shows that at 100mg/ml and above the malaria parasites could not grow. They are inactivated. This signal that a fractionate of the extract could have a biocides or biostatic effect on the malaria parasite.

CONCLUSION: The result above suggest that a malaria lead can be extracted from callandra portoricensis and could help in further works on the drug especially in the development of the drug.


Hean, A.L.1, Coetzter,T.L.1,2,3
1WITS Research Institute for Malaria (WIRIM); 2Department of Molecular Medicine and Haematology, School of Pathology, Faculty of Health Sciences, University of the Witwatersrand; 3National Health Laboratory Service.

BACKGROUND: Malaria in its most severe form is caused by the protozoan parasite Plasmodium falciparum. Programmed cell death (PCD) has been characterised in eukaryotic organisms. A key regulator in the PCD pathway is ataxia telangiectasia mutated (ATM), a serine/threonine protein kinase with downstream effects ranging from facilitating homologous recombination repair to apoptosis. The proposed PCD pathway in Plasmodium falciparum is currently uncharacterised. Bioinformatic analysis identified a Plasmodium falciparum ATM homologue (PF3D7_0311300), annotated as a putative phosphatidylinositol kinase (P13K or PI4K) in the Plasmodium data base. The aim of the project is to characterise this Plasmodium falciparum ATM
P284: Haemoglobin loss and its association with protection at relatively low parasitaemia is influenced by a host genetic factor in semi-immune mice infected with Plasmodium berghei ANKA

Gideon K Heleebbe1, Nguyen T Huy2, Tetsuo Yanagi2, Mohammed N Shalbai2,3, Mihoko Kikuchi2,3 Mahamoud S Cherif2 and Kenji Hirayama2,3

1Department of Biochemistry and Molecular Medicine, University for Development Studies, School of Medicine and Health Sciences, Tamale, Ghana; 2Department of Immunogenetics, Institute of Tropical Medicine (NEKKEN), Nagasaki University, 1-12-4 Sakamoto, Nagasaki 852-8523, Japan; 3Global Center of Excellence, Institute of Tropical Medicine (NEKKEN), Nagasaki University, 1-12-4 Sakamoto, Nagasaki 852-8523, Japan

BACKGROUND: Human studies and our previous animal studies have shown that there are individual responses towards malarial infection even under the same malarial transmission intensity. These studies also show that individuals with low haemoglobin (Hb) are protected. We, therefore hypothesize that host genetic factors is/are influencing these differences affecting the extent to which Hb is synthesized during malaria.

METHODS: In testing this hypothesis we crossed two mice strains (Balb/c of low parasitaemia) and CBA (moderately high parasitaemic) to generate semi-immune through 6-7 cycles of infection (with Chloroquine/Pyremethamine) to generate semi-immune BALB/c (of low parasitaemia) and CBA (moderately high parasitaemic) to study the effect of infection.

RESULTS: Significant survival (>70%), mean %Hb loss (45%) and mean parasitaemia (50%) was observed in Balb/c and F1, while 0% survival, mean %Hb loss (80%) and mean parasitaemia of 15% was observed in CBA. IgG subtypes were two times higher in Balb/c and F1 than CBA. While IL1a, IL2, IL10, IL12a, IFNγ and TNFα were similar in the three mice strains, IL17 was 4.5 times higher in Balb/c and F1 than CBA. Increasing trend of cytokines levels was observed in CBA whilst a maximum cytokine level was observed at D16 (point at which recovery from parasitaemia occurs, with lowest Hb) in the Balb/c and F1. CD4+CD25+ Treg cells in CBA were similar on days measured, but lower than those of Balb/c and F1.

CONCLUSION: In conclusion, innate mechanism of Hb loss in controlling parasitaemia level, hence survival is similar in Balb/c and F1. Autoimmunity is implicated in Balb/c and F1 due to high IL17 levels. A genetic factor controlling this Hb loss in Balb/c is passed onto the F1 progeny. Further studies of F2 between the F1 and Balb/c will be informative to evaluate if these genes are segregated or further apart.

P285: Study of antibody and cellular responses to plasmodium vivax variant vir proteins during pregnancy

Pilar Requena1,2, Edmilson Rui3, Alex Umbers2, Anna Rosanas4, Maria Ome5, Dhiraj Hans5, Azucena Bardají2, Michaela Menegoni6, Myriam Arévalo-Herrera3, Norma Padilla7, M. Eugenia Castellanos8, Flor E. Espinosa9, Camila Boto-Menezès2,10, Swati Kohar9, Sanjay K. Kochar9, Dhanpat K. Kochar9, Llorence Quintó11, Sergi Sanz12, Francesca Mateo13, Peter M. Siba5, Chetan Chitnis14, Carlo Severini15, Alfredo Mayor16, Carmen Fernández-Becerra17, Stephen Rogerson18,19, H. Mueller20,21, Clara Menéndez22, Hernando del Portillo23,24, Carlota Dabó9,25

1Barcelona Centre for International Health Research (CRESIB)-Hospital Clinic, Universitat de Barcelona, Barcelona, Spain; 2Papua New Guinea Institute of Medical Research (PNG IMR), Goroka, Papua New Guinea; 3International Center for Genetic Engineering and Biotechnology (ICGEB), New Delhi, India; 4Istituto Superiore di Sanità (ISS), Rome, Italy; 5Centro Internacional de Vacunas (CIV) / Instituto de Inmunología, Cali, Colombia; 6Universidad del Valle de Guatemala (CES-UVG), Guatemala, Guatemala; 7Fundación de Medicina Tropical Dr. Heitor Vieira Dourado (FMT-HVD), Manaus, Brazil; 8Sardar Patel Medical College (SPMC), Bikaner, India; 9University of Melbourne, Melbourne, Australia; 10Liverpool School of Tropical Medicine (LSTM), Liverpool, United Kingdom; 11Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain; 12Fundación de Hematología y Hemoterapia del Amazonas (HEMOMAM), Manaus, Brazil; 13Walter and Eliza Hall Institute (WEHI), Parkville, Australia.

BACKGROUND: VIR antigens may be considered as promising candidates for a Plasmodium vivax malaria vaccine. We set out to characterize naturally acquired cellular and antibody responses to VIR proteins in pregnant and non-pregnant women heavily exposed to malaria.

METHODS: This work is part of a multicenter cohort study (Preg/vax) conducted in pregnant women in five P vivax endemic countries: Brazil, Colombia, Guatemala, India, and Papua New Guinea (PNG) funded by European Commission (under grant agreement FP7-HEALTH-201588). 2000 plasma samples collected during pregnancy and 200 plasmas collected after puerperium were included in this study. IgG levels against 5 recombinant proteins and two long synthetic peptides (PvLP1 and PVL2) covering different VIR sequences were measured by multiplex suspension array technology using the Bioplex platform. Cytokine production was assessed in peripheral blood mononuclear cells (PBMC) from women with a malaria-exposed women. PBMCs from women with a naturally acquired cellular and antibody responses.

RESULTS: Antibody responses were detected to all VIR antigens tested, with the highest responses found in PNG. The lowest levels of antibodies were found at delivery, when comparing with mid-pregnancy or post-partum. PvLP1 and PVL2 positive infection and IFN-γ in response to PvLP2, and had a significantly lower percentage of CD8* T cells producing IFN-γ than non-infected women.

CONCLUSIONS: P. vivax variant VIR proteins are targets of naturally acquired antibody and cellular immune responses. P. vivax-infected women produced less pro-inflammatory and more immuno-modulatory cytokines in response to PVL2 than non-infected women.
P286: The larval habitat choice of Anopheles gambiae s.l. on Rusinga Island, western Kenya

Manuela Herrera-Varela1,2, Jenny Lindh1, Steve W. Lindsay1, Ulrike Filling1,2,3
1School of Public Health and Tropical Medicine, London, UK; 2International Centre for Insect Physiology and Ecology, Mbita, Kenya; 3Royal Institute of Technology, Stockholm, Sweden.

BACKGROUND: New tools for attacking Anopheles gambiae s.l. are urgently required as insecticide resistance in these major malaria vectors gathers pace. The cues used by gravid mosquitoes to find an egg-laying site remain unknown, but could be used to target breeding sites or develop mosquito traps to control malaria. Here we examine if environmental factors can be used to recognize habitats in the field that are preferred oviposition sites of An. gambiae s.l.

METHODS: A case–control approach using aquatic habitats with larvae (cases) and without larvae (controls) was used to study differences in natural habitats colonised and not colonised by early instar Anopheles larvae in Rusinga Island, on the shores of Victoria Lake, Kenya. Factors evaluated include biological characteristics of the sites, zooplankton, invertebrate fauna, physical parameters, nutrients, bacteria communities and volatile chemicals released from the water. Characteristics of 120 habitats (74 cases and 46 controls) were analyzed between March and July 2012.

RESULTS: Early An. gambiae s.l. larvae were associated with turbid habitats located near to the lake shore, high abundances of late instar culicines and small crustaceans of the orders Cyclopoida and Cladocera. Invertebrate predators were common in anopheline habitats. Sites preferred by anophelines exhibited high content of phosphate and increased conductivity. Volatile chemicals released from the water headspace were less diverse and released in lower concentration from cases than from controls.

CONCLUSION: Further analyses of the chemical profiles are ongoing with the aim to identify putative oviposition chemicals. Bacterial communities were diverse but no significant associations were found with cases and controls. Results of this study challenge the understanding that An. gambiae s.l. avoids ovipositing in mature habitats heavily colonised by competitors and predators.

P287: Seasonal prevalence of malaria in a district hospital of N'Djamena, Chad

Ali H.D., Bigoga D.J.1, Kera C., Esemi L.F., Fodjo Y.A.1, Njeambosay B.A.1, Zeukeng, F.2, Seumen T.C., Leke, R.F.G.2,3
1Molecular Parasitology And Disease Vector Research Laboratory, Biotechnology Center, University Of Yaounde I. 2National Malaria Control Program Chad

BACKGROUND: In Chad, malaria still remains the leading public health issue despite the enormous effort put in place by the government to curtail this burden. The current study thus aimed at collecting baseline data on malaria from a health district of N’Djamena in order to help predict the natural dynamics of this disease and improve upon the existing control strategies.

METHODOLOGY: Capillary blood was collected from 320 individuals in two seasons (120 during the rainy season and 200 during the dry season). A portion of the blood collected was used to prepare thick and thin blood smears for Plasmodium spication and to calculate the parasite densities by microscopy while another portion was used to measure the hematocrit. A total of 53 out of 120 (44.2%) individuals were screened to be slide positive for malaria during the rainy season and 74 out of 200 (37%) were slide positive during dry season. Also, 98% of all infections were due to Plasmodium falciparum in both seasons. The remaining 2% were co-infections of P. falciparum and P. malariae. Children under the age of five carried the highest parasite load. Hematocrit results revealed a negative correlation between parasite density and anemia during both seasons.

CONCLUSIONS: This study shows that malaria is more prevalent during the rainy season than during the dry season and this should be taken into account by the NMCP while elaborating control programs for this locality.

P288: In Vivo antiplasmodial activities of Echnops kebericho Mesfin and Zingibir officinale Roscoe against Plasmodium berghei in Swiss albino Mice.

Abdissa Biruksew Hordofa1, Ahmed Zeynudin2, Lamu Golassa1, Moti Yohannes3, Asfaw Debella4, Sultan Suleiman5

Echinos kebericho Mesfin and Zingibir officinal Roscoe are traditionally used for the treatment of malaria and other ailments in Ethiopia. The root parts of Echinos kebericho Mesfin and Zingibir officinal Roscoe are claimed to have medicinal value. The objective was to evaluate in vivo antiplasmodial activities of 70% ethanol crude root extracts of Echinos kebericho Mesfin and Zingibir officinal Roscoe against Plasmodium berghei in adult Swiss albino mice.

MATERIALS AND METHODS: The 70% ethanol crude root extracts were obtained from both Echinos kebericho Mesfin and Zingibir officinal Roscoe. Oral acute toxicity test for Echinos kebericho Mesfin was determined in mice by single administration of the crude extract. The in vivo assays were done by administering mice infected with Plasmodium berghei for four consecutive daily doses of the extracts through the intragastric route following Peters’ 4-Days suppressive test.

RESULTS: The acute toxicity study showed no significant toxic effects of the extract of Echinos kebericho Mesfin on the organs of animals up to the dose level 5000mg/kg. It was observed that Echinos kebericho Mesfin (1000mg/kg/day) showed the highest antiplasmodial activity and suppressed parasitaemia by 49.53% and 34.66% at dose 500mg/kg/day. Zingibir officinale Roscoe (1000mg/kg/day) suppressed parasitaemia by 32.83%.

CONCLUSIONS: Acute oral toxicity studies showed the safety of the 70% ethanol root extract of E. kebericho Mesfin in mice with minimal sign of toxicity at higher doses. The 70% ethanol root extracts of E. kebericho Mesfin and Z. officinale Roscoe possess antiplasmodial activity as seen in their ability to suppress P. berghei in mice in a dose dependent manner. The antiplasmodial activities of these plant extracts support the ethnomedicinal studies and reason for the use of the plant material by the traditional medicinal practitioner in Ethiopia. This study thus, provides for the first time, both acute oral toxicity and antiplasmodial activities and confirms the rationale use for their application in traditional medicine for the treatment of malaria in Ethiopia.

P289: Reliability of Rapid Diagnostic Tests in The Diagnosis of Malaria amongst children in Two Communities in South West Nigeria

Roch Appollinaire Hounghin

BACKGROUND: Prompt treatment of malaria following adequate diagnosis help to reduce morbidity and mortality in children. Lack of resources and adequate manpower especially in primary healthcare facilities in many communities in Western make microscopic diagnosis which is the Gold standard difficult. Rapid Diagnostic Test Kit despite its availability and ease of use has not been incorporated into primary healthcare because of paucity of data in the region as to its effectiveness leading to over-prescription of ACTs.

METHOD: A total of 132 children age range 1-9 years, mean age of (5 years) were screened for malaria routinely with rapid diagnostic test kits (RDTs HRP 2) at the general outpatient department of OAU Teaching hospital as well as at comprehensive health centre at Imesi Ile, South West Nigeria during the world malaria day exercise 2013. Needle prick was used to collect blood sample for thick and thin smear. Giemsa stain was used before microscopic examination was carried out on the slide.
RESULTS: From a total of 132 children who had rapid diagnostic test done, only 98 of the children had both RDTs and microscopy done. A total of 28(28.6%) children tested positive while 70(71.4%) children tested negative for malaria. In the Microscopy category, 27(27.6%) children tested positive while 71(72.4%) tested negative for malaria parasite. Out of the 27 children tested positive, RDT picked 25 as positive and 2 as negative, (sensitivity = 92.6%). While out of the 71 that tested negative for microscopy, RDT picked 68 as negative and 3 as positive (specificity = 95.8%). The positive predictive value and negative predictive values are 89.3% and 97.1%.

CONCLUSION: Rapid Diagnostic Test is an effective diagnostic tool for malaria amongst children in the study population. Primary and secondary health centres in the region should adopt Rapid Diagnostic Test in malaria diagnosis before administration of ACTs to avoid unnecessary treatment.

P290: Role of human gamma-delta T cells in the instruction of the adaptive immunity against P. falciparum infection

Jennifer Howard1, Séverine Loizon2, Marianne Guenot1, Cécile Le Scanff-Terrien1, Julien Dalloens1, Maria Mamani Matsuda1, and Charlotte Behr2.1 Université Bordeaux 2, Bordeaux, France; 2C.I.R.I.D. unit, CNRS UMR 5164, Bordeaux, France.

A non-conventional T cell, the γδ T cell, is involved in the early immune response to the erythrocyte stage of Plasmodium falciparum infection. γδ T cells possess TCRs made of the γ and δ chain. A subset unique to primates and expressing Vγ9Vδ2 TCR is activated in a non-MHC dependent manner by small non-peptidic phosphorylated molecules called phosphoantigens. In vivo Vγ9Vδ2 T cells are activated and expanded during primary P. falciparum infection. Recent data from our lab has shown that Vγ9Vδ2 T cells inhibit erythrocyte stage growth in vitro by granulysin release targeting the extracellular invasive parasite stage (merozoites). Besides cytotoxic effector functions Vγ9Vδ2 T cells have been shown to be able to present antigens and induce adaptive T cell responses in the context of both infection and tumours. Our aim is to investigate if antigen presenting Vγ9Vδ2 T cells play a role in the instruction of the adaptive immune response to P. falciparum infection.

P291: Consistently high estimates for the proportion of human exposure to malaria vector populations occurring indoors in rural Africa

Bernadette Huho1,2, Olivier Briet1,2, Akilu Seyoum3, Chadwick Sikaala4, Nabile Bayoh5, John Gimnig3, Fredros Okumu6,7, Diadier Diallo4, Salim Abdulla1, Thomas Smith8,9, Gerry Killeen10,11,12

1Environmental Sciences Thematic Group, Ifakara Health Institute, Dar es Salaam, United Republic of Tanzania; 2Swiss Tropical Public Health Institute, Basel, Switzerland; 3University of Basel, Basel, Switzerland; 4Liverpool School of Tropical Medicine, Vector Biology Department, Liverpool, UK; 5National Malaria Control Centre, Chainama Hospital College Grounds, Lusaka, Zambia; 6Centre for Global Health Research, Kenya Medical Research Institute, Kisumu, Kenya; 7Centers for Disease Control and Prevention, Kisumu, Kenya; 8Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, GA, USA; 9London School of Hygiene and Tropical Medicine, Disease Control and Vector Biology Unit, London, UK; 10Centre National de Recherche et de Formation sur le Paludisme, Ouagadougou, Burkina Faso.

BACKGROUND: Insecticide-treated nets (ITNs) and indoor residual spraying (IRS) are highly effective tools for controlling malaria transmission in Africa because the most important vectors, from the Anopheles gambiae complex and the An. funestus group, usually prefer biting humans indoors at night.

METHODS: Matched surveys of mosquito and human behaviour from six rural sites in Burkina Faso, Tanzania, Zambia, and Kenya, with ITN use ranging from 0.2% to 82.5%, were used to calculate the proportion of human exposure to Anopheles gambiae sensu lato and An. funestus s.l. that occurs indoors (PI) as an indicator of the upper limit for the personal protection that indoor vector control measures can provide. This quantity was also estimated through use of a simplified binary analysis (Pi*) so that the proportions of mosquitoes caught indoors (Pi) and between the first and last hours at which most people are indoors (Pi) could also be calculated as underlying indicators of feeding by mosquitoes indoors or at night, respectively.

RESULTS: The vast majority of human exposure to Anopheles bites occurred indoors (Pi* = 0.79 – 0.97). Neither An. gambiae s.l. nor An. funestus s.l. strongly preferred feeding indoors (Pi = 0.40 – 0.63 and 0.22 – 0.69, respectively) but they overwhelmingly preferred feeding at times when most humans were indoors (Pi = 0.78 – 1.00 and 0.86 – 1.00, respectively).

CONCLUSIONS: These quantitative summaries of behavioural interactions between humans and mosquitoes constitute a remarkably consistent benchmark with which future observations of vector behaviour can be compared. Longitudinal monitoring of these quantities is vital to evaluate the effectiveness of ITNs and IRS and the need for complementary measures that target vectors outdoors.

P292: Development of a new data processing tool – making standardisation painless

Georgina Humphreys, Andrew Payne, Clarissa Moreira, Muan Hong Ng, Carol Sibley, Philippe J Guerin


BACKGROUND: Standardising heterogeneous data sets is critically important for conducting meta-analyses, but requires substantial resources. In the context of malaria clinical trials, WWARN has designed such a system and currently hosts a consolidated database of clinical malaria studies of 85,400 individual patient records.

METHODS: Data sets are submitted in any digital format. Our tool transforms diverse source datasets into a standard set of relational tables. A detailed XML configuration file defines the structure of these tables specifying what parameters are collected and their permitted ranges, normalises the units used and imposes a controlled vocabulary. A rich graphical user interface developed with Java and Google’s Web Toolkit empowers data managers to make decisions needed to transform an input data set into a standard output format. An audit trail is preserved for all transformations carried out within the tools. The tool has processed individual clinical, pharmacology and molecular patient data from trials of antimalarials. After standardisation, bespoke tools summarize and analyse the data and build detailed reports based upon an open access data management and statistical analysis plan. Companion tools are used to upload raw data, manage the standardisation process, perform initial data cleaning and visualise results.

RESULTS: Using these tools we have pooled and standardised data from complex relational databases, statistical tools such as Stata, Access, SPSS, SAS, EpiData and Excel. During the transformation, checks are performed on the validity of data such as out of range temperatures, invalid dates and times, or duplicate contradictory measurements for the same patient at the same time. After some initial data cleaning, the data transformation step can be completed in one to two hours.

CONCLUSIONS: Harmonising large amounts of heterogeneous data is a challenge, but the benefits of powerful analysis justify the resources needed. WWARN has created a robust and flexible set of curation tools that standardise data allowing statistically powerful pooled analyses. Development and validation of our tools has required considerable effort, but adaptation for other diseases could now prove a cost-effective approach.
P293: The population dynamics of *Plasmodium falciparum* parasites in the Lake Victoria Islands

**Huja C, Aman R, Culleton R, Kaneko A**

1Centre for Research in Therapeutic Sciences (CREATES), Strathmore University, Kenya; 2Institute of Tropical Medicine, Malaria Unit, Nagasaki University, Japan; 3Department of Microbiology, Tumour and Cell Biology (MTC) Karolinska Institutet, Stockholm, Sweden; 4Department of Molecular Protozoology, Osaka University, Japan

**BACKGROUND:** Malaria is responsible for extensive mortality and morbidity with the current statistics recording approximately 660,000 deaths and 220 million malaria cases globally in 2010. Today, 11 countries in Africa have embarked on malaria elimination. Although the continent is witnessing an epidemiological transition with plummeting malaria risks, the feasibility of malaria elimination in settings of high transmission tropical Africa, remains unclear. A molecular epidemiological survey aimed at generating baseline data in a malaria endemic site to reveal patterns of disease transmission across the Lake Victoria islands is currently ongoing. The contribution of inter-island human and mosquito migration in the spread of these parasites will be investigated for effective planning of malaria interventions. Following the application of malaria control measures, the possibilities of malaria resurgence by human or mosquito migration can then be taken into consideration. In addition, reductions in malaria endemicity may lead to the emergence of clusters or foci of malaria transmission. These can also be identified and control measures scaled up for effective and sustained malaria elimination.

**METHODS AND ANTICIPATED RESULTS:** Data is currently being obtained from *Plasmodium falciparum* positive blood isolates collected from 4 Lake Victoria islands and one mainland. Following DNA extraction, the genetic structure of malaria parasites obtained by comparisons between parasites obtained from each of the islands and also between the islands and the mainland, will be performed to establish gene flow patterns across these sites. This will be carried out using 8 neutral microsatellite loci randomly distributed across the parasite genome. In addition, the multiplicity of infection (MOI) using the standard polymorphic markers located in *P. falciparum* merozoite surface protein 1 & 2 (*Pfmsp1* & *Pfmsp2*) genes will also be determined. This will be done to establish the genetic diversity of *P. falciparum* infections in these regions prior to application of intervention strategy. Following intervention, the MOI will be determined again to reveal the effects of the strategy on parasite genetic diversity. Reductions in MOI levels indicate successful control measures.


**Victoria C Ibhebi, Olufemi Ajumobi, Godwin Ntabom**

National Malaria Control Programme, Federal Ministry of Health, Abuja, Nigeria

**BACKGROUND:** Malaria is endemic in Nigeria. About 97% of the population is at risk and it accounts for 25% under-5 mortality. Artemisinin-based Combination Therapy and Intermittent Preventive Treatment are current curative and preventive interventions. Clinical trials using malaria vaccine were conducted in Enugu and Jos, Nigeria. The RTS,S/AS01 coadministered with Expanded Programme on Immunization (EPI) vaccines provided modest protection against both clinical and severe malaria in young infants. Meetings were held in-country to discuss the pros and cons, in preparation for the adoption of malaria vaccine in Nigeria.

**METHODS:** Nigeria officials held Stakeholder and advocacy meetings with facilitators from MVI PATH in 2011 and 2012. Key informants were interviewed about national policy decision making processes on adopting new malaria control interventions and new vaccines in Nigeria and the readiness of the health system in adopting the candidate vaccine. The results were analyzed by content analysis.

**RESULTS:** Progress is being made with adoption of malaria vaccine in Nigeria. There was no National Immunization Technical Advisory Group/Committee for all vaccine preventable diseases in Nigeria, and no standard documented guideline for decision making process for the adoption of new vaccines. The existing public-private partnership for the adoption of Human Papilloma Virus vaccine and Expanded Programme on Immunization in Nigeria was proposed for Malaria vaccine.

Challenges foreseen with the decision-making process include large population size, weak health system, resistance to change by health staff, inadequate trained staff, high cost of implementation, budgetary deficits, inadequate cold chain and storage supply. A Ministerial memo has been sent to the National Council of Health and the Federal Executive Council.

**RECOMMENDATIONS:** A proposal presentation would be made to the Technical Working Group on Malaria and relevant stakeholders. Lessons will be learnt from neighbouring countries with functional health system. Funding will be harnessed from World Health Organization and Global Alliance for Vaccine and Immunization. A proper cost benefit analysis would be done to ascertain cost-effectiveness. High level advocacy will be carried out at all levels.

P295: Impact of Allelic Variation in P450s on Patterns of Pyrethroid Resistance in Field Populations of Major Malaria Vector *Anopheles funestus* in Africa

**Sulaiman Ibrahim, Jacob Riveron, Mark Paine, Charles Wondji**

1Vector Biology Department, Liverpool School of Tropical Medicine, UK
2Department of Biochemistry, Bayero University Kano, Nigeria

**INTRODUCTION:** Control of malaria relies heavily on the use of insecticides and resistance to insecticides in malaria vectors such as *An. funestus* is a serious threat to the success of these control tools.

**HYPOTHESIS:** Recent studies have shown that the duplicated cytochrome P450s P450a and P450b shown to confer pyrethroid resistance vary extensively in their allelic distributions across Africa and this is possibly impacting distribution of pyrethroid resistance in this specie.

**METHOD:** Polymorphism in CYP6P9a/b in resistant mosquitoes from Benin, Uganda, Malawi, Mozambique and susceptible strain (FANG) was mapped. Models of these genes were constructed using CYP3AA4-1TQN and 3D insecticides docked using GOLD. Membranes from these genes co-expressed with CPR were screened with a panel of fluorescent probes. Kinetic parameters and IC50 against ten insecticides were established with the probe diethoxyfluorescein. Metabolism assay was carried out against pyrethroids; time- and NADPH-dependent depletion and kinetic parameters determined.

**RESULTS:** Docking results predicts higher activity in alleles from Southern Africa compared with those from other regions. FANG alleles have the lowest activity. With the exception of FANG, the membranes metabolise diethoxyfluorescein very well, with the lowest Km and 50% rate of inhibition. The significant differences observed in the metabolic activity of different alleles of CYP6P9a and CYP6P9b probably explain the variation in distribution of pyrethroids resistance in Africa. Efforts are currently being put to identify causative mutations responsible for the resistance pattern and to design possible diagnostic tools to easily detect this resistance.
P296: Toxicological evaluation of combination therapy of artemisinin derivative (artemether and lumefantrine) with ivermectin in male albino rats (rattus novergicus)

Emmanuel T IDOWU1, Olubunmi A OTUBANJO1, Abdurrahmon B. TALABI2, Gideon C. ALUMBA2.
1Department of Zoology, University of Lagos, Akoka, Lagos State, Nigeria; 2Department of Zoology, University of Ibadan, Oyo State, Nigeria.

BACKGROUND: Combined therapies in the co-endemic treatment of parasitic infections are ongoing in sub-Saharan Africa. Artemisinin based Combination therapy (ACTs) is the drug of choice recommended by World Health Organization in the treatment of malaria while annual rounds of ivermectin (IVM) administration is ongoing in areas of Africa where onchocerciasis is endemic. Combined therapies pose potential drug toxicity, rare adverse events and complications.

METHODS: Behavioural biochemical and histopathological effects of artemisinin derivative ACT and IVM administered using normal and double human therapeutic doses (HTDs) in male albino rats were investigated. Rats were exposed daily to IVM for 15 days while, ACT exposure was undertaken for 3 days prior to rats sacrifice. The doses of these drugs were calculated based on the mean average weight of animals assigned to the group and administered orally. The estimations of L-asparagine amino transferase (AST), L-alanine amino transferase (ALT), alanine phosphatase (ALP), total albumin, total protein and cholesterol were determined using the automated chemistry analyzer. Histopathological alterations of liver and kidney were also assessed.

RESULTS: The result showed no significant loss or gain of body weight (p>0.05) of the animals in all the treated groups. Biochemical assessment also showed no significant alteration (p>0.05) in values of biochemical parameters including ALT, AST, ALP, Total protein, cholesterol, creatinine, total bilirubin and urea analyzed compared to the control. Histopathological damage of the kidney was minimal, however mild to moderate congestion of the liver were recorded in rats exposed to HTD of IVM and HTDs of IVM+ACT.

CONCLUSION: The findings of this study validate previous findings that co-administration of IVM+ACT is safe and the two drugs can be co-administered where the diseases are co-endemic.

P297: Transfusion Malaria: Infectivity and Multiplication of Plasmodium berghei Stored at 4°C for various time intervals.

Idowu, O.A.*, Jimoh, W; Obe V.O
Department of Biological Sciences, University of Agriculture, Abeokuta, Nigeria.

INTRODUCTION: Blood transfusion is a life-saving venture; it also poses problems if not well managed as there is the risk of transmission of blood-borne pathogens such as malaria parasite. This study was designed to document the prevalence of Plasmodium infection in human blood stored in blood bank. Infectivity and multiplication of Plasmodium berghei stored at 4°C was also assessed in albino mice.

METHODOLOGY: Human bloods stored at the blood bank were examined for malaria parasite and in-vivo multiplications of P. berghei stored at 4°C were assessed with high and low parasite inocula in albino mice.

RESULTS: Malaria parasite prevalence of 34% was observed in banked human blood examined, with parasitaemia ranging from 1 x 103 to 2.5 x 103 parasites/μl of blood. P berghei infected blood samples from albino mice stored at 4°C for different time periods were observed to initiate new infections in mice with high and low inoculums of parasites, though with varying prepatencies. Longer prepatencies were observed in blood samples stored for 28 days and mice inoculated with low inoculum. There was no significant difference in the multiplication rate of Plasmodium berghei in low and high inoculum of the parasite.

CONCLUSION: This study revealed that Plasmodium berghei retains its infectivity despite storage at 4°C for up to 28 days; this has great implications for transfusion malaria.

P298: Impact Of Malaria Rapid Diagnostic Tests On Artemisinin Combination Therapy Prescription Patterns In Selected Primary Health Care Centres In A South Western Nigerian State.

O Ige

BACKGROUND: In the era of valuable and costly Artemisinin Combination Therapy (ACT) for malaria, it has been recommended that ACTs be restricted to only those with confirmed malaria diagnoses. The potential benefits of Rapid diagnostic tests (RDTs) on anti-malarial consumption have been demonstrated in some clinical trials. It is however yet to be seen if the introduction of RDTs in Nigeria has achieved the desired goal of reducing ACT consumption. This paper assesses the impact of a state-wide roll-out of RDTs on ACT prescription in a South western Nigerian State.

METHODS: ACT prescribing patterns for febrile patients were compared pre-RDT introduction and post-RDT implementation period in 106 supported primary health care facilities. Routine data from the national malaria control programme monthly facility summary forms were extracted for three months pre and post the RDT intervention and compared using a ‘before and after’ design.

RESULTS: RDT testing rates for fever patients revealed no trend, mean testing rate was 64.5% among patients seen in the post RDT period. Mean test positivity rate was 70.6% which equalled a proportional morbidity rate of 45.3% of all fever cases. ACT treatment to confirmed case ratio was consistently above the expected value of one and the ratio of treatment to tested patient exceeded one (mean ratio of 1.1) for the three month post RDT period. The absolute doses of ACTs prescribed increased remarkably after the introduction of RDTs and ACTs revealing an extra utilization of 14,199 ACT doses, 5534 (±179.29) versus 10267 (±2452.59) [p<0.001]. Relative Risk was 1.71 (1.33-2.25).

CONCLUSION: There is notable non-adherence to RDT results with increase in ACT prescriptions after the initial introductory period for RDTs. This over reliance on ACTs for the management of non-malaria illness would compromise gains from reducing malaria morbidity and mortality and needs to be addressed urgently.

P299: Integrated mosquito larval source management reduces larval numbers in two highland villages in western Kenya

Imbahale, S.Susan1,2,*, Githeko, Andrew3, Mukabana, W. Richard4 and Takken, Willem5
1Laboratory of Entomology, Wageningen University, P.O. Box 8031, 6700 EH Wageningen, The Netherlands; 2Kenya Medical Research Institute, Centre for Global Health Research, P.O. Box 1578–40100, Kisumu, Kenya; 3International Centre of Insect Physiology and Ecology, P.O. Box 30772 – 00100-GPO, Nairobi, Kenya; 4School of Biological Sciences, University of Nairobi, P.O. Box 30197– 00100 GPO, Nairobi, Kenya; 5School of Pure and Applied Sciences, Technical University of Kenya, P.O Box 52428–00200, Nairobi, Kenya

BACKGROUND: In western Kenya, malaria remains one of the major health problems and its control remains an important public health measure. Malaria control is by either use of drugs to treat patients infected with malaria parasites or by controlling the vectors. The most commonly applied control strategies rely on pyrethroids and target only indoor resting mosquitoes. However, due to development of resistance to the commonly used pyrethroids, targeting the aquatic stages can complement well with existing adult control strategies.

METHODS: Larval source management (LSM) of malaria vectors was examined in two villages i.e. Fort Ternan and Lunyere, with the aim of testing strategies that can easily be accessed by the affected communities. Intervention strategies applied include environmental management through source reduction (drainage of canals, land levelling or by filling ditches with soil), habitat manipulation (by provision of shading from arrow root plant), application of Bacillus thuringiensis var israelensis (Bti) and the use of predatory fish, Gambusia affinis. The abundance of
immature stages of Anopheles and Culex within intervention habitats was compared to that within non-intervention habitats.

RESULTS: In Fort Ternan no significant differences were observed in the abundance of Anopheles early and late instars between intervention and non-intervention habitats. In Lunyere, the abundance of Anopheles early instars was fifty five times more likely to be present within non-intervention habitats than in habitats under drainage. However, late instars had 89% and 91% chance of being sampled from non-intervention rather than habitats under drainage and those applied with Bti respectively.

CONCLUSION: Most habitats were either man made or arose due to human activities. Therefore involvement of community members in control programs is crucial in the long term once they understand the role they play in malaria transmission. The proposed LSM strategies target outdoor immature mosquitoes, hence can complement well with measures that target indoor resting vectors.

P300: Community involvement for malaria elimination in Ruhuha Sector: application of an open space methodology
1Chantal Ingabire, 2Jane Alaii, 3Emmanuel Hakizimana, 4Fredrick Kateera,
5Ingmar Nieuwold, 6Karsten Bezoijjen, 7Leon Mutesa, 8Bart Van Den Borne.
1Department of Health Promotion, Maastricht University, the Netherlands;
2Family Health International, Nairobi, Kenya; 3Department of Vector Control, National Malaria Program, Rwanda Biomedical Center, Rwanda; 4Academic Medical Center, Amsterdam, the Netherlands; 5100 Village, Amsterdam, the Netherlands; 6Department of Health Research, Rwanda Biomedical Center, Rwanda

BACKGROUND: Despite the significant reduction of malaria transmission in Rwanda, Ruhuha sector is still a highly endemic area for malaria transmission. Horizontal participatory approaches such as Open Space have been deployed to explore local priorities, stimulate community engagement the local community, enabled them exploring issues related to malaria in the area and the approach was deemed to be used to look for solutions. The latter seemed suitable for local community if one aims at sustainable malaria elimination gains.

CONCLUSION: This bottom up approach was found useful in engaging the local community, enabled them exploring issues related to malaria in the area and the approach was deemed to be used to look for solutions. The latter seemed suitable for local community if one aims at sustainable malaria elimination gains.


Inyama, P.I., Samdi, L., 1 Nsa, H., Iwuchuku, N., Kolyada L. and Dereje, D.
1PMI | AIRS Project, Abt Associates, Lafia, Nigeria; 2Nigerian Institute of Medical Research, Maiduguri Outstation, Borno State; 3Abt Associates, Bethesda, USA

BACKGROUND: The President’s Malaria Initiative | Africa Indoor Residual project (PMI | AIRS), IRS 2 Task Order 4, executed the year 2 spray operation in Nasarawa Eggon and Doma Local Government Areas (LGA) of Nasarawa State, Nigeria. The objectives of the program being the reduction of malaria associated morbidity and mortality, a total of 62,592 structures were sprayed. To measure the impact of the IRS program on the malaria vectors the proportion of parous mosquitoes in the vector population was determined before and after Indoor Residual Spraying.

METHODS: Nine hundred (900) female Anopheles gambiae s.l. specimens drawn from a pool of 2,123 Female Anopheles mosquitoes captured by Human Landing Catches from three LGAs of Nasarawa Eggon, Doma (intervention areas) and Lafia (Control) of Nasarawa State Nigeria were dissected using WHO-recommended techniques for parity. The degree of coiling of ovarian tracheoles was observed pre-IRS intervention in March 2013 and post IRS intervention May 2013.

RESULTS: Of the 900 ovaries of An. gambiae s.l. dissected before intervention, the parity rate of An. gambiae s.l. was 71.43% in Nasarawa Eggon, 76.70% in Doma and 77% in the control area. After IRS in May 2013, it was found that the parity had declined dramatically to 17.69% in Nasarawa Eggon, 27.98% in Doma (p <0.05) while in the control area (Lafia) Parity remained as high as 68 percent in the month of May.

CONCLUSION: This study has shown a reduction in the longevity of Anopheles mosquitoes and their ability to transmit malaria as evidenced by the presence of less parous mosquitoes after Indoor Residual spraying than before spraying when compared to areas that were not sprayed (Lafia). The Federal Ministry of Health plans to reduce malaria morbidity and mortality by 50% at an affordable cost by scaling up Indoor Residual Spraying (IRS). This study shows the need for a scaling up of IRS across Nigeria.

P302: Assessment of Capacity for Parasitological Diagnosis of Malaria in Primary and Secondary Health Facilities in Nigeria

Oladipupo Ipadeola1, Abidoun Ojo1, Bolatito Ayienigba1, Uwem Inyang1,
Elizabeth Streit2, Abba Umar3
1PMI/MAPS, Nigeria; 2PMI/USAID, Nigeria; 3Malaria Consortium, Africa Regional Office, Uganda

BACKGROUND: The Nigeria malaria treatment guideline recommends parasitological testing of all fever cases before administration of ACT in line with the WHO guideline. However, findings from the 2010 Nigeria Malaria Indicator Survey show that only 5.4% of fever cases were tested for malaria before treatment. This study presents the baseline finding of assessment of malaria parasitological diagnosis capacity in selected Health Facilities (HF) in 5 President Malaria Initiative (PMI) supported states in Nigeria.

METHODS: A cross sectional study was carried out in 2012 in Benue, Ebonyi, Nasarawa, Oyo and Zamfara. These states have a total of 96
Local Government Areas and about 384 HFs receiving support from PMI. A total of 186 HFs made up of one primary and one secondary public HF per LGA was purposively selected for the study. Data was collected by trained laboratory scientists through key informant interview using a semi-structured questionnaire. Laboratory, facility management and/or clinical staff of selected HFs were interviewed. Data entry and analysis was performed using SPSS.

RESULTS: Out of 186 HFs assessed, 17.2%(16 of 93) of primary and 17.2%(16 of 93) of secondary HFs conducts mRDT, 35.5% of primary and 63.4% of secondary HFs conducts microscopy while about 26.9% of both levels of facilities conducts RDTs and Microscopy. About 67.7% of the secondary HFs had functional microscope, 23.7% performed specie identification while 25.8% performed parasite count. Ninety-six percent present diagnosis report using the plus system. Of the 40S laboratory scientist/technicians in the selected facilities, 12% had been trained on RDT; 14% on malaria microscopy. Over 50% of the HFs reported stockout of malaria diagnosis commodities lasting more than seven days within the last three months. Job aides for laboratory diagnosis were available in only 22% of the facilities. Slide validation and quality assurance for malaria diagnosis was non-existence and 47% used standardized form to document and report result.

CONCLUSIONS: Poor individual and institutional/infrastructure capacity for malaria parasitological diagnosis is a big gap that needs to be filled in Nigeria health facilities. Intervention for malaria control should focus on strengthening malaria parasitological diagnosis for successful implementation of the national policy.

P304: Prevalence of Malaria and Adherence to RDT Test Results among Customers Receiving Treatment for Malaria at Private Formal and Informal Outlets in Nigeria

Jennifer Anyanti1, Jenny Liu2, Chinwolke Isiguzo2, Ernest Nwokolo1, Anna De La Cruz2, Eric Schatzkin3, Sepideh Modrek2, Chizano Uuju3, Dominic Montagu3

1Society for Family Health, Nigeria; 2University of California, San Francisco, Global Health Group; 3Stanford University

BACKGROUND: Results from small-scale studies suggest lower prevalence of malaria in parts of Nigeria. Rapid diagnostic tests for malaria provided at privately owned formal and informal drug outlets may aid in improving diagnosis for malaria and reduce overtreatment using first-line artemisinin combination therapy (ACT) drugs. The study aims to assess malaria prevalence and examine adherence to RDT results among adult purchasers of antimalarials at private drug shops which are the main sources of care for management of uncomplicated malaria in Nigeria.

METHODS: In urban and peri-urban areas in Oyo State, a survey was conducted for participants exiting either a Patent Medicine Vendor’s shop or a pharmacy. A follow-up phone survey was conducted four days after to assess self-reported treatment adherence. At enrollment, the eligible participant was offered a free mRDT performed by a nurse, after which a detailed survey and inventory of drugs purchased was conducted. Contact information was collected to facilitate later follow-up.

RESULTS: Of the 427 participants who sought malaria treatment in the 53 enrolled outlets, only 18 participants (4%) tested positive for malaria using RDTs. Only 2% of participants reported to have ever had an RDT; 92% reported they figured out they had malaria by themselves. Among participants who had RDT-negative results and were successfully followed-up by phone, 72% did not take the antimalarials they purchased at the drug shop. Of those that were positive for malaria, 75% took the free course of ACTs given to them by the study nurse. Over 97% of study participants reported they felt better during the follow-up survey.

CONCLUSION: There was a high degree acceptance of test results by participants. Deployment of RDTs among private formal and informal outlets for malaria diagnosis should be further explored to improve malaria case management in Nigeria.

P303: Declining burden of malaria over two decades in a rural community of Muheza district, North-eastern Tanzania

Deus S Ishengoma1, Bruno P Mmbando1, Method D Segeja1, Michael AlIfrangisi, Martha M Lemnege1 and ib C Bygbjerg2

1National Institute for Medical Research, Tanga Medical Research Centre, P.O. Box 5004, Tanga, Tanzania; 2Centre for Medical Parasitology at the Department of International Health, Immunology and Microbiology, University of Copenhagen and Department of Infectious Diseases, National University Hospital (Rigshospitalet), Copenhagen, Denmark

BACKGROUND: The recently reported declining burden of malaria in some African countries is attributed to different interventions; although in some areas, these changes started before implementation of major interventions. This study assessed the trends of malaria burden for 20 years in Magoda and for 15 years in Mmapayu village of Muheza district, North-eastern Tanzania; in relation to different interventions as well as changing National control policies.

METHODS: Blood smears for detection of malaria parasites by microscopy were collected during repeated cross-sectional surveys involving individuals aged 0 – 19 years. Prevalence and density of Plasmodium falciparum infections and other indices of malaria burden (prevalence of splenomegaly and gametocytes) were compared across infections and other indices of malaria burden.

RESULTS: In Magoda, the prevalence of P. falciparum infections initially decreased between 1992 and 1996 (from 83.5 to 62.0%), stabilized between 1996 and 1997, and further declined to 34.4% in 2004. A temporary increase between 2004 and 2008 was followed by a progressive decline to 7% in 2012, i.e. -10-fold decrease since 1992. In Mmapayu (from 1998), the highest prevalence was 81.5% in 1999 and it decreased to 25% in 2004. After a slight increase in 2008, a steady decline followed, reaching <5% from 2011. Bed-net usage was high in both villages from 1999 to 2004 (≥97%) but it decreased between 2008 and 2012 (range, 28 to 68%). After adjusting for the effects of bed-nets, age, fever and year of study, the risk of P. falciparum infections decreased by ≥97% in both villages between 1999 and 2012 (p<0.001). The prevalence of splenomegaly (≥40% to <1%) and gametocytes (23% to <1%) also decreased markedly in both villages.

CONCLUSIONS: The burden of malaria declined remarkably between 1992 and 2012 and the initial decline (1992 – 2004) was possibly due to the deployed interventions while the steady decline observed from 2008 (with low bed-net coverage) suggests that other factors contributed to these changes. These results provide evidence that could potentially lay the foundation for exploring opportunities for elimination of malaria in the region.

P305: Identification of novel HDAC inhibitors against the malaria parasite, Plasmodium falciparum

Nabila Ismail, Pieter Burger, Abraham i. Louw and Lyn-Maree Birkholtz
Department of Biochemistry, University of Pretoria, Private Bag x20, Pretoria

BACKGROUND: The malaria parasite, Plasmodium falciparum, undergoes a complex developmental cycle, which requires strict regulation of the cell cycle and gene expression simultaneously. One of the regulatory mechanisms involved is epigenetic; through the modification of histones and chromatin. The proteins involved in these processes are thus ideal anti-malarial drug targets, as inhibition of such proteins would have consequences on gene expression within P. falciparum parasites, ultimately leading to parasite death. One such family of proteins is the histone deacetylase (HDAC) family, which is the focus of this work.

METHODS: Three histone isolation methods were compared and used to isolate histones for mass spectrometry analysis, in order to detect the post-translational modifications (PTMs) on the lysine and arginine residues of Plasmodium histones. Following this, treated histones were investigated to determine whether mass spectrometry techniques could
be used to identify the effect of HDAC inhibitors on histone PTMs. *In silico* screening of the MMV Malaria Box against PfHDAC1 was used to identify 10 compounds which were putative HDAC inhibitors. These compounds were tested for their inhibitory activity against *P. falciparum* proliferation, after which they were tested in an adapted HDAC assay to confirm their inhibitory activity.

**RESULTS:** The three histone isolation procedures were successful for obtaining high protein yields from which over 200 modifications on the eight *Plasmodium* histone protein variants could be detected. Ten MMV compounds were identified as hits from *in silico* screening and of these, four significantly inhibited parasite proliferation. Moreover, at least two of the compounds (100 μM) significantly decreased *PfHDAC1* activity, comparable to that of the known HDAC inhibitor, SAHA.

**CONCLUSION:** This work involves the adaptation of methods which can be used for the identification of HDAC inhibitors and their effects. At least 2 compounds with drug-like properties were shown to have HDAC inhibiting effects. The use of such inhibitors against *P. falciparum* could be promising as the HDAC family is one of many attractive epigenetic drug targets.

**P306: Msp3 genotypes diversity in malaria symptomatic and asymptomatic children under five years living in malaria seasonal setting of Burkina Faso.**

**Issiaka Soulama1, Edith Bougouma1, Amidou Diarra1, Souleymane Sanon1, Alfred Tiono2, Alphonse Ouédraogo1, Jean Baptiste Yaro2, Éspérance Ouédraogo1, Adamou T. Konaté1, Issa Nébih Ouédraogo1, and Sidoniiom B. Sirima12**

1Centre National de Recherche et de Formation sur le Paludisme, Ouagadougou, Burkina Faso; 2Groupe de Recherche Action en Santé, Ouagadougou, Burkina Faso

**BACKGROUND:** Most of the *P. falciparum* merozoite surface antigens have been shown to elicit polymorphic regions. Like the sequences of several other malaria surface proteins, MSP3 sequences can be divided into 2 allelic families: the K1 and 3D7 types, named after *P. falciparum* lines with these allelic types. Given the importance of MSP3 as a vaccine candidate, little is known about the relative importance on the natural distribution of these two parasites allelic families in malaria seasonal setting as well as their association to clinical malaria profile. The present study was designed to analyze the overall prevalence’s of the two msp3 allelic forms substantially increases the complexity of *P. falciparum* as a vaccine. However, there is some indication that the inclusion of 2 allelic families suggests that it may be better to include 2 allelic forms of MSP3 in a vaccine. However, there is some indication that the inclusion of 2 allelic forms substantially increases the complexity of a vaccine, especially and probably may not be sufficient to overcome antigenic diversity.

**METHOD:** Cross sectional surveys was conducted in 2008 during malaria transmission season. Blood filter papers were collected from 228 and 199 southern region of Burkina Faso.

**RESULTS:** The prevalence of msp3_ K1 (43.6 %) and msp3_3D7 (49.88 %) was associated with the age of the infected children. In contrast the msp3_ K1 alleles were statistically more prevalent in symptomatic malaria cases in children under five year living in the southern region of Burkina Faso. In contrast the msp3_ K1 alleles were statistically more prevalent in symptomatic malaria cases in children under five year living in the southern region of Burkina Faso. In contrast the msp3_ K1 alleles were statistically more prevalent in symptomatic malaria cases in children under five year living in the southern region of Burkina Faso.

**CONCLUSIONS:** These results showed that the msp3 *P.falciparum* alleles may play a role in malaria pathogenicity. The comparable prevalence of two msp3 allelic families suggests that it may be better to include 2 allelic forms of MSP3 in a vaccine. However, there is some indication that the inclusion of 2 allelic forms substantially increases the complexity of a vaccine, especially and probably may not be sufficient to overcome antigenic diversity.

**P307: Isolation, Fractionation and Evaluation of the Antiplasmodial Properties of Phyllanthus niruri Resident in its Chloroform Fraction.**

**Obidike Ifeoma1-2, Okhale Samuel2, Aboh Mercy Iyoho2, Salawu Olukwanyinsola Adeola1**

1Department of Pharmacology and Toxicology, National Institute for Pharmaceutical Research and Development, P.M.B. 21, Abuja, Nigeria; 2Combi Chem Bio Resource Centre, Organic Chemistry Division, National Chemical Laboratory, Pune 411008 India; 3Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development, P.M.B. 21, Abuja, Nigeria; 4Department of Microbiology and Biotechnology, National Institute for Pharmaceutical Research and Development, P.M.B. 21, Abuja, Nigeria.

**BACKGROUND:** The increasing burden of malaria has contributed significantly to poverty, decreased productivity and slow economic growth in malaria endemic regions. In 2010 alone, an estimated 655 000 cases of malaria-associated deaths were recorded, especially in very young African children. This trend is attributed mainly to growing resistance of the Plasmodium parasite to drugs like chloroquine and sulphadoxine/pyrimethamine. The dried aerial parts of *Phyllanthus niruri* have been used to stimulate appetite and to treat malaria in Thailand and the West Indies. Our study is aimed at investigating the antiplasmodial activities of *P. niruri* methanol extracts and fractions, to identify the most active fractions using mouse models of infection.

**METHODS:** *P. niruri* methanol extract and its chloroform, ethanol and aqueous portions were tested against chloroquine-sensitive *Plasmodium berghei* in early, established and repository models of infection using Knight and Peter’s 4-day suppressive model, Ryley and Peters curative model and Peters prophylactic model respectively.

**RESULTS:** Chemosuppression of parasitaemia (37.65 %–50.53 %) was elicited by 100-400 mg/kg (b.w) of ME. At doses of 100 mg/kg b.w., the chloroform fraction (F1) significantly (P<0.01) suppressed parasitaemia by 85.20 %, while ethanol and aqueous fractions (F2 and F3, respectively) elicited 67.06 % and 51.8 % chemosuppression. The most active fraction, F1 was selected for further antiplasmodial screening. In established infection, ME reduced parasitaemia (15.81 % - 62.96 %) while F1 significantly (P<0.01) reduced parasitaemia (44.36 % - 90.48 %), with effects comparable to that of chloroquine (96.48%). The prophylactic antiplasmodial activity of ME (92.5 % suppression) was also significant (P<0.01) and was more effective than pyrimethamine (85.00 %). Additionally, cell membrane integrity of non-parasitized erythrocyte incubated with 125 – 500 mg mL⁻¹ F1 was maintained.

**CONCLUSIONS:** The findings indicate the antiplasmodial efficacy of *P. niruri* methanol extract, and the localization of this effect in its chloroform fraction.
METHODS: Three histone isolation methods were compared and used to isolate histones for mass spectrometry analysis, in order to detect the post-translational modifications (PTMs) on the lysine and arginine residues of Plasmodium histones. Following this, treated histones were investigated to determine whether mass spectrometry techniques could be used to identify the effect of HDAC inhibitors on histone PTMs. In silico screening of the MMV Malaria Box against pHDAC1 was used to identify 10 compounds which were putative HDAC inhibitors. These compounds were tested for their inhibitory activity against P. falciparum proliferation, after which they were tested in an adapted HDAC assay to confirm their inhibitory activity.

RESULTS: The three histone isolation procedures were successful for obtaining high protein yields from which over 200 modifications on the eight Plasmodium histone protein variants could be detected. Ten MMV compounds were identified as hits from in silico screening and of these, four significantly inhibited parasite proliferation. Moreover, at least two of the compounds (100 μM) significantly decreased P.fHDAC1 activity, comparable to that of the known HDAC inhibitor, SAHA.

CONCLUSION: This work involves the adaptation of methods which can be used for the identification of HDAC inhibitors and their effects. At least 2 compounds with drug-like properties were shown to have HDAC inhibiting effects. The use of such inhibitors against P. falciparum could be promising as the HDAC family is one of many attractive epigenetic drug targets.

INTRODUCTION AND BACKGROUND: The adverse impact of Malaria on health and socio-economic structure of the affected countries around the globe is a major concern. Traditional medicinal system of Ayurveda is successfully used for years by the practitioners to treat patients of fever similar to malaria. We investigated in-vivo anti-plasmodial activity of aqueous extracts of plants selected based on the symptomology mentioned in Ayurveda.

METHODOLOGY: The aqueous extracts of Holarrhena antidysentrica (Kutaja) and Azadirachta indica (Neemb) for their antiplasmodial potential in Plasmodium berghei infected mice was assessed using Peters four day suppressive test. Both the extracts were administered at 2 dose levels, full dose (1000mg/day) and minimized dose (200mg/ day). 10 P.bergiei infected RBCs were injected on day ‘0’ and treated from day ‘0’ till day ‘3’ post-infection. Tail blood smears were collected, giemsa stained and analyzed. The mice were observed for survival and parasitemia was assessed till 50% of mice in control survived.

RESULT: It was observed that the % parasitemia increased gradually in all the groups, with maximum in control group (Day 3-35, Day 9-46.98) and minimum in Chloroquine arm (Day 3-14.06, Day 9-19.92). The % parasitemia was compared using Mann-Whitney U test depicting that all test groups exhibited reduction in parasitemia as compared to control (p-value< 0.002 for all groups). These groups showed similar % survival as Chloroquine.

CONCLUSION: The present investigation demonstrated the anti-plasmodial effects of Holarrhena antidysentrica and Azadirachta indica, which are two most commonly used medicinal plants in Ayurved for treatment of fever.
control group. Luteinizing Hormone and Follicle Stimulating Hormone levels significantly increased at 70 mg/kg, testosterone level significantly increased at 35 and 70 mg/kg. Histopathology revealed mild congestion and oedema at the interstitium at all doses in testes. The epididymis showed enlarged interstitium, few fibrocytes and inflammatory cells.

CONCLUSIONS: Free radicals produced by artemisinin were able to overwhelm the activities of the antioxidant defense system of the testes, adversely affected sperm parameters and hormonal profile at all the doses investigated. Recommendation is that drug containing artemisinin must be taken with cautions.

P312: Randomised village-scale evaluation to compare the efficacy of alternative insecticides (Chlorfenapyr SC (240g ai/l) at 2 different doses (150 mg/m2 and 250 mg/m2), Pirimiphos-methyl CS (300g AI/L), Pirimiphos-methyl EC (500gAI/L) with DDT for indoor residual spraying for malaria vector control in The Gambia.

Musa Jawara1, Elhadji kaba Sylla1, Mathurin Ditta1, Lamin Jarju2, Balla kande2, Margaret Pinder1, Adam Jagne Sonko2 and Umberto D’Alessandro1
1 Medical Research Council Unit, The Gambia, Atlantic Boulevard, P. O. Box 273, Fajara NR Banjul The Gambia; 2National Malaria Control Programme (NMCP), Ministry of health, The Gambia; 3Département de Biologie animale, Faculté des Sciences et Techniques, Université C.A. Diop, Dakar, Sénégal

BACKGROUND: Malaria transmission in the Gambia is highly seasonal, occurring mainly during the rainy season (June-November). Since 2005, there has been a renewed interest in large-scale IRS programs in Sub-Saharan Africa (SSA), with 25 out of 42 malaria endemic countries including IRS in their national strategy for malaria control. However, IRS campaigns are threatened by the development of resistance to DDT and pyrethroids, and therefore a more effective, longer acting and user-friendly alternatives to the insecticides mostly used for IRS throughout SSA, including The Gambia, must be used. Recommendation is that drug containing artemisinin for IRS an alternative to DDT.

METHOD: Pirimiphos-methyl, an organophosphate compound, is among 12 insecticides recommended by the World Health Organization (WHO) for IRS. A new formulation of micro-encapsulated pirimiphos-methyl (CS) having 300 g AI/L has been developed as well as a new compound chlorfenapyr, an insecticide which belongs to pyrrole class and has shown promise for the control of agricultural pests and could be an alternative to DDT. This is a phase III randomised village scale trial (sponsored by the WHO Pesticide Evaluation Scheme, WHOPES) aiming at determining the residual effect of formulations of pirimiphos-methyl CS, pirimiphos-methyl EC and Chlorfenapyr SC (at 2 doses) and their impact on local malaria vectors in The Gambia compared with DDT. In addition safety and acceptability of the insecticidal treatment has also been assessed by the local community and their perception of its effect.

RESULTS: The results of these trials are being analysed and will be discussed to help inform the Gambian Medical and Health Department and other African Departments of Health of the potential benefits of having for IRS an alternative to DDT.

P313: Agricultural activities and epidemiology of malaria in Soudano-Sahelian zone in Cameroon

Jean

BACKGROUND: Malaria is a major public health problem in Cameroon. Unlike in the southern forested areas where the epidemiology of malaria has been better studied prior to the implementation of control activities, little is known about the distribution and role of anophelines in malaria transmission in the northern areas.

METHODS: We have comparatively studied the dynamics of malaria transmission in the villages of Mokolo-Douvar located in the rural area with traditional agriculture and Gounougu irrigated rice area, in 2007 August and November, 2009 May and October, to assess vectors biting habits, and malaria inoculation rate and malaria parasite prevalence in cohort of children from 0 to 15 years. Mosquitoes captured by landing catches on volunteers and by pyrethrum spray collections were identified morphologically. Species of the Anophelidae gambiae complex were identified using the polymerase chain reaction (PCR). Mosquito infectivity was detected by the enzyme-linked immunosorbent assay and PCR. Malariometric indices (plasmodic index, parasite species prevalence) were determined in three age groups (<5 yrs, 5-9 yrs, 10-15 yrs).

RESULTS: A total of 5961 Anopheles were collected. Seven Anopheles species were identified: Anopheles gambiae s.s., Anopheles arabiensis, Anopheles funestus, Anopheles pharoensis, Anopheles rufipes, Anopheles ziemanni and Anopheles squamosus. A. arabiensis was the major species (56.2%) and the main malaria vector in both study sites, followed by A. funestus (32.6%). Malaria transmission was high in the irrigated area of Gounougu (1.42 infection bites per man per night) whereas in the non-irrigated zone of Mokolo-Douvar, it was below detection level during the rainy season (0,245 ib/h/n). In Gounougu, a total of 655 children were examined. The mean plasmodic index was 21.1%. Two Plasmodium species were identified: P. falciparum and P. malaria P. falciparum was the major species (94.9%).

CONCLUSION: Our findings confirm that changes in irrigated rice agriculture influence malaria transmission dynamics, and call for control measures that are readily adapted to local eco-epidemiological settings.

KEY WORDS: Entomological inoculation rate, Anopheles, Plasmodium species, Plasmodic index, Cameroon.
treatment failures and high in vitro IC₅₀ for artemisinin, related compounds and partner drugs in some subjects. We therefore expect in this study, subjects will harbor parasite with a wide range of clearance rates. Our hypothesis is that parasite genotype correlate with parasite clearance rates. We also expect archived parasites collected at baseline will contain different baseline genetic profile from those parasites obtained the in vivo efficacy study. The success of this study is predicated on assumption that genetically indistinguishable parasites (multicus parasite genotypes) infecting >1 person will be identified.

CONCLUSION: Tracking of genetically determined artemisinin resistance in P. falciparum is critical in monitoring emergence/spread of resistant parasites in Kenya. Findings will inform authorities to develop containment strategies as artemisinin resistance emerges in Kenya.

P315: A PCR Method for Estimating Clonal Dynamics of Plasmodium falciparum

Josaphat N Nyataya, Beth K Mutai, and John N Waitumbi
Walter Reed Project, Kenya Medical Research Institute, Kisumu, Kenya

BACKGROUND: P. falciparum clonal assessment is traditionally done by polymerase chain reaction of MSP-1, MSP-2 and glurp genes of P. falciparum followed by alleleic discrimination by gel electrophoresis. This method has limitations such as subjectivity of size scoring and inability to quantify the alleles. Due to its high resolution, capillary electrophoresis (CE) can resolve PCR fragments to one base pair and when the amplicons carry a fluorescent label, alleles have two other parameters: height and area. In this report, we present an optimized method for estimating the relative abundance of P. falciparum alleles using peak height and area.

METHODS: P. falciparum laboratory strain 3D7, was grown to 13,200 parasite/μl and then log diluted to 1 parasite/μl. DNA was extracted from each dilution and amplified in replicate by primary and nested PCR that targeted msp-1 (K1, MAD20 and RO33) and msp-2 (FC27 and IC3D7) alleles. The second PCR was performed using fluorescently labeled reverse primers and amplicons resolved by high resolution CE. PCR were terminated at the exponential phases (10, 15, and 20 cycles) and at plateau phase (30 cycles). Peak height and area of amplified alleles were correlated to parasite density.

RESULTS: Peak height and area were correlated to parasite density when PCR cycles were terminated at 20 cycles. K1 peak height and area respectively reduced from 2,775 and 19,757 at 13,200 parasites/μl to 260 and 1798 at 132 parasites/μl, respectively reduced from 2,775 and 19,757 at 13,200 parasites/μl to 260 and 1798 at 132 parasites/μl. Similar trend was seen for IC3D7: 5205 and 69109 for 13,200 parasites/μl, 594 and 8511 for 1,320 parasites/μl, and 102 and 1356 for 13,200 parasites/μl. A positive correlation was observed between peak height and area with parasite density: K1 peak height R²=0.8133, K1 peak area R²=0.8151. IC3D7 peak height R=1.000, IC3D7 peak area R=0.9999. When PCR was terminated at 30 cycles, the change in peak height and area for the same alleles did not correlate with parasite density.

CONCLUSIONS: When PCR is terminated at the exponential phase, the relative abundance of alleles can be quantified. This is important for assessing P. falciparum clonal dynamics in culture or host.

P316: A historic overview of published data on insecticide resistance in malaria vectors

Tessa B Knox1, Helen Pates Jamet2 and Elijah O Juma1
1Vestergaard Frandsen (Ltd.) East Africa, PO Box 66889-00800, Nairobi, Kenya
2Vestergaard Frandsen SA, Lousanne, Switzerland

BACKGROUND: Chemical-based malaria control through indoor residual spraying has been conducted for well over half a century with pyrethroid-impregnated bednets introduced in the late 1980s, originally as a complementary strategy. Increased insecticide use in malaria control has been accompanied by a corresponding increase in insecticide resistance. Reporting of data from insecticide susceptibility tests gained currency in the 1950s rapid development of resistance to DDT was observed for malaria vectors. We will review the trends in reports of insecticide resistance in Anopheles since 1950.

METHODS: Data from a systematic search of peer-reviewed and published literature on insecticide susceptibility and resistance mechanisms for malaria vectors were assessed by year of mosquito collection, mosquito species or complex, geographic area, type of insecticide or mechanism tested to identify trends and gaps in data collection and outcomes.

RESULTS: Following the earliest publication on insecticide susceptibility in the 1960s and on resistance mechanisms in the 1980s, there were very few publications released up until 1998. Thereafter there was an exponential increase in publications, with highest number in 2012 expected to be exceeded in 2013. There was a focus on testing of organochlorines and pyrethroids and while the majority of testing was conducted in the high-burden malaria countries, for some key countries (such as the Democratic Republic of Congo) very few data were available. Testing for mechanisms in An. gambiae s.s. and An. arabiensis focused on target site mutations, whereas for An. funestus very few data were available and the majority of testing was for metabolic mechanisms. There was a clear increase in the prevalence of both DDT and pyrethroid resistance in An. gambiae s.l. throughout sub-Saharan Africa.

CONCLUSION: The momentum on increasing testing and reporting of insecticide susceptibility must be maintained in order to safeguard the efficacy of the currently available malaria vector control tools through the design of informed insecticide resistance management strategies. Critical data gaps need to be addressed, such as the paucity of data for certain species or complexes (eg. An. funestus), resistance mechanisms (eg. metabolic-based) and specific areas of high malaria burden (DRC).

P317: IR Mapper: extended utility for evidence-based malaria vector control

Tessa B Knox1, Helen Pates Jamet2 and Elijah O Juma1
1Vestergaard Frandsen (Ltd.) East Africa, PO Box 66889-00800, Nairobi, Kenya
2Vestergaard Frandsen SA, Lousanne, Switzerland

BACKGROUND: Over the past decade, coverage with insecticidal interventions – namely, insecticide-treated nets and indoor spraying of residual chemicals - contributed to substantial reductions in malaria burden particularly in sub-Saharan Africa. However, rapid development of insecticide resistance in Anopheles poses a threat to effective malaria control. Pragmatic and informed use of insecticides is critical, although a publically-accessible repository of up-to-date insecticide resistance data has been lacking.

METHODS: IR Mapper, an online geospatial application, was developed to collate published reports on insecticide susceptibility and resistance mechanisms in Anopheles. The database was established through a systematic search of peer-reviewed data sources and extraction into Excel worksheets. Additional unpublished datasets were sourced from the President’s Malaria Initiative, African Network on Vector Resistance (via IRBase) and University of the Witwatersrand. IR Mapper was built on ArcGIS API for JavaScript platform, with a user-friendly interactive mapping interface allowing display and print of tailored maps. The recent 2013 release of IR Mapper includes the facility for users to temporarily view and produce maps of their own data alongside the online dataset, and to displaying Plasmodium parasite endemicity data layers.

RESULTS: As of June 2013, the IR Mapper application contains data from 207 publications from 49 peer-reviewed journals for a total of 7,724 unique field records for 1,402 geo-referenced localities in 52 countries for 57 Anopheles species or species complexes. Phenotypic resistance to at least one insecticide was reported in 43 of the 52 countries. Resistance to organochlorines was the most frequently reported, followed by pyrethroids, carbamates and organophosphates. Resistance mechanisms data were obtained for 39 countries with resistance to at least one detected in 36 countries. Kdr mutations were detected in 30 countries; insensitive acetylcholinesterase (Ace-2) detected in 9 countries, with metabolic mechanisms detected in 20 countries.

CONCLUSION: IR Mapper leverages geospatial information technology to create an application that collates data on insecticide resistance and allows easy display via a free online platform. IR Mapper facilitates rapid assessment of historic and up-to-date data relevant to the formulation of vector control strategies incorporating insecticide resistance management plans.
P318: Molecular Marker Trends in Plasmodium falciparum dihydrofolate reductase (DHFR) and dihydro Folate reductase synthase (DHPS) genes in the Kenyan Isolates between the years 2008 to 2012.

Dennis Juma, Angela Achieng', Luise Ingasia, Redemptah Yeda, Agnes Cheruiyot, Charles Okudo, Chelagat Cheruiyot, Joseph Ndegwa, Peninah Muuiru, Jemosp Lorna, Stephen Bidii, Hoseah Akala, Ben Andagalu, Edwin Kamau

Global Emerging Infections Surveillance Program, United States Army Medical Research Unit-Kenya –Kenya Medical Research Institute, Kisumu, Kenya

BACKGROUND: Sulfadoxine-pyrimetamine (SP), an antifolate was replaced by artemether-lumefantrine (AL) as the first-line malaria drug treatment in Kenya in 2006 due of the widespread resistance of Plasmodium falciparum. In addition, over the counter SP drugs and other antifolate drugs such as Trimethoprim/sulfamethoxazole (TMP/SMZ) used for treatment of opportunistic infections in HIV patients are still widely used. This study assessed the prevalence of mutations in dihydrofolate reductase (pfdhfr) and dihydropteroate synthase (pfdhps) genes which are associated with SP resistance in samples collected in Kenya between 2008 and 2012.

METHODS: Malaria positive field isolates (340) collected from Kisumu, Kisii, Kericho and Malindi district hospitals between 2008 and 2012 were assessed for genetic polymorphism at various loci within the two genes by Sanger sequencing.

RESULTS: Point mutation frequencies in the pfdhfr gene at codons 16, 51, 59, 108 and 164 were 9.3%, 95.6%, 91.7%, 97.9% and 1.5% respectively. The frequency of the triple pfdhfr NS51, C59R, S108N mutant was 86.4%. This triple mutation is linked with high levels of pyrimethamine resistance. Point mutation frequencies in the pfdhps gene at codons 437 and 540 were 82.89% and 72.37% respectively. The frequency of double pfdhps (A437G, K540E) mutants was 55.26%. This double mutation is associated with increased resistance to sulphadoxine. Additional point mutations at codons 436, 581 and 613 at frequencies of 6.58%, 34.21% and 1.32% respectively were observed. These additional mutations cause greater decrease in sensitivity to sulphadoxine. The pfdhfr/pfdhps quintuple, 511S/59R/108N/437G/540E mutant has been shown to be the most clinically relevant marker for SP resistance and was observed in 38.16% of the samples.

CONCLUSIONS: The prevalence of SP resistance seems to be persistently high even after SP was removed as the first-line malaria treatment. This indicates that the pressure on the parasite from this class of drug is still high. This is disconcerting since SP is still being used as IPT, and it is a candidate partner drug for artemisinin combination therapy.

P319: Efficacy of artemether-lumefantrine and amodiaquine-artesunate for the treatment of uncomplicated Plasmodium falciparum infection in Tanzania


1Ifakara Health Institute, Dar es Salaam, United Republic of Tanzania, 2Centers for Disease Control and Prevention, USA

BACKGROUND: Sulfadoxine-pyrimethamine (SP) has been shown to prevent to adverse outcomes associated with MIP, although increasing resistance to SP threatens its efficacy. We conducted a prospective, randomized trial at two clinics in Ifakara, Tanzania from November 2003 to February, 2007 to assess the efficacy of two doses of IPTp-SP (IPTp-SP2) compared to monthly IPTp with either SP (IPTp-SPm) or SP-artesunate (IPTp-SPAS). Consenting primi- and secundigravidae were enrolled at antenatal care (ANC) visits between 16-36 weeks gestation and followed monthly. At delivery, maternal peripheral blood and placenta smear samples were collected, and infant birth weight was recorded.

RESULTS: 1201 women were enrolled and 1144 followed until delivery: 386 in IPTp-SP2, 388 in IPTp-SPm, and 370 in IPTp-SPAS. 88.7% of women reported sleeping under an ITN on the preceding night with no significant difference between arms (p=0.71). The mean number of doses per study arm was 2.6, with no significant differences between arms. Both peripheral and placental parasitemia were infrequent in all arms with no significant differences between study arms. At the time of delivery (placental parasitemia was 2.1% in IPTp-SP2, 1.5% in IPTp-SPm and 1.6% in IPTp-SPAS (p=0.83) while peripheral parasitemia was 1.9% in IPTp-SP2, 0.5% in IPTp-SPm, and 1.6% IPTp-SPAS (p=0.23). There were no significant

P320: Monthly Intermittent Preventive Treatment in pregnancy (IPTp) with sulfadoxine-pyrimethamine (SP) provides added benefit over a two dose regimen

AM Kabanywanyi', S Abdulla', J Gutman', A Baja', PB Bioland, SP Kachur1,2 and JR MacArthur2

1Ifakara Health Institute, Tanzania; 2Centers for Disease Control and Prevention, USA

BACKGROUND: It is estimated that 30.3 million pregnancies occur annually in malaria endemic areas of sub-Saharan Africa, with approximately 100,000 infant deaths as a result of malaria in pregnancy (MIP). Intermittent Preventive Treatment in pregnancy (IPTp) with sulfadoxine-pyrimethamine (SP) has been shown to prevent to adverse outcomes associated with MIP, although increasing resistance to SP threatens its efficacy.

METHODS: We conducted a prospective, randomized trial at two clinics in Ifakara, Tanzania from November 2003 to February, 2007 to assess the efficacy of two doses of IPTp-SP (IPTp-SP2) compared to monthly IPTp with either SP (IPTp-SPm) or SP-artesunate (IPTp-SPAS). Consenting primi- and secundigravidae were enrolled at antenatal care (ANC) visits between 16-36 weeks gestation and followed monthly. At delivery, maternal peripheral blood and placenta smear samples were collected, and infant birth weight was recorded.

RESULTS: 1201 women were enrolled and 1144 followed until delivery: 386 in IPTp-SP2, 388 in IPTp-SPm, and 370 in IPTp-SPAS. 88.7% of women reported sleeping under an ITN on the preceding night with no significant difference between arms (p=0.71). The mean number of doses per study arm was 2.6, with no significant differences between arms. Both peripheral and placental parasitemia were infrequent in all arms with no significant differences between study arms. At the time of delivery (placental parasitemia was 2.1% in IPTp-SP2, 1.5% in IPTp-SPm and 1.6% in IPTp-SPAS (p=0.83) while peripheral parasitemia was 1.9% in IPTp-SP2, 0.5% in IPTp-SPm, and 1.6% IPTp-SPAS (p=0.23). There were no significant
different in prevalence of low birth weight (p=0.73), or mean birth weight in the three arms (mean (95% CI) grams: IPTp-SP=2836 (2783-2890), IPTp-SPm= 2856 (2805-2907), IPTp-SPAS=2796 (2744-2849)).

CONCLUSIONS: No significant difference was found in the prevalence of peripheral and placental parasitemia at delivery, LBW, or mean birth weight between arms, likely due to the fact that there were similar number of documented infections. This study was unable to substantiate advantage of SPAS over SP alone. Efforts should be made to improve uptake of IPTp-SP at each ANC visit following the recently released WHO guidelines.

P321: The impact of insecticide-treated bed nets on malaria parasite transmission potential in Kamuli district, Uganda: where is Uganda on the road to elimination?

Fredrick Kabbale1, Anne Akol1, Enoch Matovu1 and Ambrose Onapa2
1Department of Biological Sciences, College of Natural Sciences, Makerere University, Kampala, Uganda; 2Department of Molecular Biology, College of Veterinary Medicine and Animal Biosecurity, Makerere University, Kampala, Uganda; 1Envision/NTD Programme, RTI, Uganda.

The main entomological justification for use of insecticide-treated bed nets (ITNs)/Long Lasting Insecticide-treated bed nets (LLINs) as the main malaria vector control method in Uganda is that most biting by Anopheles gambiae sensu lato and Anopheles funestus group, the principal vectors, is believed to occur between 10:00pm and 5:00am when most people are in bed and under nets. Hypothetically, this biting pattern changed following prolonged use of ITNs/LLINs, rendering this intervention less effective, explaining the continued morbidity and mortality due to malaria in endemic Uganda. A longitudinal study was conducted to determine the Plasmodium falciparum sporozoite-infective biting hours of the night and the parasite transmission intensities under prolonged use of ITNs/LLINs in Kamuli district. A P.f. circum-sporozoite protein ELISA was carried out on 551 (112 pools) and 3640 (351 pools) Anopheles gambiae s.l. and An. funestus group caught at different hours of the night in intervention (with ITNs) and non-intervention (without ITNs) zones respectively. The circumsporozoite positivity of the vectors was related to the time of biting humans, while the annual entomological inoculation rates (AERIs) were obtained by multiplying the average annual human biting rate by the sporozoite rate. Results showed no impact of ITNs/LLINs on the sporozoite-infective biting hours of the night and seemingly reduced sporozoite infection rates. Infective biting by the vectors occurred throughout the night, with peak infection bites occurring between 20:00 and 04:00hours in both zones, indicating protective effectiveness of ITNs/LLINs against malaria sporozoite-infective biting by the vectors. In both zones, the malaria transmission potential was higher outdoors than indoors, and was several fold higher in the non-intervention than in the intervention zone, indicating that ITNs/LLINs may have reduced the EIRs in the intervention zone. The AERIs in both zones exceeded one, placing Kamuli district far from malaria elimination phase like most of the country. An integrated approach to malaria control, including integrated vector management and effective case management should be adopted in Kamuli District and other parts of the country to reduce the transmission intensity to levels that could interrupt P. falciparum malaria transmission, and possibly driving Uganda closer to the malaria elimination phase.

P322: Distribution of Anopheles gambiae s.l. and its insecticide resistance profile in Tanzania: implications for malaria vector control

Bilal Kabula1, Patrick Tungu2, Robert Malima3, Bernard Batengan4, William Kisima5, Stephen Magesa6, Martin Donnelly7 and Franklin Mosha8
1Kilimanjaro Christian Medical University College (KCMUC), Moshi, Tanzania; 2National Institute for Medical Research, Amani Research Centre, Muheza, Tanzania; 3Liverpool School of Tropical Medicine, Liverpool, UK; 4RTI International, Nairobi, Kenya

BACKGROUND: Members of the Anopheles gambiae complex are important malaria vectors in Tanzania. The species complex exhibits an enormous diversity in its biology which impacts greatly on its importance as a vector of malaria. This study investigated the distribution of members of the An. gambiae complex and their insecticide resistance profile relative to the ecological differences found across Tanzania.

METHODS: Indoor-resting Anopheles mosquitoes were collected from 12 districts located across various ecological zones of Tanzania. These were morphologically identified as An. gambiae and tested for resistance to deltamethrin, lambdacyhalothrin, permethrin and DDT using standard WHO methods. Molecular diagnostics were used to genotype mosquitoes and to detect resistance mechanisms.

RESULTS: A total of 7,596 mosquitoes were morphologically identified as An. gambiae s.l. of which, 2,947 were identified to their species level. Out of these, 68.7% and 31.3% were An. arabiensis and An. gambiae s.s respectively. Both species occurred in sympathy in 30.8% of the sites, while An. arabiensis occurred alone in 69.2%. Overall An. arabiensis predominated and was distributed widely across all ecological zones. The distribution of resistance was not homogenous. The species complex was resistant to the three pyrethroids tested (mortality rate < 80%) in Moshi, Arumeru, Muheza and Mulela. Resistance to DDT was also recorded in one site, Dar es Salaam (mortality rate of 65% [95% CI, 54–74]). Presence of kdr-L1014S was recorded in some parts of the country, occurring in both species with varying allelic frequencies (4%-35%). kdr-L1014S genotypes were found most frequently in An. gambiae s.s. (p<0.05). The cytochrome P450s (CYP6P1, CYP6P2, CYP6P3, CYP6P4, CYP6P5) were previously associated with resistant phenotypes were significantly overexpressed in An. gambiae s.s resistant to DDT.

CONCLUSION: This study demonstrates the predominance and wide distribution of An. arabiensis in Tanzania. It also demonstrates that Anopheles gambiae s.l. is becoming resistant to pyrethroids in several parts of the country. This appearance of resistance mandates close monitoring and adoption of rational resistance management strategies in the country if the gains so far made in malaria control are to be sustained.

P323: Prevalence of antibodies to the surface of P. falciparum infected red blood cells among two cohorts in different parts of Tanzania

Mwanaidi Kafuye1, 2, Magdalena Kasya3, Doto Kalovya, Odamira Ngerageza4, Eric Lyimo, Joseph Mziray, Jeffrey Dorfman2
1National Institute for Medical Research, Dar es Salaam, Tanzania; 2International Centre for Genetic Engineering and Biotechnology, Cape Town, South Africa; 3National Institute for Medical Research, Dar es Salaam, Tanzania; 4National Institute for Medical Research, Dar es Salaam, Tanzania

BACKGROUND: This is a preliminary data for monoclonal antibody isolation and studies of effects of helminths infections upon immunity to P. falciparum. Malaria in pregnancy causes significant maternal and infant morbidity and mortality. Effective adhesion blocking antibodies developed with successive pregnancies and this is associated with protection from disease. However in tropic areas, pregnant women are susceptible to helminths co-infections with malaria and disease severity increase with decreasing parity. Helminths induce immunomodulatory activities during malaria infections and can influence vaccine efficacy. This study aims to generate protective human monoclonal antibodies and use them to identify high priority candidate antigens for vaccine development. We also aim to look at the prevalence and effects of helminths on malaria immunity.

METHODS: Peripheral blood mononuclear cells were collected from immune women for generation of monoclonal antibodies using myeloma fusion technique. These samples were collected from women living in Misungwi district and Rufiji districts, Tanzania. RBC from pregnant women currently infected with malaria will be collected from Bagamoyo district, Tanzania for isolation of pregnancy-associated P. falciparum to screen binding antibodies. Furthermore prevalence of helminths and malaria infections will be determined from sera of the study population using soluble egg antigen ELISA and HRP-2 based rapid diagnostic tests.
new ones, to readily detect disease outbreaks and predict future disease trends. We established a longitudinal adult mosquito surveillance system in rural Tanzania, to provide essential data necessary for examining malaria transmission patterns and existing and new interventions. The intention was to be able to identify transmission hotspots and the dominant factors. From a population of 2433 households in 3 villages (Kivukoni, Minepa and Mavimba) in southern Tanzania, 1600 households were randomly selected and spatially assigned, based on latitudes, to 16 clusters each consisting of 100 households. Monthly mosquito collections were performed using CDC-Light traps inside 6 households randomly selected from each cluster. The mosquitoes were sorted by taxa and abdominal status, after which a sub-sample of the malaria vectors were examined by (PCR) to distinguish between sibling species. The vectors were also examined by (ELISA) to detect Plasmodium sporozoites in their salivary glands. The distribution of *Anopheles gambiae* s.l and *Anopheles funestus* was spatially clustered, mostly in a set of adjoining clusters centered on the middle of the study area. At least 75% of *An. gambiae* s.l and 86% of *An. funestus* were collected in adjoining clusters centered in Minepa village. PCR and ELISA analyses are yet to be completed. These preliminary results show that most of the malaria vectors were collected from a set of contiguous clusters in an area centered in Minepa village, suggesting suitability of spatially targeted intervention. Further assessments are underway to determine risk factors associated with this distribution pattern and mosquito house entry.

**BACKGROUND:** The role of Tregs in malaria is under active investigation. Although several surface markers were defined for the identification of Tregs, further investigation has constantly been hindered by their lack of discriminatory surface markers. Despite this lack of specificity, fluorochrome labeled CD4, CD25, CD127 and FoxP3 antibodies are common flowcytometric reagents used in the identification of these immunosuppressive cell types. To facilitate large studies on host immune function in malaria, both batching of laboratory work and cryopreservation of PBMC are an essential part in these studies. This helps to reduce operator dependent inter-assay variability and also optimizes the use of the available resources. However, there are a few conflicting data presently available on the effects of cryopreservation of PBMCs, especially in HIV patients and, little is known in malaria. In this study both the effects of cryopreservation on the selective recovery of CD4*+*CD25*+*FoxP3*+*CD127-/lo (Tregs) T cells and therefore studies can be performed on the individuals produced, and is often ignored. *Anopheles gambiae* eggs normally hatch within three days under optimal conditions. However, some eggs may hatch up to three weeks after oviposition increasing their risk of desiccation. Anopheline eggs do not normally cope well with desiccation. The ability to delay or stagger time-to-hatch may be an adaptive trait that increases reproductive output despite the increased risk of desiccation in an unstable environment. It is proposed that staggered time-to-hatch occurs because a proportion of the individuals are genetically predisposed to hatch late which also offers a wider platform for the selection of insecticide resistance. This study aimed to investigate the mechanism of staggered larval time-to-hatch by examining embryo development and metabolic rate in *An. gambiae* eggs from early and late hatching parents. METHODS: The metabolic rates of groups of 150 eggs from early and late hatching parents were measured using an infrared CO2 analyzer. Results were compared and analysed statistically. Observational comparisons based on developmental differentiation seen in embryos were also made between eggs from early and late time-to-hatch parents at various age intervals.

**RESULTS:** Egg metabolic rates peaked at two to three days post oviposition then declined with age. Preliminary results showed no difference between early and late eggs. Most embryos were fully developed two to three days post oviposition. No clear differences between early and late eggs were observed.

**CONCLUSIONS:** Although some eggs are able to delay hatching, the developmental rate and metabolic requirements of eggs from early and late hatching parents is similar. It appears that while most eggs hatch once fully developed after two to three days some eggs are able to wait once fully developed, possibly in a diapause mechanism, until they hatch. It is likely that there are triggers required for hatching of these late hatching eggs. These potential triggers require further investigation.

**P324: Frequency of CD4⁺CD25⁺FOXp3⁺CD127⁻/LO cells in children with asymptomatic malaria infection**

**Oscar K Kai¹, Kevin N Couper², Brett S Lowe¹,², Britta C Urban¹,³**

¹KEMRI Centre for Geographic Medicine Coast P.O Box 230, Kilifi, Kenya; ²Centre For Tropical Medicine, Nuffield Department of Clinical Medicine John Radcliffe Hospital, Oxford OX3 9DU, UK; ³Immunology Unit, Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, UK; ⁴Liverpool School of Tropical Medicine, Liverpool, UK

**BACKGROUND:** The frequency of T-regulatory cells (Tregs), CD4⁺CD25⁺FOXp3⁺CD127⁻/LO, in children with asymptomatic malaria was examined to guide the design of future studies of Tregs in malaria. **METHODS:** Nineteen healthy adults were recruited and nine of them were bled at more multiple time points as controls. Expression levels of CD4, CD25, FoxP3 and CD127 were compared between their fresh and frozen/thawed PBMC samples. There was no significant difference in the FoxP3+ expressing Tregs in children with asymptomatic malaria were examined to guide the design of future studies of Tregs in malaria.

**RESULTS:** There was no significance variation observed in FoxP3+ expressing Tregs in children with asymptomatic malaria. CD4⁺CD25⁺FOXp3⁺CD127⁻/LO Tregs in fresh compared to freeze/thawed PBMC samples even after two or twelve weeks post freezing. There was no significant difference in the median proportion of FoxP3+ expressing Tregs between malaria negative and asymptomatic children.

**CONCLUSION:** Cryopreservation does not affect the recovery of total CD4⁺CD25⁺FoxP3⁺CD127⁻/Lo (Tregs) T cells and therefore studies can still rely on the results produced by use of cryopreserved PBMCs. However, even though differences of Tregs expression is not apparent in asymptomatic malaria infections there is need to further enumerate Tregs cells proportions in children with different malaria syndromes.

**P325: Longitudinal surveillance of disease-transmitting mosquitoes in rural Tanzania: creating an entomological framework for evaluating existing and new interventions**

**Emmanuel Kaindoa, Gustav Mkandawile, Godfrey Lingamba, Gerry Killeen and Fredros Okumu**

High quality mosquito surveillance data is necessary to: assess spatial and temporal disease trends, evaluate new and existing interventions, identify transmission hotspots, identify dominant vectors and pathogens or detect
P327: Examining the Larvicidal activity of Solanum elaeagnifolium against the Predominant Indian rural malarial vector, Anopheles culicifacies

Kalimuthu Kovendan1, Kaliyaperumal Karunamoorthy2, Kadarkarai Murugan1, Savarar Vinath1
1Division of Entomology, Department of Zoology, School of Life Sciences, Bharathiar University, Coimbatore - 641 046, Tamil Nadu, India; 2College of Public Health & Medical Sciences, Jimma University, Ethiopia; Centre for Environmental Research and Development, P.G. Research & Department of Advanced Zoology and Biotechnology, Loyola College, Nungambakkam, Chennai – 600 034, Tamil Nadu, India.

BACKGROUND: Anopheles culicifacies is one of the major vectors of human malaria in the resource-poor settings of rural India. It is a complex of five sibling species and controlling them is quite challenging due to their biochemical and molecular differences in activated nitric oxide synthase (ACNOS) gene. However, vector control remains considered to be a cornerstone to contain malaria related death and illness. The continuous application of chemical insecticides imposed serious negative impact on human health, environment and insecticide resistance. Therefore, identifying novel green pesticides is extremely important to sustain the ongoing malaria control efforts. This is an attempt to assess the mosquitocidal properties of Solanum elaeagnifolium leaf extracts against An. culicifacies.

METHODS: The S. elaeagnifolium plants were collected in and around Vadavalli village, Coimbatore, Tamil Nadu, India and the fresh leaves were washed thoroughly with tap water. The extracts were concentrated at reduced temperature on a rotary vacuum evaporator and stored at a temperature of 4°C. From this stock solution various concentrations were prepared. The An. culicifacies mosquitoes were reared in the entomology laboratory, Department of Zoology, Bharathiar University, Tamil Nadu. The larvicidal activity was assessed by using the procedure of WHO (2005).

RESULTS: The larval and pupal mortality was observed after 24 h of exposure; no mortality was observed in the control group. S. elaeagnifolium had values of An. culicifacies (LC50 = 109.68, 142.34, 249.58, 320.40 and 379.20 ppm; LC90 = 494.43, 645.22, 846.36, 928.87 and 997.01 ppm). Statistical software package 16.0 version was used. Results with P< 0.05 were considered to be statistically significant.

CONCLUSION: The results clearly suggesting that the leaf extract of S. elaeagnifolium could be a best alternative to replace the existing toxic synthetic insecticides. The emergence of insecticide resistance against all the four classes of chemical insecticides is considered to be a potential threat to the global public health. Therefore, the use of plant-based insecticides could substantially inhibit the insecticide resistance considerably. This ideal eco and user-friendly vector control strategy could minimize the malaria burden and eventual elimination in the near future.

P328: Increasing Rapid Diagnostic Test (RDT) Uptake and Adherence through Improved Malaria Case Management Training in Kavango Region, Namibia

Lourenco, C.1,2, Usisku, P.3, Haidula, L.2, Ward, A.1, Kandula, D.1 and Cohen, J.1
1Clinton Health Access Initiative, Boston, USA; 2National Vector-borne Disease Control Programme, Windhoek, Namibia; 3Global Health Group, University of California San Francisco, San Francisco, USA.

INTRODUCTION: Proper malaria diagnosis is essential for any country. In elimination settings, diagnosis is even more critical since it is the basis for identifying the final foci of transmission and effectively targeting interventions. Despite its importance, diagnosis rates tend to be quite poor in many African countries. An operational research pilot was conducted in Namibia to identify the key barriers to appropriate diagnosis of malaria and to demonstrate the effectiveness of training approaches on the uptake and adherence to RDTs.

METHODS: After identifying weaknesses of the current case management programme through focus group discussions and key informant interviews with health care workers (HCWs), prospective training interventions were designed to address these barriers. The study had three intervention arms and one control, using four districts within the Kavango region of Namibia where clinical diagnosis and wide scale overtreatment practices were observed. Three interventions were designed and tested over a 6 month period: an enhanced training model/curriculum, clinical mentorship and SMS reminders to HCWs.

RESULTS: All intervention arms produced substantial improvements in case management practices compared to the control where no interventions were implemented. The enhanced training plus mentorship arm resulted in a significantly greater proportion of fevers receiving RDTs compared to the district receiving enhanced training alone, increasing from 27% to over 90% at endpoint. No ACTs were prescribed to untested patients after receiving mentorship or SMS reminders. These changes show almost a complete reversal of improper case management practices over the 6 month study period. Findings from this work have informed the national malaria programme’s roll out of a more robust case management training across Namibia that is aligned with their national strategic plan for malaria elimination.

CONCLUSIONS: This study showed that implementing simple training interventions can have a significant impact on the uptake of and adherence to malaria RDTs and that the approaches described here in the context of Namibia can be carried out and brought to scale in many resource-constrained countries. Findings from this operational research can also extend worldwide as a growing number of low endemic countries are looking for practical guidance on sustainable approaches to febrile illness management.

P329: Mapping Malaria Parasite Flows to Inform Optimal Strategies for Elimination

Tatem A.J.1,2, Huang Z.3,4, Kumar U.3,4, Pindolia, D.1,4, Usisku, P.3, Narib, C.1, Graupe, B.5, Kandula, D.1,4 and Lourenco, C.6
1Department of Geography and Environment, University of Southampton, UK; 2Fogarty International Center, National Institutes of Health, Bethesda, USA; 3Department of Geography, University of Florida, Gainesville, USA; 4Emerging Pathogens Institute, University of Florida, Gainesville, USA; 5Department of Computer Science, University of Florida, Gainesville, USA; 6National Vector-borne Disease Control Program, Windhoek, Namibia; 7Mobile Telecommunications Limited, Windhoek, Namibia; 8Clinton Health Access Initiative, Boston, USA; 9Global Health Group, University of California San Francisco, San Francisco, USA.

INTRODUCTION: Sub-Saharan Africa has experienced a dramatic reduction in malaria over the past decade, positioning several countries for transition from control to elimination. Swaziland, Namibia, Botswana and South Africa are at the forefront of these elimination efforts, where targeting and intensifying attack on transmission foci and managing importation risk are increasingly becoming strategic priorities. This study demonstrates how high resolution malaria risk maps can be integrated with anonymized mobile phone records to inform elimination planning, using the example of Namibia.

METHODS: Following approaches developed for Swaziland, geolocated malaria cases were combined with datasets describing weather, geography, and population in a regression tree modeling framework to produce a high resolution risk map. Anonymized mobile phone records were then used to quantify movement patterns across Namibia by examining changes in tower locations where calls were routed through. By linking movements with malaria risk, weighted movement networks were constructed to identify the relative connectivities between high risk zones and the likely human and parasite movement routes. Distinct communities were identified using modularity optimization algorithms, and the relative weights of infections exported or imported were analyzed to characterize locations as “sources” (net parasite exportation) or “sinks” (net parasite importation).
RESULTS: Malaria risk was most notably associated with vegetation, population density and distance to water bodies. The heterogeneity in malaria risk across Namibia means that parasite movement patterns vary greatly from that of human movement. The quantification and mapping of sources and sinks was completed to inform scenario planning on the impact of targeting interventions on likely parasite movements.

CONCLUSIONS: Elimination of an infectious disease is often complicated by human movement bringing in new infections. The parasite flows highlighted here suggest that any elimination effort will require strategies designed to minimize re-importation of infections. As countries begin to look at the introduction of new strategies for elimination, such as mass drug administration, it is important to assess the potential effectiveness in areas where infections migrate in. Here we show tools built on integrated, readily available datasets can provide useful evidence for elimination planning through an improved understanding of malaria risk and its relationship with human movements.

P330: Demonstrating resistance-mitigating effect of *artemisia annua* phytochemical blend with in-vitro cultures of *plasmodium falciparum* and in-vivo with *plasmodium berghei* anka in mice

Lucy N. Kangethe 1,2, Hassanali Ahmed 1, Sabah Omar 1, Kimani Francis 3, Joseph K. Nganga 3, Johnson Kinyu 1

1Department of Biochemistry Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya; 2Department of Chemistry Kenyatta University, Nairobi, Kenya; 3Kenya Medical Research Institute, Nairobi, Kenya

Resistance of *Plasmodium falciparum* to drugs such as chloroquine and sulfadoxine-pyrimethamine is a major problem in malaria control. Artemisinin derivatives, particularly in combination with other drugs, are thus increasingly being used to treat malaria, reducing the probability that parasites resistant to the components will emerge. Although stable resistance to artemisinin has yet to be reported from laboratory or field studies, its emergence would be disastrous because of the lack of alternative treatments. The project was designed to demonstrate resistance-mitigating effects of phytochemical blend of *Artemisia annua* relative to pure artemisinin against the malaria parasite *Plasmodium falciparum* and on rodent malaria parasite *Plasmodium berghei* Anka. For the in vitro experiments selection was undertaken on two cultures of *P. falciparum* D6 (CQ-sensitive strain from Sierra Leone) and W2 (CQ-resistant strain from Indochina), by exposing them to *A. annua* phytochemical blend and the pure artemisinin over 50 cycles at doses initially required to give 50% mortality (IC50) of the parasites. Dose-response effects of the blend and the pure compound were determined after 10, 20, 30, and 40, cycles and compared to see if significant difference developed in their efficacy in causing mortality of the parasites. The in vivo experiments mice have been done by inoculating the Albino Swiss mice with the *P. berghei* ANKA parasite and thereafter treated them with the test drugs. After 4 days the mice were passaged and parasitaemia determined to calculate the ED90 and the ED50. The ED90 and ED50 got for artemisinin with *P. berghei* ANKA was 1.43 and 7.18 mg/kg/day respectively while the ED90 and ED50 got for the blend with *P. berghei* ANKA was 34.5 and 118 mg/kg/day. The molecular basis of resistance is ongoing where GFM were calculated. After 4 days the mice were passaged and parasitaemia determined to calculate the ED90 and the ED50. The ED90 and ED50 got for artemisinin with *P. berghei* ANKA was 1.43 and 7.18 mg/kg/day respectively while the ED90 and ED50 got for the blend with *P. berghei* ANKA was 34.5 and 118 mg/kg/day. The molecular basis of resistance is ongoing where GFM were calculated.

P332: Contribution of integrated community case management in reduction of malaria and childhood illnesses in Rwanda

F. Ngabo1, C. Karem1,1, J.P. Habimana2, C. Mugenzi1, A. Binagwaho1

1Maternal and Child Health Unit- Ministry of Health-Rwanda; 2Malaria & Other Parastic Diseases Division-RBC, Ministry of Health-Rwanda; 3Ministry of Health-Rwanda

INTRODUCTION: Rwanda Progress in child survival has come thanks to a variety of efforts such as malaria control interventions, integrated community case management (ICCM) and immunization. Community Health Workers (CHW) are providing countrywide basic diagnostic -treatment of malaria, pneumonia, diarrhea and malnutrition since 2008. In order to assess impact of integrated community case management, the Maternal and child health Unit and partners have measured the trends in main causes of under five mortality from 2008 to 2011.

METHODS: Trend analysis of out-patients cases and deaths of childhood illnesses was conducted using pre-existing data from the Rwanda Health information system and the community health information system SIS-Com. All causes of deaths in under five children in all districts were ascertained from surveillance records. All-cause deaths in children under-five in household surveys of 2007-8 and 2010 were also reviewed to corroborate with the trends of deaths observed in hospitals.

RESULTS: The top six of causes of death among the under five children in 2011were: prematurity with 32%, respiratory tract infection with 12%, malaria with 5%, malnutrition with 4% the last are congenital abnormalities and diarrhea with 3%. From 2008 to 2011, there was a decline in the 4 diseases managed by CHWs, where malaria has shown a rapid decrease from 18% to 5% in 2011, as well as respiratory infection decreased from 20% to 12% in 2011,diarrhea from 10% to 3% in 2011 and malnutrition decreased from 6% to 4%. The proportion of under-five with malaria/fever, diarrhea, and cough receiving appropriate treatment within 24h (community) has increased from 62% in 2008 to 94% in 2011.

CONCLUSION: Our analysis suggests that ICCM has essentially an impact on the reduction of the 4 main causes of death in Rwandan children under five although other reasons such as permanent good coverage of Vitamin A, use of ITNs and immunization. These successes show that the reduction of mortality and morbidity in children at an accelerated rate is possible if interventions with high impact are applied to large-scale integrating community level.
P333: Increase coverage and use of key malaria control interventions in Rwanda

C. Karena1, J. Habimana1, F. Ngabo2, M. Mulindahabi1, I. Umulisa1, R. Hong1, A. Binagwaho3, A. Rukundo1
1Malaria & Other Parasitic Diseases Division-RBC, Ministry of Health-Rwanda; 2ICF International-Macro DHS Measure; 3Ministry of Health

BACKGROUND: In 2005, the demographic and health survey (RDHS) estimated that 14% of households in Rwanda owned an Insecticide treated net (ITN).17.2% of pregnant women aged 15-49 years and 13% of children under five slept under an ITN the night preceding the survey. Among children who had a fever, 12% received antimalarial drugs and 40% of them took them early. The Government of Rwanda set an ambitious national goal in 2005 to scale-up 85% coverage in ITN and universal access to ACTs.

METHODS: A nationally representative malaria indicator survey (MIS) was conducted in Rwanda between February and April 2013 to provide information on malaria indicators and to assess coverage, use and access to scaled-up malaria control interventions. The survey used a two-stage random cluster sample of 4,772 households in 159 villages (enumeration areas). Data was collected using worldwide Demographic and Health Surveys (DHS) household and women’s questionnaires.

RESULTS: Of 4766 surveyed households, 83% of households own at least one ITN. 43% of household own at least one net for every two persons who stayed in the household last night. 74.1% children <5 years of age and 74% of pregnant women 15 - 49 years of age had slept under an ITN prior night of the survey. Among children with fever, 12% took antimalarial drugs of which 58.3% children took antimalarial drugs the same or next day after developing a fever. 92% who took antimalarial drugs took ACT. Nearly 9 in 10 women (88%) know that fever is a symptom of malaria infection and 2/3 of women (67%) know that malaria can be prevented by sleeping under a mosquito net.

CONCLUSIONS: Since mid-2005, the Rwanda National Malaria Control Programme has considerably scaled-up its malaria control interventions. The MIS showed, however, that besides sustaining and expanding malaria intervention coverage, efforts have to be made to increase intervention access and use for Rwanda to achieve its targets and move towards malaria pre-elimination.

P334: Prevalence and Risk Factors Associated to Malaria in Rwandan Children, 2010-2011

C. Karena1, M. Murindahabi1, N. Umulisa, J. Habimana, A. Binagwaho2, A. Rukundo1
1Malaria & Other Parasitic Diseases –RBC, Ministry of Health-Rwanda; 2Ministry of Health

BACKGROUND: Rwanda has scaled up malaria control interventions successfully and has set the ambitious goal of achieving pre-elimination status by 2017. Rwanda was one of the first countries in sub-Saharan Africa to achieve universal coverage of Long Lasting Insecticide Nets, increased access to diagnostic and treatment using Artemisinin Combination Therapies (ACTs) and integrated community case management (ICCM) countrywide. In 2010, the Rwanda Demographic and Health Survey was conducted to determine the health indicators including malaria prevalence, malaria prevention and control coverage. Blood smears were collected from 4,046 children aged between 6 and 59 months.

METHODS: Secondary data analysis was carried out to determine malaria prevalence and risk factors associated to malaria in children under five. Multinomial logistic regression models were used to identify individual, household and environmental risk factors.

RESULTS: Prevalence of malaria infection in children was 1.4% (95% CI: 1.0% - 1.7%). Analysis was performed for twelve risk and protective factors for malaria infection. In multivariate model, Eastern Region (OR=11, 95% CI: 1-120), the age group of 48-59 months (OR=10.2, 95% CI: 1.1 – 94) contributed to a significantly higher risk of malaria infection among under five years children. Parent’s exposure to mass media (OR= 0.44, 95% CI: 0.20 - 0.97), measles immunization (OR=0.29, 95% CI: 0.10-0.84), sleeping under treated mosquito net (OR=0.72 95% CI: 0.37 - 1.37), and vitamin A (OR= 0.67 95% CI: 0.21 - 2.08) were associated with a reduced risk of malaria infection.

CONCLUSIONS: Eastern Province and age independently influence the malaria infection. Our study suggests that an effective mobilization through mass media of the population of endemic area on malaria prevention is the key intervention in malaria prevention in Rwanda.

P335: Scaling Up Malaria Control in Rwanda: Progress and Impact 2005–2011

C. Karena1, J. Umulisa1, A. Rukundo1, A. Binagwaho2
1Malaria & Other Parasitic Diseases Division-RBC, Ministry of Health-Rwanda; 2Ministry of Health-Rwanda

INTRODUCTION: Rwanda first comprehensive National Malaria Control Strategic Plan covered the period from 2005 to 2010; setting ambitious goals to scale up a package of malaria control interventions: insecticide-treated mosquito nets (ITNs), case management with diagnosis using microscopy and rapid diagnostic tests (RDTs) and prompt effective treatment with artemisinin-based combination therapy (ACT), increased behaviour change communication and integrated community case management (ICCM).

METHODS: Available information was reviewed from national surveys, program data, special studies, and in-country reports to assess malaria control progress in Rwanda. Data available from the standard published and activity reports, surveys, and special studies were reviewed and compared over time; no additional within-survey analyses were undertaken.

RESULTS: From 2005 to 2011, the Rwandan HMIS has shown remarkable reductions in malaria indicators: 86 % reduction in malaria incidence, 87% reduction in malaria morbidity, 74% reduction in malaria mortality, and a 71% reduction in malaria test positivity rate. 82% of households own at least one ITN and more than 70% of children under five and pregnant women sleeping under a ITN. Rwanda has also achieved universal treatment of malaria cases using ACTs both at health facilities and community level using CHWs which were trained to test malaria with RDTs with more than 94 % of all children sick are treated within 24 hours and 99% of malaria cases are lab diagnosed before receiving the correct treatment. The Malaria prevalence has decreased from 2.6 in 2008 to 1.4 in 2010 in children US and from 1.4 in 2008 to 0.7 in 2010 in pregnant women. Key of Rwanda Malaria control success has been an aggressive Government-led roll out of an integrated mix of prevention, treatment and mosquito control activities, with a strong emphasis on strengthening our health system.

CONCLUSIONS: For the last decade, Rwanda has made tremendous progress in malaria control. However, history has taught Rwanda that malaria control is fragile. Transitioning from malaria control to pre-elimination requires new innovative ideas, leadership, constant vigilance, resources, and commitment in order to move the country to pre-elimination phase of malaria and zero deaths by 2017.

P336: Antibody activity against Plasmodium falciparum in a region of declining malaria transmission in southern Zambia

Ben Katowa1, Kasapo Musonda1, Tamaki Kobayashi, Harry Hamapumbu1, Philip E Thuma1, Sungano Mharakurwa1,2 and William J Moss3
1Macho Research Trust, Choma, Zambia, 2Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

BACKGROUND: With the decrease in malaria transmission in southern Zambia, partial immunity to the parasite may be lost due to the fact that exposure to the parasite has also been reduced. This study was designed to understand the relationship between antibody activity and the decline in malaria transmission in southern Zambia.

METHOD: Households were randomly selected from a satellite image and participants were enrolled in two different study designs in the Macha Hospital catchment area: rolling cross-sectional surveys (where participants were visited only once) and a longitudinal cohort (where participants were visited repeatedly) between 2008 and 2012. A dried blood spot collected on Whatman 903 Protein Saver paper was soaked in 5% skim milk-PBST
for one hour and the eluted sample was used in an enzyme immunoassay (EIA) to detect immunoglobulin G (IgG) antibodies against whole parasite P. falciparum asexual blood stages.

RESULTS: Individuals whose EIA optical density (OD) value exceeded a pre-specified cut-off value (defined as the mean plus three standard deviation of ten samples from individuals who were never exposed to malaria) were considered seropositive. The proportion of individuals who were seropositive did not change significantly from 2009, 2010 and 2011, with seroprevalence of 69% to 68% and 67%, respectively. During this period, the prevalence of malaria by rapid diagnostic test declined from 8.1% in 2008 to 0.5% in 2011. Seropositivity increased with age but preliminary data for 2012 showed evidence of decreasing OD values consistent with the loss of malaria immunity.

CONCLUSION: Despite the reduction in malaria prevalence in Macha, seropositivity was relatively high from 2009 to 2011 indicating antibody persistence over this time period. However, more recent findings indicate that by 2012 antibody levels began to decrease, suggesting loss of immunity and a population increasingly susceptible to malaria resurgence.

P337: Assessment of the intermittent preventive treatment for prevention of malaria in pregnancy (IPTp) strategy for malaria control using sulphadoxine-pyrimethamine (SP) through focused antenatal care (FANC)

U Katsayal

BACKGROUND: Intermittent preventive treatment for prevention of malaria in pregnancy (IPTp) using sulfadoxine-pyrimethamine (SP) through focused antenatal care (FANC) and using direct-observed therapy (DOT) is a key component of malaria control strategy in Nigeria. Despite the evidence of the effectiveness of IPTp strategy using SP in reducing the adverse effects of malaria during pregnancy, the coverage of the programme in Nigeria seems to be low over a decade now. The present study therefore assessed the use of IPTp as a strategy for malaria control in pregnancy using SP in Giwa and Sabon Gari Local Government Areas (LGAs) of Kaduna State, Nigeria.

METHODS: A cross-sectional design was employed in this study using a key-informant interview targeted at the heads of primary health care department at the Local Government headquarters as well as semi-structured questionnaire targeted at the heads of the primary health care centres (PHCs) in the two LGAs and another well-structured questionnaire targeted at all the pregnant women attending the PHCs for ANC services.

RESULTS: All the PHCs in the two LGAs provide ANC and IPTp-SP services free of charge as directed by the Nigerian Federal Ministry of Health under the malaria control programme with high utilization rates. However, 40 % of the PHCs in Giwa LGA reported an irregular supply of the drug compared with Sabon Gari LGA which reported only 20 %, which could be as result of remote location of most of the PHCs in Giwa LGA. Though, 80 % of the PHCs in the two LGAs provide pharmacy services, only 15 % of them were staffed by pharmacy technicians. More than half of the PHCs in the two LGAs provide FANC but only about 30 % of them are practicing DOT. A significant gap was noted in the practice of the IPTp strategy at the PHCs due to poor understanding of the program by most of the heads of the facilities, whereby only 50 % of them had a satisfactory understanding of what IPTp is all about and only 60 % of them attended a training on IPTp programme at least once since its inception and only 20 % of the PHCs have a written guideline on the IPTp programme. Although all the heads of the PHCs correctly administer 3 tablets of SP to the pregnant women, it was observed that 50 % of them incorrectly did so in the first trimester, a big problem which could directly be related to lack of proper training on the programme.

CONCLUSION: For the programme to succeed there should be a regular training of the health workers, especially those heading the PHCs. A reliable SP supply system should be set up from procurement stage through national manufacture or importation channel to storage, distribution and ultimately to the end users as conveniently and successfully practiced during the Nigeria’s Petroleum Trust Fund (PTF) era.

P338: Trend of Malaria morbidity in Kersa, Southwest Ethiopia

Seleshi Kebede1, Abraham Asefaw2, Jihad Kemal1, Nasir Abdo1 and Girmay Medhin1

1Addis Ababa University and 2Armvaen Hansen Research Institute and 3Jimma Zone Health offices

Malaria is a highly infectious disease, causing the major cause of disease and death in Ethiopia, especially among children and pregnant women. The aim of the study is to assess the trend of malaria morbidity in Southwest Ethiopia. This paper assessed the trend of malaria cases from September 2005 to August 2011. A retrospective analysis of daily outpatient consultation records was obtained from Jimma Health Bureau. Moreover, One year cross-sectional blood film examination was performed in Bulbul, Serbo and Bala Wajo health centers in Southwest Ethiopia. Data were entered and checked, thereafter analyses were performed using excel and SPSS version 16 software. Descriptive statistics was used to assess the trend of malaria cases detected over six year period. We assessed 6 years trend of malaria case in Kersa area between September 2005 to August 2011. A total of 57482 malaria cases were diagnosed in the three Health centers. Among these, a total of 15865 were children under five years of age. The majority (88.80%) of malaria cases were reported in 2006/2007. Moreover, the percentage of P.falciparum and P vivax were 57.37% and 42.63% respectively. To minimize reliability and validity of secondary data, one year cross sectional analysis was performed in Kersa Woreda from three health centers. Concerning the one year cross-sectional study, males were more affected (60.1%) than females (39.9%). In the same year 33.8% of the positive cases were children. The proportion of malaria cases detected among clinical suspects over the 5 year period was 51.13% On the other hand the proportion of malaria cases was during one year blood film examination was 25.32%. Despite recent decline in malaria consultation rates, malaria was a problem in Kersa. Furthermore results presented in this study suggest that the burden of malaria in children <5 years of age is still significant. Our assessment indicated that annually, malaria consultations peaked during September to December which coincides with the end of the rainy season.

P339: Genetic Polymorphism of Apical Membrane Antigen-1, Malaria Vaccine Candidate, in Plasmodium falciparum isolates from Niété, South-Cameroon

Jude D. Bigoga1,2, Rose G.F. Leke1,2, Arnaud D. Kaze3,4

1Department of Biochemistry, Faculty of Science University of Yaoundé I, Yaoundé, Cameroon; 2The Biotechnology Center, University of Yaoundé I, Yaoundé, Cameroon
3Faculty of Medicine and Biomedical Sciences, University of Yaoundé I, Yaoundé, Cameroon; 4Annex Regional Hospital, Ayos, Center Region, Cameroon

BACKGROUND: The apical membrane antigen-1 (AMA-1) of P falciparum is one of the most promising malaria vaccine candidates. Nevertheless, like most blood stage antigens, AMA-1 is highly polymorphic, posing challenges for vaccine development. Recent studies suggested that the allelic diversity in ama-1 could be covered by vaccination with a combination of allelic types. Therefore, it is important to know how many alleles will need to be included in an AMA1-based vaccine to induce antibodies with specificity broad enough to recognize the existing antigenic diversity. Thus, this study was designed in order to assess the feasibility of incorporating the AMA-1 in a future malaria vaccine in Cameroon.

METHODS: A cross-sectional study was carried out in January 2011 in Niété, South-Cameroon. Blood samples showing P. falciparum mono-infections upon microscopy and Nested PCR were genotyped to check for ama-1 polymorphism by PCR-RFLP using the restriction enzymes Msel, BfuI, and Spl. The ama-1 K1, 3D7, and HB3 alleles were identified as single fragments of 285, 400, and 335 base pairs, respectively, when subjected to digestion with Msel, Spl, and BfuI, respectively. The CME-1 allele was identified in absence of digestion with all the three enzymes.

RESULTS: Overall, 61 P. falciparum isolates were successfully scored for ama-1 genotyping based on PCR-RFLP method. Four allelic families were
observed, namely H83, K1, 3D7 and CMP-1, based on classification from Marshall et al. H83 was the most prevalent in the study area (45.9% of the isolates) followed by K1 (36.1%) and 3D7 (23%). The prevalence of CMP-1 allele was 13.1% in the parasite population. Two new unidentified alleles were found mixed with H83 and K1.

CONCLUSIONS: Our results suggest that P. falciparum isolates from Niété exhibit a higher ama-1 polymorphism than previously reported. To the best of our knowledge, this is the first study to report the presence of CMP-1 family in sub-Saharan Africa. Sequencing and immunological studies might throw more light on the extent to which variation in ama-1 may compromise its inclusion in a subunit vaccine against P. falciparum in this region of the world.

P340: Exploring the Potential to Enhance Malaria Diagnosis and Treatment by Qualified Health Workers: A Qualitative Study

Sarah V. Kedenga1, Sophie Githinji2, Ahmeddin H. Omar3, Dejan Zurovac4, Sassy Molyneux5, Catherine A. Goodman6.

1KEMRI-Wellcome Trust Research Programme, Kenya; 2Division of Malaria Control, Kenya; 3London School of Hygiene and Tropical Medicine, United Kingdom

INTRODUCTION: Current WHO guidelines stipulate that prompt parasitological confirmation in all patients suspected of having malaria be carried out before any treatment is commenced. Despite this recommendation, studies have demonstrated that even where diagnostics are available, they are not always used, and that health workers do not always prescribe on the basis of test results. Moreover, prescription of antibiotics often increases when malaria diagnostics are introduced. This study therefore seeks to understand why this is so and how malaria case management can be improved in the context of Kenya, which has been gradually rolling out rapid diagnostic tests (RDTs) in public health facilities since mid 2011.

METHOD: We conducted a qualitative study, drawing on the perspectives of a wide range of health workers and other stakeholders with a role in malaria diagnosis and treatment. Data were collected in four districts, with varying length of experience with RDTs and varying malaria transmission levels. In each district, clinical and laboratory staff from two dispensaries and two health centres were interviewed, and curative consultations and laboratory activities were observed to understand patient flow and the process of care. Further interviews were conducted with district and national level stakeholders.

RESULTS: Results will be presented on health workers’ views on RDTs and microscopy; their perceptions of the guidelines on use of diagnostics; their reasons for adhering or not adhering to these guidelines; and criteria they use to determine whether to prescribe antibiotics or other medicines. Views will be presented from stakeholders at all levels on the adequacy of malaria diagnostic training, supervision and supply systems, and their recommendations on how case management and adherence to guidelines can be improved.

DISCUSSION AND CONCLUSION: These data will provide information to policy makers on key barriers to guideline adherence, contrasting findings from settings with longer and shorter experience of RDT use. The data will inform strategies to develop and enhance strategies to optimize malaria case management in public facilities in Kenya and elsewhere.

University of New Mexico Laboratories of Parasitic and Viral Diseases, Centre for Global Health Research, Kenya Medical Research Institute, Kisumu, Kenya. 2Department of Medical Biochemistry, Maseno University, Maseno, Kenya. 3Center for Global Health, University of New Mexico, Albuquerque, New Mexico, USA. 4Department of Pathology, Kenyatta University, Nairobi, Kenya. 5Centre for Global Health Research, Kenya Medical Research Institute, Kisumu, Kenya. 6Department of Psychology, College of Charleston, Charleston, SC, USA.

BACKGROUND: Cyclo-oxygenase (COX; prostaglandin-endoperoxide H synthase)-2-mediated production of prostaglandins regulates the host-immune response to invading pathogens. Although several studies showed that functional COX-2 promoter variants regulate clinical outcomes in autoimmune and inflammatory diseases, the effect of COX-2 promoter variants on development of severe malarial anemia [SMA, hemoglobin (Hb<5.0g/dL)] and erythropoietic responses remain undefined. The relationship between four COX-2 promoter variants (-512 CT [rs20429], -608 T>C [rs20419], -765 G>C [rs20417] and -1195 A>G [rs689466]) and SMA and inefficient erythropoiesis (reticulocyte production index [RPI] <2.0) was therefore examined in children with clinical malaria.

METHODOLOGY: Parasitic children (age, 3-36 months; n=842) were recruited at Siaya District Hospital, western Kenya after the parents/guardians provided written informed consent. Children were stratified according to non-SMA (Hb<5.0g/dL) versus SMA, and RPI<2.0 versus RPI≥2.0 (inefficient erythropoiesis). Complete hematological measurements were obtained and COX-2 promoter variants were genotyped by high throughput TaqMan™ 5′ allelic discrimination Assay-By-Design (Applied Biosystems).

RESULTS: Binary logistic regression analyses, controlling for covariates, demonstrated increased risk of developing SMA in individuals with the -512 TT genotype (P=0.026), and reduced risk of SMA associated with carriage of the -765 GC genotype (P=0.035). Similarly, carriage of the following COX-2 promoter variants were associated with increased risk of inefficient erythropoiesis (RPI<2.0): -512 CT (P=0.037), -608 TC (P=0.033), -765 GC (P=0.029) and -765 CC (P=0.001) genotypes. Additional analysis of haplotype constructs demonstrated enhanced risk of developing SMA for the -512C/-608T/-765G/-1195A [CTGA; (P=0.034)] and TTCA (P=0.025) haplotypes. Furthermore, carriage of the TTCA (P=0.001) and TTGG (P=0.050) haplotypes were associated with an enhanced risk of inefficient erythropoiesis, while presence of the CTCA (P=0.039) and CTGA (P=0.030) haplotypes protected against inefficient erythropoiesis.

CONCLUSION: These results suggest that polymorphic variation at the -512, -608, -765 and -1195 loci of the COX-2 promoter influence the development of SMA and erythropoietic responses in children residing in a holoendemic Plasmodium falciparum transmission region of western Kenya.

P341: Cyclo-oxygenase-2 Gene Polymorphisms Condition Clinical Outcomes of Childhood Severe Malarial Anemia and Erythropoietic Responses in Plasmodium falciparum Holoendemic Region of Western Kenya

Samuel B. Anyona1, Prakash Kemapia1, Evans Raballah1, Tom Were1,3, Gregory C. Davenport4, John M. Vulule1,3, James B. Hittner3, John M. Ong’echa1, and Douglas J. Perkins1,3

1University of New Mexico Laboratories of Parasitic and Viral Diseases, Centre for Global Health Research, Kenya Medical Research Institute, Kisumu, Kenya. 2Department of Medical Biochemistry, Maseno University, Maseno, Kenya. 3Center for Global Health, University of New Mexico, Albuquerque, New Mexico, USA. 4Department of Pathology, Kenyatta University, Nairobi, Kenya. 5Centre for Global Health Research, Kenya Medical Research Institute, Kisumu, Kenya. 6Department of Psychology, College of Charleston, Charleston, SC, USA.

BACKGROUND: Heat shock protein (Hsp) 70 is known to play a key role in the regulation of pro-inflammatory cytokines such as IL-1β, IL-6 and TNF-α that have been shown to influence the pathogenesis of severe malarial anemia [SMA, hemoglobin (Hb<5.0g/dL)]. Several studies demonstrated that glutamine (GLN), a non-essential amino acid, up-regulates the expression of HSP70 both in-vitro and in-vivo, and provides beneficial clinical outcomes in patients with acute illness, chronic inflammation, and inflammatory injury. However, the relationship between GLN, Hsp70, and clinical outcomes has not been explored in individuals with malaria. Towards this end, we first determined circulating GLN levels in P. falciparum (+) children (n=109, age 3-36 mos.), devoid of common

P342: Glutamine ameliorates hemozoin-mediated suppression of heat shock protein 70 and over-expression of pro-inflammatory cytokines in peripheral blood

Prakash Kemapia1, Karol Dokladny2, Gregory C. Davenport3, Zachery S. Karim3, Samuel B. Anyona4, Dukens LaBaze4, John M. Ong’echa5,6, Pope L. Moseley7, Douglas J. Perkins1,3

1Center for Global Health, Department of Internal Medicine, University of New Mexico Health Sciences Center, Albuquerque, NM, USA; 2Department of Internal Medicine, University of New Mexico Health Sciences Center, Albuquerque, NM, USA; 3University of New Mexico/KEMRI Laboratories of Parasitic and Viral Diseases, Kisumu, Kenya.

Heat shock protein (Hsp) 70 is known to play a key role in the regulation of pro-inflammatory cytokines such as IL-1β, IL-6 and TNF-α that have been shown to influence the pathogenesis of severe malarial anemia [SMA, hemoglobin (Hb<5.0 g/dL)]. We conducted a qualitative study, drawing on the perspectives of a wide range of health workers and other stakeholders with a role in malaria diagnosis and treatment. Data were collected in four districts, with varying length of experience with RDTs and varying malaria transmission levels. In each district, clinical and laboratory staff from two dispensaries and two health centres were interviewed, and curative consultations and laboratory activities were observed to understand patient flow and the process of care. Further interviews were conducted with district and national level stakeholders.

RESULTS: Results will be presented on health workers’ views on RDTs and microscopy; their perceptions of the guidelines on use of diagnostics; their reasons for adhering or not adhering to these guidelines; and criteria they use to determine whether to prescribe antibiotics or other medicines. Views will be presented from stakeholders at all levels on the adequacy of malaria diagnostic training, supervision and supply systems, and their recommendations on how case management and adherence to guidelines can be improved.

DISCUSSION AND CONCLUSION: These data will provide information to policy makers on key barriers to guideline adherence, contrasting findings from settings with longer and shorter experience of RDT use. The data will inform strategies to develop and enhance strategies to optimize malaria case management in public facilities in Kenya and elsewhere.
co-morbidities (i.e., bacteremia and HIV-1), from a holoendemic malaria transmission region of western Kenya. Children with SMA had reduced GLN levels (mean=558±M, n=43) compared to the non-SMA group (Hb≥5.0 g/dL, mean=765±M, n=66, P<0.01). In addition, children with SMA had significantly reduced peripheral blood leucocyte HSP70 transcript levels (P<0.001) and elevated levels of IL-1β, IL-6 and TNF-α (P<0.05, P=0.075 and P<0.05, respectively). To explore the molecular interactions between GLN and Hsp70 in the context of malaria, peripheral blood mononuclear cells (PBMCs) were stimulated with *Plasmodium falciparum*-derived malarial pigment, hemozoin (PfH). Treatment of PBMCs with PfH caused suppression of HSP70 transcripts and protein (P<0.001 and P<0.05, respectively) and over-expression of Hsp70-modulated inflammatory mediators (i.e., IL-1β, IL-6 and TNF-α; P<0.001 for all). The addition of GLN dose-dependently rescued the inhibitory effect of PfH on Hsp70 production (P<0.05), and suppressed the over-expression of IL-1β, IL-6 and TNF-α (P<0.001 for all). Taken together, these findings demonstrate that GLN levels and HSP70 are suppressed in children with SMA, and that GLN treatment can overcome the PfH-induced suppression of Hsp70, and over-expression of pro-inflammatory mediators in vitro.

**P343: Interferon gamma (IFN)-g promoter haplotypes influence erythropoietic responses in Kenyan children residing in a holoendemic *Plasmodium falciparum* transmission area**

**Evans Rabalabak, Gregory C. Davenport, Collins Ouma, Samuel B. Anyona, Tom Were, Prakash Kemparaiah, John M. Vulule, James B. Hittle, Douglas J. Perkins**

**University of New Mexico Laboratories of Parasitic and Viral Diseases, Centre for Global Health Research, Kenya Medical Research Institute, Kisumu, Kenya; Department of Medical Laboratory Sciences, Masinde Muliro University of Science and Technology, Kakamega, Kenya; Center for Global Health, Department of Internal Medicine, University of New Mexico, Albuquerque, NM, USA; Centre for Global Health Research, Kenya Medical Research Institute, Kisumu, Kenya; Department of Psychology, College of Charleston, Charleston, SC, USA

**BACKGROUND:** *Plasmodium falciparum* malaria is among the leading causes of morbidity and mortality among African children. In *P. falciparum* holoendemic transmission areas of western Kenya, severe malaria commonly manifests as severe splenomegaly (SMA; haemoglobin (Hb) ≤5.0 g/dL) in children. Interferon-gamma (IFN-g) is a pleotrophic cytokine associated with both protection and pathogenesis in severe malaria. Since the functional role of IFN-g variants in conditioning susceptibility to SMA is inconclusive, the relationships between IFN-g polymorphisms (-183 G/T, rs2069709 and -1616 A/G, rs2069705), their haplotypic structures, SMA and erythropoietic responses were investigated.

**METHODS:** Parasitaemic children (aged 3-36 mos.; n=744) presenting at a rural hospital in western Kenya were recruited. DNA was extracted from buccal swabs (epicentre)™. 3.0 mL of whole blood was obtained and full haemogram performed (Beckman Coulter AU 582)™. Parasitaemia was determined on Giemsa-stained blood smears. IFN-g -183 G/T was genotyped by RFLP, while IFN-g -1616 A/G was genotyped using a Taqman™ 5′ allelic discrimination assay. IFN-g concentrations were determined as part of a Cytokine 25-plex Antibody Bead Array.

**RESULTS:** Bivariate logistic regression analysis, controlling for age, gender, sickle-cell trait, parasitaemia, and HIV-1 status, demonstrated that relative to homozygous -1616 A (wild) individuals, carriage of GA genotype was associated with a reduced risk of inefficient erythropoiesis (RPI=2) (OR, 0.564; 95% CI, 0.323-0.983; P=0.043), while carriage of AG genotype showed a trend towards reduced risk of inefficient erythropoiesis (OR, 0.556; 95% CI, 0.280-1.104; P=0.094). Additionally, carriage of GA haplotype was associated with an increased risk of inefficient erythropoiesis (OR, 2.005; 95% CI, 1.573-2.555; P<0.001), while GG (OR, 0.525; 95% CI, 0.412-0.668; P<0.001) and TG (OR, 0.147; 95% CI, 0.031-0.691; P=0.015) haplotypes were associated with reduced risk of inefficient erythropoiesis. However, none of the genotypes or haplotypes were significantly associated with the development of SMA.

**CONCLUSIONS:** Although the IFN-g promoter genotypes and haplotypes investigated are significant predictors of the erythropoietic response in children with malaria, they appear only indirectly associated with the development of SMA. An explanation for these findings may be related to differences in the temporal scale of events in which inefficient erythropoiesis precedes the clinical outcome of SMA.

**P344: The ethics of returning aggregated genomic results to research participants in developing countries**

**Angeliki Kerasidou**

MalariaGEN Ethics Coordinator, The Ethox Centre, University of Oxford

One of the main ethical issues related to genetic and genomic research is informing participants of research results. The prevailing view, regarding results disclosure to research participants is that researchers have an obligation to feedback individual information to participants or provide access to findings. Depending on: (i) the type of research project (primary or secondary research); (ii) the types of results produced (aggregated or individual); (iii) the relationship between the participants and the researchers (whether the researcher is also the clinician); (iv) the nature of the findings (e.g. analytically valid, treatable, etc.); and (v) the expectations of participants (have they been promised individual results?), the level of responsibility a researcher has to inform participants and participating communities varies, some have argued. Yet, the moral obligation of returning results is widely acknowledged, and it is based on the ‘right to know’, the principle of autonomy, respect for persons and on promoting trust relationships between researchers and research communities, and the public. However, all the suggestions and guidelines so far regarding returning genetic and genomic results to participants are focusing at biobanks situated in developed countries. The practice and ethical implications of returning aggregated genomic results to research participants in developing countries has not been examined yet. Drawing from the MalariaGEN experience, I am going to look at the ethics and practice of informing research participants of aggregated genomic findings in developing countries. I will examine the moral justification for returning results and the particular issues that might arise. I will conclude that the moral obligation to return results remains, however, the implementation of this obligation could differ according to the particular circumstance of each study and research site.

**P345: Role of T Regulatory and Th17 Cell during Lethal *Plasmodium berghei ANKA* and Non-Lethal *Plasmodium yoelii* infection**

**Tarun Keswani, Bhattacharyya**

Immunology Laboratory, Department of Zoology, University of Calcutta, India

**BACKGROUND:** The outcome of malaria infection is determined, in part, by the balance of pro-inflammatory and regulatory immune responses. Host immune responses in disease including malaria are finely regulated by the opposing effects of Th17 and T regulatory (Treg) cells. **METHODS:** Male Swiss albino mice infected with *P berghei ANKA* and *P. yoelii* respectively with 1 x 10⁶ pRBC, in 100 ul PBS by intraperitoneal injection. Immunohistochemical analysis of Foxp3 and RORγt was observed in spleen tissue. Flow cytometric analysis of CD4+ and CD8+ T, CD4+CD25+ Foxp3+, CD4+IL-17+ RORγt and CD4+IL-2+ expression in splenocytes were performed on respective dpi during both the parasite infections. Western blot was performed to analyze the role of TGF-β, TNF-α, Stat-3, IL-6 and NFAT during the course of infection. ELISA and RT-PCR was further performed as confirmatory tests. **RESULTS:** Here we have examined the role of Treg cells and Th17 cells during malaria infection and find that low levels of Treg cells influence the outcome of infections with the lethal strain of *Plasmodium berghei ANKA* (PBA). In contrast, we observed that possibly high level of Treg cells influencing the outcome of non lethal *Plasmodium yoelii* (P. yoelii)
P346: Discovery of new anti-malarial drugs from African medicinal plants: The Road to El Dorado
Asaad Khalid
Medicinal and Aromatic Plants Research Institute, National Center for Research,

Just like Eldorado (legendary lost city of gold), the road to the discovery of successful drug from medicinal plants could be packed with challenges and adventures. The myriad of structurally diverse compounds found in nature makes them play an important role as a unique source for drug discovery, but they often skillfully play Hide-and-seek and/or hard-to-get. Even though, most of the FDA approved drugs are either natural products or natural product-derived compounds. Natural products are characterized by its unorthodox and often unanticipated chemical structures that offer novel leads of clinically useful drugs. Studies have demonstrated that the hit rate of natural products is on average 3-10%, compared with ~ 0.03% of that of compounds from synthetic origin. Anti-malarial drug discovery could follow any of the two approaches i.e. cell-based and/or target-based. Recent reports show that about 50% of small molecule drugs are enzyme inhibitors. For malaria, enzymes represent the major class of current anti-malarial drug targets. Moreover, medicinal plants have contributed some of the most successful anti-malarials such as quinine and artemisinin.

This lecture will give an overview of our research on the inhibition of neglected diseases-related enzymes. Our target and cell-based antimalarial drug discovery research will also be highlighted. This research has led to the identification of very interesting anti-plasmodial properties of some Sudanese medicinal plants.

P347: Sustained multiplicity among asymptomatic malaria infections in the dry season in an area of seasonal transmission
Amani Kheir1,4, Nizar Enweji 1, Susanne Kerje 1, Abdel-Muhisin A. Abdel-Muhisin2, Göte Söderberg 1, Hamza A. Babiker 2
1Department of Medical Biochemistry and Microbiology, Uppsala University, Sweden, 2Biochemistry Department, Faculty of Medicine, Sultan Qaboos University, Oman 3Tropical Medicine Research Institute, National Center for Research, Sudan, 4Afzad University for Women, Omdurman, Sudan

In areas of seasonal transmission, asymptomatic parasite carriers maintain a large parasite reservoir that raise annual malaria epidemics. Previous field studies in eastern Sudan reported that some patients, who acquired infections during the short wet season, maintained sub-patent parasitaemia throughout the lengthy dry season in the apparent absence of mosquito transmission. In the present study we monitored the dynamics of Plasmodium falciparum parasites, in a longitudinal follow up, among 83 infected patients, over a 13-month period. Analysis of microsatellites flanking pfdhfr and pfdhps genes revealed consistent fluctuation of the same alleles in some asymptomatic carriers during the dry season. However, some showed successive new infection at the beginning of the next rainy season indicative of new infection. However, dynamics of persisting parasites survival have significant association with drug resistance.

P348: A Guide to Engaging Local Communities and Stakeholders in Integrated Vector Management
Lydiah Kibe1, Joseph Mwangangi1,2, Charles Mbogo1,2
1Kenya Medical Research Institute, Centre for Geographic Medicine Research Coast, Kilifi; 2Malaria Public Health Department, Kenya Medical Research Institute - Wellcome Trust - University of Oxford Programme, GPO, Nairobi, Kenya.

BACKGROUND: The global strategic framework for integrated vector management (IVM) identifies community empowerment and inter-sectoral collaboration among its key elements. However, programmes implementing IVM face difficulties in ensuring effective community empowerment and inter-sectoral collaboration for lack of guidelines for effective community and stakeholders engagement. The objective of this study was to provide a guide to engaging local communities and stakeholders in integrated vector management programmes.

METHODS: The guide relies on the research work of KEwPA icipe IVM Programme in Malindi, Coastal Kenya. The research aimed at using the IVM approach based on its 5 elements; capacity building, integrated approach, evidence based decision making support, advocacy and social mobilization and inter-sectoral collaborations. Community empowerment and inter-sectoral collaborations was achieved by strengthening advocacy, communication and social mobilization capacities for malaria control to ensure that at least 80% of people in Malindi and coastal region have knowledge on prevention and treatment of malaria through trainings, workshops and the development of appropriate advocacy for uptake of specific malaria interventions. Three community strategies were employed to increase uptake and implementation of IVM in Malindi which are a) School Mosquito Health Clubs, b) Community based Mosquito Control and c) Annual Mosquito Field week. Further, key health stakeholders with the ministry of health taking the lead were involved in the IVM implementation strategies.

RESULTS & DISCUSSION: The process identified 8 - point elements for engaging local communities and stakeholders in integrated vector management. They are community entry, needs assessment, capacity building, communication, operational support, accountability, monitoring and evaluation and sustainability. Each of the element is highlighted stating the objectives, tools used, process and results obtained. We have also identified the strengths and challenges of the process.

CONCLUSION: Despite the challenges, the guide is useful to programmes engaging local communities and stakeholders like in integrated vector management.

P349: Malaria associated changes in Syncytiotrophoblast function
Winifreda B. Kidima1, Rose G. F. Leke2, James M. Burns3, and Diane W. Taylor4
1 University of Hawaii at Manoa, Hawaii, USA; 2 University of Yaoundé, Yaoundé, Cameroon; 3 Drew University, College of Medicine, Philadelphia, USA

BACKGROUND: Plasmodium falciparum infections during pregnancy increase the risk of women having poor pregnancy outcomes, including low birth weight (LBW) babies. P. falciparum infected erythrocytes (IE) sequester in the intervillous space (IVS) of the placenta leading to an inflammatory response, creating pathology that may lead to poor neonatal outcomes. ST which line the IVS play a critical role in foetal development. It is not fully understood how PM changes ST functions. Therefore, the aim of this study was to elucidate the influence of PM on ST functions.

METHODS: In Cameroon, biopsies of 30 placenta were collected within 15 minutes of delivery, washed free of maternal blood, and foetal cells were placed in RNAlater. The biopsies contained foetal ST, stroma, macrophages and blood vessel endothelial cells. Microarray analysis was done on biopsies from 3 PM+ positive samples compared with a pool of
P350: The impact of malaria on haemoglobin levels of pupils at different altitudes in the Mount Cameroon area

H Kimbi

BACKGROUND AND AIDS: Anaemia is one of the most important complications of malaria in Sub-Saharan Africa. Altitude also plays an important role in the prevalence and transmission of malaria and consequently, malaria-related anaemia. This work was aimed at assessing the impact of malaria on the haemoglobin levels of pupils at different altitudes in the Mount Cameroon area.

METHODS: A total of 728 pupils aged 4-15 years were enrolled in the study. Demographic and socio-economic data were recorded on each child. Blood samples were collected by venepuncture. Haemoglobin (Hb) levels and other red cell indices were determined using the couter counter. Malaria parasite density and prevalence were determined from Giemsa-stained blood smears.

RESULTS: The overall malaria parasite prevalence was 33.8%. Children at lowlands had a significantly higher (P < 0.001) malaria parasite prevalence (60.6%) when compared with their counterparts at higher altitudes. Malaria parasite prevalence was significantly higher (P = 0.015) in febrile pupils (40.4%) and highest (42.8%) in the youngest age group (P = 0.003). The overall prevalence of anaemia was 19.8%. The mean haemoglobin (Hb) was 11.84 ± 1.37g/dl. Hb value was significantly lower (P = 0.01) in the youngest age group while anaemia prevalence was significantly higher (P = 0.02) in the same age group when compared with the older age groups. Malaria positive participants had a significantly lower (P < 0.001) mean Hb than their malaria negative counterparts. The mean Hb level of subjects was significantly lower (P < 0.001) in pupils in the lowlands when compared with those from higher altitudes. A statistically significant negative correlation (r = -0.188, p = 0.003) was observed between mean Hb level and parasite density. Amongst the anaemic subjects, malaria parasite negative pupils had a higher mean Hb level when compared with their parasite positive counterparts.

CONCLUSION: Malaria leads to lower haemoglobin levels in pupils and contributes enormously to the prevalence of anaemia. Anti-malarial measures need to be intensified in order to reduce the prevalence of malaria-related anaemia in pupils in the Mount Cameroon region.

P351: Space-time modelling of malaria and its co-distribution with malnutrition risk among children under the age of five years in Somalia

Damaris Kinyoki1, Ngianga-bakwin Kandala2, Jay Berkley1 Abdisalan Noor2
1Kenya Medical Research Institute-Wellcome Trust Research Programme; 2University of Warwick

BACKGROUND: Several studies in developing countries have shown that malaria is associated with greater malnutrition morbidity and mortality and that there is a transient weight loss in young African children following a malaria attack. The majority of studies that have investigated the association of malaria and malnutrition were done in clinical settings and there are limited population-wide investigation of the co-epidemiology of malaria and malnutrition. In 2011, rate of acute malnutrition in Somalia was cited to be the highest worldwide. Plasmodium falciparum prevalence in Somalia also varies from very low to moderate transmission. This study seeks to map the distribution of malaria and malnutrition among children under the age of five years at similar spatial and temporal resolutions over the period 2007-2010 in Somalia and determine their spatial and temporal co-distribution and risk factors.

METHODS: The main objective is to map space-time distribution of both malaria and malnutrition and determine their co-distribution among children under the age of five years in Somalia. This will be accomplished using geostatistical modelling of malaria and malnutrition using SPDE methods in INLA Bayesian algorithm. The co-distribution of the two health problems will be compared using several methods namely; shared component method, multinomial method and Multivariate intrinsic conditional autoregressive (ICAR).

RESULTS: Preliminary results show that the shared component distribution (representing social economic and environmental determinants) has a larger effect on malaria and malnutrition in the southern and central part of Somalia.

CONCLUSION: In conclusion, multivariate mapping models provide a better understanding of co-morbidity between health outcomes than using separate univariate models. In particular, the analyst of multiple disease outcomes can assess the underlying common and divergent spatial distributions of the diseases to optimally integrate disease management required to address the multiple burden of diseases in the Sub-Saharan African region.

P352: Novel Approach to Malaria Vaccine Discovery to Eradicate and Control Malaria

Zelalem Kiros

The two developmental stages of human malarial parasite Plasmodium falciparum, asexual and sexual blood stages, were continuously cultivated in vitro. Both asexual and sexual stages of the parasites were assayed for mitochondrial oxygen consumption by using a polarographic assay. The rate of oxygen consumption by both stages was found to be relatively low, and was not much different. Furthermore, the mitochondrial oxygen consumption by both stages was inhibited to various degrees by mammalian mitochondrial inhibitors that targeted each component of complexes I-IV of the respiratory system. The oxygen consumption by both stages was also affected by 5-fluoroorotate, a known inhibitor of enzyme dihydroorotate dehydrogenase of the pyrimidine pathway and by
an antimalarial drug atovaquone that acted specifically on mitochondrial complex III of the parasite. Moreover, antimalarials primaquine and artemisinin had inhibitory effects on the oxygen consumption by both stages of the parasites. Our results suggest that P. falciparum in both developmental stages have functional mitochondria that operate a classical electron transport system, containing complexes I-IV, and linked to the pyrimidine biosynthetic pathway.

**BACKGROUND:** About 3.3 billion people – half of the world’s population – are at risk of malaria. In 2010, 90% of all malaria deaths occurred in the sub Saharan African, mostly among children under five years of age. This is a major priority for the Public Health field.

**METHOD:** Safe and Effective vaccines are responsible for Plasmodium Malaria. Cure of these diseases rests heavily on vaccines but anti Malaria vaccines cure faces multiple challenges. I have established new techniques for antibodies interaction and binding approach to the parasite antigen to control and eradicate Malaria. The goal of this application is to unravel the complexity of this Zelk-M1 in mechanistic detail. Further I believe that discovering and characterizing this Zelk-M1 in its molecular detail will lead me to important insights into the biochemical of Zelk-M1, and ultimately to novel targets for Malaria vaccines interference.

**RESULT:** Zelk-M1 is important human antibodies responsible for Malaria disease. These include the various forms of Malaria (Plasmodium Falciparum, Vaxax, Malaria, and Ovule). Zelk-M1 is unique to the host cell and its function is essential to host cell survival. The structure and biochemistry of the Zelk-M1 is remarkably complex as it is derived from the two bio chemicals.

**CONCLUSION:** Evaluating the Zelk-M1 activity of liver stage and blood stage of different specification will identity those antibodies most effective against malaria antigen binding. This Malarial vaccine which is discovered believe that the design of future malaria Vaccine.

**P535: Long lasting insecticide treated blanket for protection against Anopheles arabiensis: an experimental hut evaluation in Tanzania**


**INTRODUCTION:** Insecticide treated blankets or bed sheets are a potential alternative to LLINs, particularly in disaster or emergency situations and to the pyrimidine biosynthetic pathway. FACTORY methods for antibodies interaction and binding approach to the parasite antigen to control and eradicate Malaria. The goal of this application is to unravel the complexity of this Zelk-M1 in mechanistic detail. Further I believe that discovering and characterizing this Zelk-M1 in its molecular detail will lead me to important insights into the biochemical of Zelk-M1, and ultimately to novel targets for Malaria vaccines interference.

**RESULT:** Zelk-M1 is important human antibodies responsible for Malaria disease. These include the various forms of Malaria (Plasmodium Falciparum, Vaxax, Malaria, and Ovule). Zelk-M1 is unique to the host cell and its function is essential to host cell survival. The structure and biochemistry of the Zelk-M1 is remarkably complex as it is derived from the two bio chemicals.

**CONCLUSION:** Evaluating the Zelk-M1 activity of liver stage and blood stage of different specification will identity those antibodies most effective against malaria antigen binding. This Malarial vaccine which is discovered believe that the design of future malaria Vaccine.

**P354: Hematological Changes in Children with Severe and Uncomplicated Malaria**

Grace Kitonyi1, George Awinda2, and John N Waitumbi

1Department of Haematology, University of Nairobi, 2Walter Reed Project, Kenya Medical Research Institute, Kisumu, Kenya;

**BACKGROUND:** Infection with malaria triggers haematological abnormalities that play a major role in the disease outcome. In this study, quantitative and qualitative haematological parameters in children with severe malaria (SM) and those with uncomplicated malaria (UM) were compared.

**METHODS:** Children presenting at Kisumu District hospital with SM malaria (Hb ≤ 6g/dL) were enrolled as cases and matched by age and gender to children with UM (Hb ≥ 6g/dL). Parent/guardian provided information on illness duration. Thin and thick Giemsa stained blood films were examined for malaria parasites and morphological changes by a haematologist who was blinded to the category of the patient. Total blood counts including red blood cells (RBC), white blood cells (WBC), platelets and differential counts were made on Act 5 Diff haematology analyzer (Beckman Coulter).

**RESULTS:** Haematological parameters from 44 cases and age matched controls were analyzed. The mean age for children with SM was 17.1±4.4 months and 16.7± 5.6 months for UM. The mean duration of illness was higher in SM (4.0, CI 3.0-7.0) compared to UM (3.0, CI 3.0-5.0, P=0.026). The mean hemoglobin in SM group was 4.5 g/dL compared to 8.7 g/dL in the UM. The following cell counts were higher in SM: WBC (15.8 X 10^9/L, CI 9.1- 22.9X10^9/L) compared to UM (10.2 X 10^9/L, CI 8.0-16.4, P= 0.01), lymphocytes (7.0, CI 4.1-9.3) compared to 4.8, CI 3.0-7.4, P=0.022), monocytes (2.2 CI 1.4-5.3) compared to 1.6 1.0-2.9, P=0.017. Mean platelets count were lower in SA (121.5, CI 80-165) compared to UM (173, CI 108-263, P=0.004). There was no difference in the occurrence of anisocytosis, target cells, burr cells, schistocytes, bite cells, hypochromia and nucleated RBC's in the 2 groups. Polychromasia and spherocytes were more frequent in SM than UM. Striking photomicrographs showing blood cells morphological changes will be presented and discussed.

**CONCLUSION:** It is concluded that, the long duration of illness before reporting to hospital in children with SM probably accounts for deterioration of illness. Lymphocytosis, monocytecytosis, thrombocytopenia, spherocytosis and polychromasia were features more commonly associated with SM group.

**P355: Profiling PfEMP1 variants associated with severe malaria and low host immunity over time**

Cheryl Andisi1, Martin Hunt1, Greg Fegan1, Thomas Dan Otto4, George Githinji1, George Warimwe1, Richard Rance2, Kevin Marsh3, Matthew Berrian1 and Pete Bull1

1KEMRI/Wellcome Trust Research Programme P.O. Box 230-80108 Kilifi, Kenya, 2Wellcome Trust Sanger Institute, Hinxton, Cambridge, CB10 1SA, UK; 3Nuffield Department of Clinical Medicine, John Radcliffe Hospital, University of Oxford, Oxford, UK, 4The Jenner Institute, University of Oxford, Old Road Campus Research Building, Roosevelt Drive, Oxford OX3 7DQ, UK.

**INTRODUCTION:** The feasibility of having a Plasmodium falciparum erythrocyte membrane protein 1 (PfEMP1) based intervention against...
malaria is based on the idea that there might be a limited subset of var gene encoded PfEMP1 variants that are associated with severe malaria. In studies in African children, several previous studies have found an association between “group A-like” var genes and severe malaria and low host immunity. For these variants to be considered viable targets for an intervention against severe disease, information is required on the stability of disease associations over time.

**METHODS:** We investigated this by sequencing the DBLα tag of var genes from 923 clinical isolates from children with different degrees of malaria severity over a 16-year period (1994 to 2012). The study was done within Kilifi District Hospital, located along the Kenyan coast. To estimate the amount of antibodies these children had at the time of disease, we used parasite schizont extract ELISA and flow cytometry on the infected erythrocyte surface as markers of host immunity.

**RESULTS:** We will present a preliminary analysis of the associations of specific subsets of var genes with severe malaria over this period.

**P356: Simplified models of vector control impact upon malaria transmission by zoophagic mosquitoes**

Samson Kiware1, Nakul Chitnis1, Sarah Moore1, Gregor Devine1, Silas Majambere1, Stephen Merrill2 and Gerry Killeen1

1Biomedical and Environmental Thematic Group, Ifakara Health Institute, Ifakara, Tanzania; 2Department of Mathematics, Statistics, and Computer Science, Marquette University, Milwaukee, WI 53201; 3University of Basel, Basel, Switzerland

**BACKGROUND:** High coverage of personal protection measures that kill mosquitoes dramatically reduce malaria transmission where vector populations depend upon human blood. However, most primary malaria vectors outside of sub-Saharan Africa can be classified as “very zoophagic”, meaning they feed occasionally (<10% of blood meals) upon humans, so personal protection interventions have negligible impact upon their survival.

**METHODS:** We extended a published malaria transmission model to examine the relationship between transmission, control, and the baseline proportion of bloodmeals obtained from humans (human blood index). The lower limit of the human blood index enables derivation of simplified models for zoophagic vectors that: (1) Rely on only three field-measurable parameters. (2) Predict immediate and delayed (with an intervention against severe disease, information is required on the stability of disease associations over time.

**RESULTS:**

**P357: Evaluation of *in vitro* sensitivity of field *P. falciparum* isolates to chloroquine, monodesethylamodiaquine, lumefantrine and dihydroartemisinine in three villages in Mali.**

Aly Kodio, Souleymane Dama, Mamadou Tekete, Aminatou Kone, Hamma Maiga, Oumar Yattara, Bakary Fofana, Bakary Sidibe, Cheick P.O. Sangare, Ogboru Dounmo and Abdoulaye Djimde.

Malaria Research and Training Center, University of Bamako, Bamako, Mali.

**BACKGROUND:** The purpose of this work was to assess the in vitro sensitivity of field *P. falciparum* isolates to chloroquine, monodesethylamodiaquine, lumefantrine and dihydroartemisinine in three villages in Mali.

**METHODS:** During in vivo clinical studies, isolates of *P. falciparum* were collected in Kollé, Bancoumana and Faladjé. The sensitivity of these isolates to chloroquine, monodesethylamodiaquine, lumefantrine and dihydroartemisinine were measured by pLDH ELISA. The cut-offs for resistance to chloroquine, monodesethylamodiaquine, lumefantrine and dihydroartemisinine were estimated to be 100 nM, 80 nM, 150 nM and 10 nM, respectively. We used nested PCR followed with restriction enzymes to detect polymorphisms of *PfK76T*, *PFATPase 6 S769N* and *PFMDR1 N86Y*.

**RESULTS:** 59 isolates of *P. falciparum* collected in Kollé, Bancoumana and Faladjé were included and we had 47 successful pLDH test. Geometric means IC_{95} were for chloroquine 143.94, 156.53, 163.76 nM in Bancoumana, Faladjé and Kollé respectively (P = 0.630); for monodesethylamodiaquine 34.26 nM 33.25 nM, 36.68 nM in Bancoumana, Faladjé and Kollé respectively (P = 0.575); for lumefantrine 10.65, 7.95, 16.79 nM in Bancoumana, Faladjé and Kollé respectively; for dihydroartemisinine in Bancoumana, Faladjé and Kollé respectively 0.69; 0.81; 0.85 nM (P = 0.551). The rates for Pfcrt 76T were in Bancoumana, Faladjé, Kollé 75%, 78.6%, 76.2%, respectively (P = 0.975). The prevalence of Pfmdr1 R691K was in Bancoumana, Faladjé and Kollé were 91, 7% and 100%; 90% respectively. No point mutation was observed at PfATPase 6 5769N and Pfmrdr 1 N86Y.

**CONCLUSIONS:** Monodesethylamodiaquine, dihydroartemisinine, and lumefantrine were efficacious against *P. falciparum* isolates but chloroquine was ineffective in these three villages.

**P358: Assessing options for maintaining universal coverage with LLIN in Tanzania**

Hannah M Koenker1, Joshua O Yuki1, Alex Mkindii, Renata Mandike1, Nick Brown3, Albert Kilian1, Karen Kramer3 and Christian Lengeler2

1Johns Hopkins University School of Public Health, Center for Communication Programs, Baltimore, Maryland USA; 2Tulane University, New Orleans, Louisiana, USA; 3National Malaria Control Program, Dar es Salaam, Tanzania; 4Tropical Health LLP; 5Swiss Tropical and Public Health Institute, Basel, Switzerland

**BACKGROUND:** Tanzania achieved universal coverage with long-lasting insecticidal nets (LLINs) in October 2011, after three years of free mass net distribution campaigns and is now faced with the challenge of maintaining high coverage as nets wear out and the population grows. A process of exploring options for a continuous or “Keep-Up” distribution system was initiated in early 2011. This was the first time a comprehensive national assessment was conducted to review the major considerations, findings and recommendations for the implementation of a new strategy.

**METHODS:** Stakeholder meetings and site visits were conducted in five locations in Tanzania to gather stakeholder input on the proposed distribution systems. Coverage levels for LLINs and their decline over
time were modelled using NetCALC software, taking realistic net decay rates, current demographic profiles and other relevant parameters into consideration. Costs of the different distribution systems were estimated using local data.

RESULTS: LLIN delivery was considered via mass campaigns, Antenatal Care-Expanded Programme on Immunization (ANC/EP), community-based distribution, schools, the commercial sector and different combinations of the above. Most approaches appeared unlikely to maintain universal coverage when used alone. Mass campaigns, even when combined with a continuation of the Tanzania National Voucher Scheme (TNVS), would produce large temporal fluctuations in coverage levels; over 10 years this strategy would require 63.3 million LLINs and a total cost of $444 million USD. Community mechanisms, while able to deliver the required numbers of LLINs, would require a massive scale-up in monitoring, evaluation and supervision systems to ensure accurate application of identification criteria at the community level. School-based approaches combined with the existing TNVS would reach most Tanzanian households and deliver 65.4 million LLINs over 10 years at a total cost of $449 million USD and ensure continuous coverage. The cost of each strategy was largely driven by the number of LLINs delivered.

CONCLUSION: The most cost-efficient strategy to maintain universal coverage is one that best optimizes the numbers of LLINs needed over time. A school-based approach using vouchers targeting all students in Standards 1, 3, 5, 7 and Forms 1 and 2 in combination with the TNVS appears to meet best the criteria of effectiveness, equity and efficiency.

P359: Insecticide resistance status of malaria vectors in Cote d’Ivoire (West Africa): an updated data base

Alphonsine A Koffi1,2, Ludovic P Ahoua Alou1,3, Jean-Paul K Kablan1,4, Pierrick Labbé5, Fabrice Chandre6, Raphael N’Guessan1,2, Cédric Pennetier1,4
1 Institut Pierre Richet (IPR), BP 47 Abidjan, Côte d’Ivoire; 2 Laboratoire de Zoologie et Biologie Animale, Université FHB, 22 BP 582 Abidjan 22, Côte d’Ivoire; 3 Institut des Sciences de l’Évolution de Montpellier (UMR 5554, CNRS-UJM2-IRD), CC65, Université Montpellier 2, 34095 Montpellier cedex 05, France; 4 Institut de Recherche pour le Développement (IRD)/UMR 224 MIVEGEC, Montpellier, France; 5 London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK; 6 Institut de Recherche pour le Développement pour le Développement (IRD)/UMR 224 MIVEGEC, Montpellier, France; 7 Centre de Recherche Entomologique de Cotonou, Cotonou 06 BP 2604, Bénin

BACKGROUND: Insecticide resistance is the major threat that might undermine the efficacies of malaria vector control programs in endemic countries. National Malaria Control Programs (NMCPs) need as much information as possible about the resistance status of the malaria vectors, the underlying mechanisms and the forces driving these mechanisms evolution to implement the most relevant control strategy. In this context, we investigated the whole country across different ecological settings and agricultural practices to generate an updated baseline data on insecticide resistance and the underlying mechanisms driving resistance among malaria vector populations.

METHODS: Anopheles gambiae s.s. larvae from 15 localities including the six sentinel sites were collected in breeding sites and reared until emergence. Resistance status of the populations to conventional insecticides was assessed using WHO bioassay test kits for adult mosquitoes with four classes of insecticides used in public health with and without pre-exposure to an inhibitor pyreonyl butoxyde (PBO). Molecular and biochemical assays were carried out to identify resistance genes (kdr and ace-1) in individual mosquitoes and to detect potential increase in mixed function oxidase (MFO) levels, non-specific esterases (NSE) and glutathione S-transferases (GST) activities.

RESULTS: Result showed high levels of resistance to three of the four classes of insecticides (organochlorides, pyrethroids and carbamates) widespread across the country. This resistance is multi-factorial including target site mutation and metabolic base. kdr and ace-1 mutations were detected in almost all localities. Significantly higher quantities of MFO, NSE and GST activities were detected in An. gambiae from all most localities than in the Kismu susceptible strain. The involvement of MFO and NSE in the phenotypic resistance, investigated through bioassays after a pre-exposure with PBO, was highly significant against both pyrethroids and carbamates.

CONCLUSION: The relationship between this resistance status, the presence of the target-site and metabolic resistance mechanisms and a potential failure in vector control strategies (LLIN and IRS) ongoing in the country is still unclear. Additional studies of the impact of such mechanisms on vector control efficacies must be run. This updated data base gives authorities crucial information for decision making for insecticide resistance management as recommended by the global plan.

P360: Plasmodium parasites interaction with reactive oxygen species during uncomplicated and complicated malaria infection in Ghanaian children

Richard Asmah1, Litowell Asare1, Isaac Sarshah1, Charles Brown2, Selorme Adupko3, M Cham4, Ben Gyan4, Micheal Ofor2, David Adjei, Edwin Wriedu5, Patrick Ayeh-Kumi6
1 Department of Medical Laboratory Sciences, University of Ghana School of Allied Health Sciences, Accra; 2 Noguchi Memorial Institute for Medical Research, College of Health Sciences, University of Ghana; 3 Comboni Hospital, Sokakope, Ghana

BACKGROUND: Malaria is an infectious disease affecting over 200 million people of which 1-2 million die yearly mainly children. The increasing and widespread resistance of malaria parasites to current anti-malarial drugs is a major problem in prevention and treatment of the disease. During malaria infection, oxidative stress in the human host is increased by production of reactive oxygen species (ROS) inducing parasite killing and tissue damage. Superoxide dismutase (SOD) represents an important enzymatic defense against oxidative stress. In this work we investigated the interactions of ROS and plasmodium parasites during complicated and uncomplicated malaria infection in children less than twelve years old.

METHODS: Blood samples were collected from 124 children between 6 months and 12 years for malaria diagnosis and parasite density by light microscopy. Haemoglobin concentration, RBC, platelet, total WBC, neutrophils count and erythrocyte lysate SOD were measured. Malaria parasite DNA damage was evaluated by Comet Assay using a commercial kit. In the uncomplicated and complicated malaria groups, there were 99 and 50 participants respectively.

RESULTS: In the complicated malaria group, cases were infected with P. falciparum parasites with mean parasite density of 127.8/µl, Hb concentration 9.81g/dl, RBC 4.3×1012/l, lymphocytes 3.2×109/l and platelets 164.6×10/l. Females cases had higher SOD activity than males. Positive correlation (r=0.222) between SOD activity and parasite density for cases was observed. DNA Comet assay showed a clear indication of parasite DNA damage. For uncomplicated malaria group, regression analysis showed a positive correlation between erythrocyte SOD activity and parasite density, and, SOD activity and neutrophil concentration.

CONCLUSION: The study demonstrates ROS-mediated damage of malaria parasite DNA in infected red blood cells and variations during complicated and uncomplicated malaria infection in the human host.

P361: Assessing malaria burden during pregnancy in Fana, Mali


INTRODUCTION: Malaria in the tropics is a public health problem among children under five years and pregnant women. The aim of the study was to assess the level of use of Insecticide-Treated bednets (ITNs) and
consequences of malaria in pregnant women and their babies. Materials and Methods: With the technique of “Rapid Assessment” we conducted a cross-sectional study from November 2005 to February 2006 at the Centre for Health Reference of Fana. The thick film and the determination of hemoglobin were diagnostic methods used. The study involved 200 women in antenatal clinics and 200 women in childbirth. Results: At the ANC, anemia (74.37%) was not associated with gravidity (p = 0.112). Peripheral infection (25.13%) was associated with age (p = 0.001) and residence of women (p = 0.005). The primigravida and secondigravida were more susceptible to malaria (p = 0.002), Kanoute B, Kayentao A K, Ongolo A, Kayentao K, Doutou M, Doumouo O. Malaria Research and Training Center, University of Bamako, BP 1805 Bamako, Mali. At delivery, peripheral infection (16, 50%) was associated with gravidity (p = 0.011). Placental infection associated with gravidity (p = 0.025) was 13, 57%. Low birth weight (11.50%) was not related to any measured factor. Prematurity (11, 11%) was associated only with LBW (p<0.001).

CONCLUSION: This work has to have data bases and build an effective strategy for malaria control.

P362: Malaria and helminth coinfection-induced oxidative stress and changes in antioxidant status among afebrile school children in Ibadan, southwest Nigeria

2Institute for Advanced Medical Research and Training (IAMRAT), 3Department of Chemical Pathology and Immunology, 4Department of Pharmacology and Therapeutics, 5Department of Biochemistry, College of Medicine, University of Ibadan, Nigeria.

BACKGROUND: Malaria and helminth infections are common tropical diseases in Sub-saharan Africa. Little is known about the effect of coinfection of the two diseases on oxidative stress and antioxidant status.

OBJECTIVE: The study was therefore conducted to determine the effect of malaria and helminth co-infection on antioxidant status in afebrile school children.

METHODS: Twenty-five afebrile school children with malaria infection, 25 school children with helminth infection, 25 school children with malaria-helminth co-infection and 24 school children negative for both malaria and helminth infections were chosen for the study. Malaria parasite was determined by microscopy while helminth infection was confirmed by Kato-Katz method. Plasma hydrogen peroxide (H2O2), malondialdehyde (MDA), protein carbonyl (PC), xanthine oxidase (XO), NADPH oxidase (NOX), myeloperoxidase (MPX), reduced glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPX), glutathione S-transferase (GST), ascorbic acid (AA) and α-tocopherol (TOC) were determined using standard protocols.

RESULTS: Plasma levels of H2O2, MDA, PC as well as activities of XO, NOX and MPX were significantly higher in children with co-infection of malaria and helminth followed by helminth infected only and malaria infected only relative to uninfected children. GST activity, GSH and AA levels were significantly reduced while SOD and GPX activities were significantly higher in co-infected children followed by malaria infected only and helminth infected only relative to uninfected children respectively. CAT activity was significantly higher in malaria only infected followed by co-infected and helminth only infected children relative to uninfected children. TOC level was however significantly lower in helminth infected followed by co-infected and malaria infected relative to uninfected children.

CONCLUSION: Malaria and helminth co-infection in afebrile school children caused a reduction in antioxidant status as evident from significant increases in oxidative stress indices in plasma namely H2O2, MDA, PC levels and activities of XO, NOX and MPX and consequent depletion of the thiol GSH, AA, TOC and GST activity. This study suggests that helminth and malaria co-infections induce considerable oxidative stress in infected asymptomatic school children.

P363: Does PermaNet® 3.0 protect better than existing PermaNet nets against pyrethroid resistant An. gambiae s.s.?

Benjamin G. Koudou1,2, Loukou Kouassi B1,3, David Malone1 and Janet Hemingway1
1Centre for Neglected Tropical Diseases, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool, L3 5QA, UK; 2Centre Suisse de Recherches Scientifiques, O1 BP 1303, Abidjan O1, Côte d’Ivoire; 3VCC, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool, L3 5QA, UK; 4Vector Biology Department, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool, L3 5QA, UK; 5UFR Science de la Nature, University Nangui Abrogoua, 02 BP 801 Abidjan 02, Côte d’Ivoire

BACKGROUND: The efficacy of the combination LLIN, PermaNet® 3.0 was compared to the pyrethroid-only LLINs, PermaNet® 2.0 and PermaNet® 2.0 Extra against pyrethroid resistant An. gambiae at the household level in two study sites in Côte d’Ivoire (Tiassale and Bouaké).

METHOD: Mosquito collections were made at each site using sentinel rooms employing exit traps (window traps) and sleeping rooms for resting catches. Mosquitoes were analyzed for species composition and resistance status. Species and molecular forms were assessed by PCR; kdr genotyping was performed using the pyrosequencing method.

RESULTS: Prior to net distribution, extremely high frequencies of kdr resistant homozygous (87.0%) and heterozygous (95.4%) individuals were recorded in Tiassale and Bouaké, respectively and WHO susceptibility tests revealed high levels of phenotypic resistance in both sites. Microarray analysis revealed the principal involvement of the L1014S kdr mutation in pyrethroid resistance in Bouaké, whereas both the kdr mutation and metabolic mechanisms were identified in the Tiassale population. In Bouaké, no difference was detected in the monthly total number of uned and blood fed An. gambiae s.s collected with each LLIN type. In Tiassale, exit trap data showed that both PermaNet® 2.0 Extra and PermaNet® 3.0 performed significantly better than PermaNet® 2.0 in terms of reduction in the total number of blood fed mosquitoes, while the mean number of blood fed An. gambiae s.s collected in resting catches, showed that PermaNet® 3.0 was significantly more effective compared with PermaNet® 2.0 and PermaNet® 2.0 Extra, which had comparable efficacy.

CONCLUSION: In Tiassale, it can be concluded that at this site PermaNet® 3.0 performed significantly better than PermaNet® 2.0 and PermaNet® 2.0 Extra. Therefore, in the Tiassalé, PermaNet® 3.0 could be considered the more effective tool for controlling resistant An. gambiae s.s. while in Bouaké site where metabolic mechanisms were not identified as involved in insecticide resistance statistically significant differences between nets were not found.

P364: High prevalence of point mutations in dhfr and dhps genes of P. falciparum isolates from Congolese pregnant women under intermittent preventive treatment with SP

Damien Bakoua1,2, Felix Koukoulila-Koussounda1,2,3, Janey C Vouvoungui1,2,3, Rode Ibara-Okabande1,2, Anissa Sidibe1,2, Lil H Iko1, Francine Ntouni1,2,3
1Fondation Congolaise pour la Recherche Médicale, Brazzaville, Republic of Congo; 2Faculty of Health Sciences, Marien Ngouabi University, Brazzaville, Republic of Congo; 3Institute of Tropical Medicine, University of Tübingen, Tübingen, Germany.

BACKGROUND: Sulfadoxine-pyrimethamine (SP) is currently the recommended drug for malaria intermittent preventive treatment (IPT) in pregnant women. However, in the Republic of Congo, the low efficacy of SP for the treatment of uncomplicated malaria as well as high prevalence of point mutations in genes associated with SP resistance namely dihydrofolate reductase (dhfr) and dihydropyrimidin synthase
P365: Adjusting for heterogeneity of malaria transmission in immune-epidemiological studies

Benno Kreuels1,2, Federica Verra3, Teun Bousema3, Nimako Sarpong4, Lutz Ehale3, Ralf Krumkamp1, Immo Kleinenschmidt1, Chris Drakeley1, Jürgen May1
1Research Group Infectious Disease Epidemiology, Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany; 2Section for Tropical Medicine, 1st Medical Department, University Medical Centre Hamburg-Eppendorf, Germany; 3Faculty of Infectious and Tropical Diseases, Department of Immunology and Infectious Disease, London School of Hygiene and Tropical Medicine, London, UK; 4Kumasi Centre for Collaborative Research, Kumasi, Ghana; 5Faculty of Epidemiology and Population Health, Department of Infectious Disease Epidemiology, London School of Hygiene and Tropical Medicine, London, UK;

BACKGROUND: While heterogeneity of malaria transmission is a well-known phenomenon, its importance in the analysis of longitudinal studies is underestimated. Particularly studies using immune-markers are sensitive to transmission-heterogeneity as antibody responses correlate both with exposure and protection. We show how a protective effect of antibody responses may be confounded by transmission-heterogeneity and present methods for adjustment.

METHODS: Using data from a trial on Intermittent Preventive Treatment in Infants in Ghana (active and passive follow-up from 3-24 months), we measured antibody responses to AMA-1, MSP-119 & MSP-2 antigens at 5 antigens) and clinical malaria (first episode in the 3 months after treatment). The screening of polymorphic codons of dhfr (51, 59 and 108) and dhps (437, 540 and 581) was achieved by PCR-restriction fragment length polymorphism while allele-specific nested PCR of the msp2 gene was used to characterize the parasite genetic diversity.

RESULTS: 246 pregnant women were enrolled. The prevalence of parasite carriage was 30%, 16% and 17% in 0 IPT, 1 IPT and 2 IPT groups respectively. In the 0 IPT group, mutations in codons 51 and 108 of dhfr were detected in 94% and 71% of isolates respectively while 100% of 1 IPT and 2 IPT isolates were found to have these mutations. All the isolates contained dhps mutations regardless of the group. With regard to the genetic diversity, both 3D7 and FC27 allele-types were detected and the multiplicity on infections ranged from 1.4 to 2.1.

CONCLUSION: These findings show a high prevalence of dhfr and dhps mutations in Congolese pregnant women in particular those who have had 1 and 2 IPT-SP doses and underline the need for further investigations assessing the impact of these mutations on the efficacy of this preventive treatment.

P366: A new approach to malaria vector control, Limpopo province: Slow release pyrethroid-impregnated indoor linings

Kruger T1, de Jager C2, Focke W3, Sibanda M4, Bornman MS4
1University of Pretoria Centre for Sustainable Malaria Control (UP CSMC), School of Health Systems and Public Health, University of Pretoria, Pretoria, South Africa; 2Institute of Applied Materials and UP CSMC, University of Pretoria, Pretoria, South Africa; 3UP CSMC, Department of Urology, University of Pretoria, Pretoria, South Africa;

BACKGROUND: Indoor Residual Spraying (IRS) and Long-Lasting Insecticide-Treated Nets (LLINs) are some of the most effective malaria vector control methods. The organochloride pesticide DDT is still used for IRS and can retain its efficacy for up to twelve months. However, research indicated that potentially harmful levels of DDT residues are present in indoor air for up to three months after spraying. IRS with DDT is still practised in Limpopo province, South Africa. LLINs only provide protection if people sleep under the net. Certain insecticide-impregnated polymers can be used as slow release carriers for vector control, possibly becoming a long term substitute for IRS.

AIMS: To assess the efficacy, durability and community acceptability and perceived efficiency of pyrethroid-impregnated linings in community homes in Vhembe district, Limpopo province, over a six month period.

METHODS: A pilot field trial was completed in 2012 in the community village of Tshilivho in Vhembe district, Limpopo province. Forty households (20 mud huts and 20 brick houses) were included. Monofilament polyethylene meshing with two deltamethrin-, two α-cypermethrin concentrates, or no insecticide (control) were attached along the inside wall of a sleeping hut or room per household. Data on acceptability and perceived efficiency was gathered with questionnaires in Tshivenda, and lining samples were tested bi-monthly for efficacy according to WHO recommended bioassays.

RESULTS: Test mosquitoes were exposed for 30 minutes to field samples, resulting in 100% knockdown (KD) within this time. Within 24 hours 100% mortality was noted. Questionnaire data show that participants welcomed the linings in their homes and they noted that fewer mosquitoes and other biting or irritating insects were present with less biting being reported. Linings will remain in the homes for a further three years to determine efficacy over a longer period.

CONCLUSIONS: Results indicate that the use of pyrethroid-impregnated indoor linings is potentially as successful as IRS. This new and potentially safer method might be a more sustainable approach for vector control.

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P367: mSpray: Using cell-phone technology to monitor population exposure to pesticides used for malaria control

Seto E1, Quirós-Alcalá L2, Lipsitt JM3, Wu LD4, Kruger PS5, Ntimbane T6, Nawn JB7, Bornman R8, Eskenazi B9
1Center for Environmental Research and Children’s Health, School of Public Health, University of California, Berkeley, CA, USA; 2Limpopo Provincial Government: Department of Health, Tzaneen, South Africa; 3National Health
BACKGROUND: DDT and pyrethroids are widely used for Indoor Residual Spraying (IRS) to combat malaria in South Africa and elsewhere. Paper-based records are used to monitor the amount and type of pesticide used, spray coverage and spray operator’s performance. These records do not incorporate the exact location of use at the household level. This method of record-keeping may lead to pesticide overuse, development of pesticide resistance, and unnecessary population exposure to potentially hazardous chemicals.

AIMS: We developed a smart phone “app” called mSpray and pilot-tested its acceptance and utility in capturing accurate GPS-linked information on IRS spray events.

METHODS: mSpray, uses internet services to determine location of pesticide applications. Data is stored in the phones and transmitted to Google data servers, allowing for remote real-time monitoring of spray operations. Ten cell phones were distributed to 13 spray workers in Vhembe, Limpopo, South Africa to pilot test the application during the 2012-13 malaria spray season. Surveys and focus groups were conducted at the end of the spray season to collect information on user acceptance and identify areas of improvement.

RESULTS: More than 1000 spray events were recorded with mSpray. These events could be readily and accurately mapped at the household level with ArcGIS. Surveys and focus groups with spray teams revealed concerns about the functioning of one cell phone and cell phone battery life. Despite this, the majority of users (>75%) felt the system was easy to use and made their jobs easier. All respondents indicated that mSpray was better than the existing paper-based forms.

CONCLUSIONS: Results demonstrate the potential for mSpray to achieve multiple benefits for efficient malaria control towards elimination and in pesticide exposure assessment and epidemiologic studies.

P368: Incidence and consequences of microscopic and sub-microscopic malaria in pregnancy in India: a cohort study

Irene Kuepfer, Anup Kumar Anvikar, Jane Bruce, Neelima Mishra, Navin Kumar, Swati Sinha, Jayne Webster, SK Mishra, Brian Greenwood, Neena Valecha, Daniel Chandramohan

Department of Immunology, Noguchi Memorial Institute for Medical Research, College of Health Sciences, University of Ghana, Legon. 2Department of Biostatistics, School of Public Health, College of Health Sciences, University of Ghana, Legon. 3Division of Malaria Vaccine Development, Walter Reed Army Institute of Research, Silver Spring, MD, USA.

We present the results of microscopic and submicroscopic infections in a cohort of 2494 pregnant women from three ecological zones in India. We describe the incidence of symptomatic, asymptomatic and submicroscopic infection during pregnancy and their effects on birth outcomes.

P369: Sporozoite antigens as alternative markers for malaria transmission intensity estimation

Kwadwo A. Kusi1, Daniel Dodoo1, Samuel Bosomparah2, Eric Kyel-Baafour3, Emmanuel K. Dickson1, Daniel A. Mensah1, Evelina Angov3, Sheetij Dutta3, Martha Sedegah3 and Kwadwo A. Koram1

1Department of Immunology, Noguchi Memorial Institute for Medical Research, College of Health Sciences, University of Ghana, Legon. 2Department of Biostatistics, School of Public Health, College of Health Sciences, University of Ghana, Legon. 3Division of Malaria Vaccine Development, Walter Reed Army Institute of Research, Silver Spring, MD, USA.

Reported malaria cases continue to decline globally, and this has been attributed to the strategic implementation of multiple malaria control strategies over the last few years. Gains made would however need to be sustained through continuous disease transmission monitoring to ensure malaria elimination and subsequent eradication. Tools such as the entomological inoculation rate (EIR) and parasite prevalence rate that are currently available for transmission monitoring are however not sensitive enough, especially in areas of very low transmission.

Transmission estimation models based on seroconversion rates (SCR) derived from the seroprevalence of antibodies to parasite antigens, especially those of the blood stage parasite, are gaining relevance as transmission monitoring tools. SCR estimates have been shown to correlate well with EIR, which is the current standard estimation tool. SCR estimates are however limited by antigenic polymorphism, and more importantly by the persistence of antibodies to blood stage antigens many years after malaria transmission has ceased. These models may thus be ideal for assessing long term changes in malaria transmission but not for short term or seasonal changes.

Prevalence of antibodies to sporozoite antigens may be a better alternative for transmission intensity estimation since these antigens are relatively conserved across Plasmodium species and usually have a very short immune exposure time. Thus measurable levels of antibodies to these antigens will only be found in individuals with recent exposure to infectious bites.

In this study, we estimate the seroconversion rates of antibodies to the sporozoite antigens CSP and CelTOS by a maximum likelihood method using reversible catalytic models and assuming a binomial error distribution. We provide proof of the non-persistence of antibodies to the two sporozoite antigens and show that the seroprevalence of antibodies to these antigens may be capable of predicting seasonal changes in malaria transmission intensity.

P370: The impact of quality systems capacity building on a clinical trial site in Western Kenya

Sylvie Kwedi Nolna, PhD

Capacity for Leadership Excellence And Research ( CLEAR, Inc.)

INTRODUCTION: Clinical research provides the data needed address weak health systems. Clinical trials have been emerging throughout the African continent and many African research sites have benefited from investments in capacity building for the conduct of high-quality clinical trials. A number of foreign and local organizations continuously allocate funds for enhancing the ability to conduct clinical trials. In order to show the impact of such capacity building efforts in a clinical trial site in Western Kenya, the historical trend of quality performance data was evaluated from 2008 to 2012.
**METHODS:** A trending analysis database was built to show a historical collection of observations and comments from various reviews and assessments of the site’s performance and its manner of conducting research. The historical data was compiled from deviation logs, audit reports, monitoring reports, and Quality Control reports of study databases. Performance was defined as the functioning of the site in relation to conformity to guidelines and standards such as Good Clinical Practice (GCPs).

**RESULTS:** The total number of findings and observations decreased by 73% (from 48 to 13) from September 2008 to January 2012 which shows significant improvement in terms of the site’s performance in terms of compliance with GCP guidelines. The historical trend comparing assessments, audits and monitoring activities since 2008 showed an upward trend in the level of compliance with GCP standards. The site showed a great deal of improvement as time went on so to have an excellent audit in the last evaluation performed 2012.

**DISCUSSION AND CONCLUSION:** To better direct resources for capacity building at clinical research sites in developing countries, it is recommended that quality assessments are conducted at these sites. Higher levels of GCP compliance directly translates to outstanding data integrity and protection of the rights of the trial volunteers. In order to be competitive in the world of clinical trials, African sites should able to withstand inspection by highly stringent regulatory authorities.

**P371: Effect of bacteria and algal biomass on Anopheles gambiae s.s aquatic stages and life history traits**

Elingleya J. Kwke,

Dirk L. Schorkopf,

Willem Takken,

Frank Magogo,

Dekker Teun,

Leonard E.G Mboera

1Tropical Pesticides Research Institute, Division of Livestock and Human Health Disease Vector Control, Mosquito Section, P.O.Box 3024, Arusha, Tanzania;

2National Institute for Medical Research, Amani medical Research Centre, Muheza, Tanga, Tanzania;

3Division of Chemical Ecology, Department of Crop Science, Swedish University of Agricultural Science PO 44, Alnarp, SE-23053, Sweden;

4Laboratory of Entomology, Wageningen University and Research Center, Wageningen, The Netherlands;

5National Institute for Medical Research, Headquarters, PO Box 9653, Dar es Salaam, Tanzania;

**BACKGROUND:** Algal biomass and bacteria are important contents for mosquito larvae development. None of the two have been concluded to be the most important food source for larvae survival and growth in natural habitats. This study is investigating the effect of bacteria and algal biomass in the survivorship of larvae, pupation rate, developmental time, sex ratio and body size of emerged adult mosquitoes.

**METHOD:** Anopheles gambiae s.s larvae were reared in three regimes; microcosms with antibiotics (removing bacteria and leave algae), with distilled water and fish food (without algae but with bacteria) and third arm is water with both bacteria and algal biomass from natural distilled water and fish food (without algae but with bacteria) and microcosms with antibiotics (removing bacteria and leave algae). Emergence rates were dosage dependant, more larvae survived in lower doses of both antibiotics. Overall sex ratio by treatment indicated that control was 0.53, gentamicine treatments was 0.54 and in tetracycline treatments was 0.36 with more males than female. Body size is still under investigation from different treatments.

**RESULTS:** Pre-liminary results showed that, control had highest larvae survival than in treatments. In treatments, survival , pupation and emergence rates were dosage dependant, more larvae survived in lower doses of both antibiotics. Overall sex ratio by treatment indicated that control was 0.53, gentamicine treatments was 0.54 and in tetracycline treatments was 0.36 with more males than female. Body size is still under investigation from different treatments.

**CONCLUSION:** More experiments are going on to give a concrete evidence on the effect of algal biomass and bacteria on life history traits of An.gambiae s.s larvae and adults. However, these pre-liminary results suggest that sex ratio is dependent on the type of antibiotic used for bacteria colonies elimination without affecting survivorship and developmental rates.

**P372: Effects of ABO Blood Groups on the Clinical Outcome of Malaria in Ghanaian Pregnant Women**

Pregnancy-associated malaria is a severe form of malaria for which over 50 million pregnant women are at risk. In sub-Saharan Africa, pregnancy-associated malaria is most common in primigravid women and is caused mainly by Plasmodium falciparum variants that have the ability to sequester in placental tissues. This can result in maternal anemia, intrauterine growth retardation, low birth weight and may even lead to maternal and foetal death. Red blood cells from different blood groups (A, B, AB, and O) may differentially support parasite growth and sequestration. Antibodies and cytokines are believed to be important mediators in pregnancy-associated malaria but little information is available on the influence of the ABO blood groups on the production of P. falciparum-specific antibodies and cytokines. This study was conducted to determine the association of ABO blood groups with the plasma levels of IgG, IgM and the cytokines TNF-α and IL10 in eighty pregnant women at various stages of gestation using an indirect ELISA with P. falciparum schizont extract as capture antigens. The results generally show that TNF-α and parasite density were different between certain blood group types. Kruskal-Wallis tests followed by pairwise comparison by Bonferroni post-hoc tests showed that median TNF-α levels (687.2 pg/ml) were higher in blood group O compared to group AB (407.4 pg/ml, p=0.0353) and group B (262.7 pg/ml p=0.0036). Group O pregnant women also had higher median parasite density (940 parasites/μl of blood) compared to the other groups [group A (40/μl of blood, p=0.0017), group AB (0/μl of blood, p=0.0005), group B (40/μl of blood, p=0.0219)]. Linear regression analysis showed levels of IgM as the only predictor of parasite density (p=0.0379). This data suggest that pregnant women with blood group O have higher plasma TNF levels and parasite densities and may be at an increased risk of PAM. The relationship between IgM and parasite density affirms the involvement of new

**P373: Assessing Antimalarial Drug Quality in Resource Poor Settings; Evidence from Afghanistan**

Mirza Lalani,

Harparkash Kaur,

Nader Mohammed,

Omar Amanzai,

Nailea Mailk,

Albert von Wyk,

Haroon Rashid,

Sakhi Jan,

Rishiya Kakar,

Khalid Mojaddidi,

Toby Leslie

1London School of Hygiene and Tropical Medicine, London, UK;

2Health Protection and Research Organisation, Kabul, Afghanistan;

3HealthNet TPO, Kabul, Afghanistan

**INTRODUCTION:** There is little knowledge of the quality of antimalarials in Afghanistan. A previous study in the region demonstrated the presence of poor quality antimalarial drugs. Good quality antimalarials are crucial to reduce the burden of disease and mortality and to limit malaria transmission. The aim of this study was to assess the quality of antimalarials available in both public and private sectors in Afghanistan.

**METHODS:** Antimalarial drug samples were collected from the public and private health sectors in rural and urban locations. Five major trading centres where antimalarials imported from neighbouring countries are available were purposefully selected. In all 7740 individual formulations of available antimalarial tablets, injections and suspensions were collected. Initial screening of 134 selected drug samples was performed using the GPHF-MiniLab®. The definitive quality of the drugs was then assessed by laboratory analysis of the dissolution profile following published United States pharmacopoeial (USP) monographs and measuring the amount of active pharmaceutical ingredient (API) using high performance liquid chromatography (HPLC).

**RESULTS:** A wide range of antimalarials were available, especially in the private sector and when tested for drug quality using the Minilab®, overall 26.2% of samples failed disintegration testing. The failing samples were
résistance moléculaire de Plasmodium falciparum en collaboration avec EPICENTRE, CERMES et FORSANI ont étudié la résistance à la pyriméthamine et de quatre mutations ponctuelles situées sur le gène de la dihydrofolate réductase conférant la résistance à la sulfadoxine-pyriméthamine. Ces mutations majeures décrites correspondent à la présence d’un haplotype, devenu le plus répandu dans le monde. Actuellement, la prévalence de l’haplotype sauvage est de 17.1% en Afrique de l’Est.

La méthodologie portait sur l’étude de séquences nucléotidiques des fragments PCR des gènes pfdhfr et pfdhps puis de reconstituer les haplotypes pfdhfr et pfdhps corrélés à la résistance à la sulfadoxine-pyriméthamine. Un échantillonnage représentatif de la population a été effectué dans le cadre de la mise en œuvre du traitement intermittent préventif (TIP) saisonnier au Niger. Les résultats ont montré que le taux de résistance à la SP avant la mise en œuvre du traitement intermittent préventif intermittent saisonnier au Niger est absente au Niger.

INTRODUCTION: Insecticide treated nets (ITNs) have proven instrumental in the fight against malaria. As distribution of ITNs throughout sub-Saharan Africa (SSA) is being scaled up, however, maintaining coverage over time will be important to sustain current gains. The effectiveness of mass distribution of ITNs, and particularly the duration of effective use, is likely to contribute to the extent of long-term impacts.

METHODS: Mass distribution of ITNs to a rural Kenyan community was performed in early 2011. Surveys collected data on ITN use both before and one year following this distribution. At both times, a complete accounting of ITNs within the home, the location of nets and ages and genders of the persons who slept under them were collected. Data on material possessions, education levels, occupations and community group membership were recorded. The presence of ceiling nets was noted. Spatial methods and regression methodologies were employed to test for associations of various factors to ITN use.

RESULTS: Less than 50% of residents reported using ITNs at the time of distribution. One year following mass distribution, 92% of >5,000 individuals representing ~2,000 households reported sleeping under an ITN the previous evening. However, ITN use varied by age, following a similar pattern both pre- and post-distribution. After infancy, ITN use sharply declined until the late teenage years then began to rise again, plateauing at ~30 years of age. Males were less likely to use ITNs than females. Prior to distribution, socio-economic factors such as parental education and occupation were associated with ITN use. Following distribution, ITN use was even across social groups. Household factors such as availability of nets and sleeping arrangement still present a barrier to consistent use, however.

CONCLUSIONS: Mass distributions of ITNs was effective in rapidly scaling up coverage, with use being maintained at a high level at least one year following. Free distribution of ITNs using a direct to household distribution method can eliminate socio-economic and spatial heterogeneities in ITN possession and use. Age is an important factor in determining consistent ITN use but problems of sleeping arrangement and ITN leakage will present a challenge to effective intervention campaigns.

P375: Insecticide treated net use before and after a mass distribution in a fishing community along Lake Victoria, Kenya

Peter S Larson (corresponding author) Email: arfangen@umich.edu, Noboru Minakawa Email: minakawa@nagasaki-u.ac.jp, Gabriel Dida Email: gdidah@gmail.com, Ed Ionides Email: ionodies@umich.edu, Mark L Wilson Email: wilsonml@umich.edu

Department of Epidemiology, School of Public Health, University of Michigan, 1415 Washington Heights, Ann Arbor, MI 48109-2029, USA; ‘Nagasaki University; ‘Maseno University; ‘Department of Statistics, University of Michigan
the later (Abong Mbang) health district, management of cases was implemented following the usual health system. Three years later, a cross-sectional survey was conducted between September and December 2012 in the three health districts, with 18,177 households, 14,870 women on Reproductive age and 9,655 guardians of children under 5 years. Kaplan-Meier Estimates were used to assess cumulative probability of survival. Chi-square tests of association were used to assess differences in treatment-seeking behavior.

RESULTS: First, the prevalence of fever and diarrhea are almost the same in the implementation districts (14.7% and 20.3%) and control district (15.3% and 25.2%). Comparing the results with the mid term evaluation that was carried out in 2010, the analysis shows that high coverage of appropriate fever and diarrhea treatment was achieved in both intervention districts at mid term (Antimalarial: 57%; ACT: 43%; ORS: 61%; ORS+ZINC: 45%), and was maintained in Nguelemendouka (Antimalarial: 61%; ACT: 51%; ORS: 57%; ORS+ZINC: 39%) but dropped in Doumé (Antimalarial: 46%; ACT: 32%; ORS: 39%; ORS+ZINC: 22%)

All causes of child mortality during the 3 years implementation period (2009 to 2012) compared to the 3 years prior, dropped by10% in Doumé (103.8/1000 vs 118.6/1000), while in Abong-Mbang it remained almost the same over time (77.3/1000 and 77.6/1000).

CONCLUSION: Where coverage is maintained over time, an effective drop of mortality is feasible. To reduce significantly infant mortality, ICCM program must ensure a good coverage of services.

P377: High prevalence of asymptomatic malaria in urban settings in Douala, Cameroon

Léopold Gustave Lehman1, Jeanne Dina Nfon Priso2, Calvin Tonga1, Hervé Nyabeyeu Nyabeyeu1, Larissa Koudjip Nono3, Arlette Linda Ngapmen Yamiadji3, Lafortune Kangam3, Antoine Mouangué2, Adolphe Dikoume Mbongo1, Loïck Pradel Kojom Foko1, Sorelle Wakam Nobou1, Peguy Assomo Ndeamba1, Nicolas Nolla1, Isabelle Matip Mbuo3.

1 University of Douala, Douala, Cameroon; 2 Cameroon Business Coalition against Malaria, Tuberculosis and HIV/AIDS (CBA/SIDA), Douala, Cameroon; 3 University of Yaoundé I, Yaoundé, Cameroon

BACKGROUND: Malaria remains a major health problem in Cameroon with 38% of consultations and 24% of deaths. The negative economical impact of malaria has encouraged a new approach targeting companies with counseling and distribution of prevention kits for workers and their families.

METHODS: A cross sectional study was undertaken from October 2012 to June 2013 in the town of Douala to collect preliminary data to assess the impact of the Exxon Mobil foundation control program in enterprises and communities through indoor spraying and distribution of Long Lasting Insecticidal Nets (LLINs). 2600 inhabitants of six neighborhoods, 829 workers of three enterprises were interviewed and screened with a mass diagnosis method based on malaria rapid blood test using pre-stained slides for fluorescence microscopy (CyScope®, Partec GmbH, Germany). Alongside, 785 children were screened in five schools. All positive cases were treated on the spot.

RESULTS: The prevalence of malaria in the 4212 participants was 37.23%, most of the infected persons (79.81%) being asymptomatic. The prevalence of malaria infection in enterprises, communities and schools was 24.49%, 38.81% and 45.47% (Chi²=83.1, p<0.0001) respectively. Children aged less than five years recorded the highest prevalence (41.09%, Chi²=22.9, p<0.0001). Only 38.18% of the 3641 respondents, possessed a LLIN of which 31.73% were damaged. The average coverage was 2.24±1.23 pers/net in households of this group. Damaged LLIN was significantly associated with increased malaria infection [Chi²=7.82, OR=0.74[0.60-0.91]; p=0.005]. Owners of LLIN were less affected (36.83% vs 40.27%); however, the difference was not statistically significant.

CONCLUSION: The impact of malaria control initiatives can be better assessed with the use of mass diagnostic tools. Coverage with LLINs is still insufficient. Given the high prevalence of asymptomatic malaria, its elimination is difficult to envisage but drastic drop can be foreseen if all detected cases with parasitaemia are promptly treated and concomitantly protected from anopheles bites.

P378: In vitro susceptibility of Plasmodium falciparum to antimalarial drugs five years after the change for treatment policy of uncomplicated malaria in Burkina Faso

In Bonkian,1,2 H Tinto,1, H Valea,1, H Zampan,1,2 Jb Ouedraogo,1,2 rt Guiguemde

1Centre Muraz, Bobo-Dioulasso, Burkina Faso; 2Institut de Recherche en sciences de la Santé/Direc tion Régionale de l’ouest, Bobo-Dioulasso, Burkina Faso.

BACKGROUND: Resistance to commonly used antimalarial drugs represents the major drawback and obstacle for controlling malaria in endemic countries. Burkina Faso has changed in 2005 its antimalarial drug policy for the treatment of uncomplicated malaria from Chloroquine to Artemether-Lumefantrine and Amodiaquine + Artesunate.

OBJECTIVE: To compare the in vitro sensitivity of the different ACT components with the results obtained various components of ACTs used in Burkina Faso and current antimalarial drugs after the implementation of effective use of ACT.

MATERIALS AND METHODS: The study was conducted in Bobo Dioulasso from July 2009 to February 2010. Blood samples were collected from patients with a parasitemia between 4000 and 200000 trophozoites /µl and cultured in presence of antimalarial drug and incubated in 5% CO₂ for 48 hours. These patients were treated at the inclusion.

RESULTS: A total of 40 blood samples were collected. We obtained, 2.78% resistant isolates to quinine, 6.06% to monodesethylamodiaquine and 52.94% to chloroquine. The geometric mean IC50 of lumefantrin, dihydroartemisinin and piperaquin were respectively 30.61 nM, 1.31 nM and 8.58 nM.

CONCLUSION: At the end of this study, we conclude that five (05) years after the adoption of policy for use of ACT in the treatment of uncomplicated malaria in Burkina Faso, there is a lower rate of in vitro resistance to quinine. Regarding dihydroartemisinin, there is no great change in the geometric mean IC50 values. And finally, we have a good antiplasmodial activity for monodesethyl amodiaquine, lumefantrin and piperaquin.
codons 86, 184 and 1246 and PfCRT codons 72, 74, 75, 76 were genotyped using PCR-RFLP. In all statistical analysis significance was assumed at p<0.05.

RESULTS: The prevalence of plasmodial infection was 22.00% (N=250) at Franceville. In vitro cultures were successfully for 103 isolates. The proportions of isolates resistant to CQ, MF and MDAQ were 44.7%, 23.4% and 56.5%, respectively. Several isolates (23.9%) had DHA IC50 values higher than 10 nM. The highest cross-resistance was observed between DHA and MF (r2=0.73). The prevalence of P184 was significantly high at Franceville (60.8%, n=152) versus rural-area (13.62%, n=13), p<0.001. The prevalence of wide type D1246 were significantly high in villages (45.26%, n=113) versus Franceville (38.38%, n=38), p=0.0017. We found that the prevalence (51.2%, n=128) of haplotypes NFD was significantly higher at Franceville compared to villages (45.7%, n=42) than Franceville (p=0.01). Strong associations were found between the PfmDr1 Y1246 and MDAQ resistance. The prevalence of CVIET haplotype of PfCRT was around 96.2% in two areas.

CONCLUSION: The high prevalence of antimalarial drug resistance markers at Franceville underlines the risk of ACT of tolerance/resistance and the need for their surveillance efficacy in Gabon.

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P380: Structural and functional validation of S-adenosylmethionine decarboxylase as novel drug target in the malaria parasite, *Plasmodium falciparum*

Dina le Roux1, Pieter Burger1, Anne Grobler3, Patricia Urbán1, Xavier Fernández-Busquets1, Robert Barker1, Abraham I. Louw1, Lyn-Marie Birkholz2

1Department of Biochemistry, Centre for Sustainable Malaria Control, University of Pretoria, Private bag X20, Hatfield 0028, South Africa; 2Unit for Drug Research and Development, North-West University, Potchefstroom 2531, South Africa; 3Biomedical Engineering Group, Institute for Bioengineering of Catalonia, Baldiri Reixac 10-12, E08028, Spain; 4Barcelona Centre for International Health Research (CRESIB, Hospital Clinic-Universitat de Barcelona), Rosselló 149-153, Barcelona E08036, Spain; 5Biomolecular Interactions Team, Nanoscience and Nanotechnology Institute (IN2UB), University of Barcelona, Martí i Franquès 1, Barcelona E08028, Spain; 6Genzyme Corporation, 153 Second Avenue, Waltham, Massachusetts 02451.

BACKGROUND: S-adenosylmethionine decarboxylase (AdoMetDC) in the polyamine biosynthesis pathway has been identified as a suitable drug target in *Plasmodium falciparum*, the parasite causing the most lethal form of malaria. An irreversible inhibitor of this enzyme, MDL73811, has poor pharmacokinetic properties and ADME toxicology profiles, therefore it was necessary to improve this compound. One of the derivatives tested against this enzyme was found to be active in vitro with high specificity some analogues to determine the optimal/minimal structural determinants of antimalarial activity and selectivity.

METHODS: Isotopic AdoMetDC protein activity assays and in vitro whole cell assays against *P. falciparum* parasites were performed.

RESULTS: MDL73811 derivatives completely inhibited PfAdoMetDC activity with high specificity some with KIC values in the low μM range. The most active compound, Genz-644131 however has low inhibitory activity against the in vitro proliferation of *P. falciparum*, with only a 2-fold observed increase in IC50 compared to MDL73811. Therefore in an attempt to improve the in vitro activity of the compound, it was included into novel nanovector drug delivery systems, which improved the uptake and in vitro inhibitory activity of the activity by 32-fold.

CONCLUSION: There were showed that compounds have an intrinsic challenge being taken up into *P. falciparum* parasites. However, the inclusion of an AdoMetDC specific inhibitor into nanovectors resulted in the identification of the most effective inhibitor targeting this enzyme in *P. falciparum*.

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P381: Quantitative Detection of Plasmodium falciparum Histidine Rich Protein 2 in Saliva of Children with Malaria

Author Block: C. E. Lekpor1, S. Amankwah1, W. Ababio1, D Dosoo1, D. F. Botchway2.

1Pathology Department, Korle Bu Teaching Hospital, Accra, Ghana; 2Chemical Pathology Department, University of Ghana Medical School, Accra, Ghana; 3Child Health Department, Korle Bu Teaching Hospital, Accra, Ghana; 4Kintampo Health Research Centre, Kintampo, Ghana.

BACKGROUND: Malaria is a global health priority with a heavy burden of fatality and morbidity. Improvements in field diagnostics are needed to support the agenda for malaria elimination. Saliva has shown significant potential for use in non-invasive diagnostics, but the development of off-the-shelf saliva diagnostic kits requires best practices for sample preparation and quantitative insight on the availability of biomarkers and the dynamics of immunoadassay in saliva. This study measured the levels of the PHRP2 in patient saliva.

METHODS: Matched samples of blood and saliva were collected between March and August 2011 from forty patients at the ER and OPD of the pediatric unit of Korle Bu Teaching Hospital. Parasite density was determined from thick-thin blood smears. Concentrations of PHRP2 in saliva of malaria-positive patients were measured using a custom chemiluminescent ELISA in microtitre plates. Forty negative-control patients were enrolled. Saliva samples were stabilized with protease inhibitor.

RESULTS: Of the forty patients with microscopically confirmed *P. falciparum* malaria, thirty seven tested positive for PHRP2 in the blood using rapid diagnostic test kits, and forty for PHRP2 in saliva. All negative-control samples tested negative for salivary PHRP2. The ELISA agreed with microscopy with 100 % sensitivity and 100 % specificity. Salivary levels of PHRP2 ranged from 15 to 1,162 pg/mL in the malaria-positive group.

CONCLUSION: Saliva is a promising diagnostic fluid for malaria when protein degradation and matrix effects are mitigated. Systematic quantitation of other malaria biomarkers in saliva would identify those with the best clinical relevance and suitability for off-the-shelf diagnostic kits.

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P382: Antimalarial activity and structure-activity-relationships of the tyrocidines, cyclic decapetides from Bacillus aneurinolyticus

Adrienne Leussa

University of Stellenbosch

Drug resistance hinders the control of malaria and is still a public health concern. There is an urgent need for the development of new drugs to which the parasite will not easily develop resistance. The tyrocidines (Trcs) are amphipathic, cyclic antimicrobial peptides (AMPs) with individual family members arising due to differences in the residues at three positions. The major natural analogues have been observed to have nanomolar concentration activity against chloroquin (CQ) sensitive *P. falciparum* 3D7 and D10. Although the Trcs show toxicity to human cells, it is possible to increase the therapeutic index through the exploitation of the structure-activity relationship (SAR).

This study aimed to investigate additional natural and synthetic analogues to determine the optimal/minimal structural determinants of antimalarial activity and selectivity.

We investigated the activity of 14 peptides against CQ sensitive and resistant strains of *P. falciparum*. The peptides were purified and characterized by HPLC and mass spectrometry. Homology modeling was carried out in situ to determine the solvent accessible areas and volumes of the peptides (SASA and SAV).

Quantitative SAR analyses showed that activity correlated with HPLC retention time (which is related to the peptide apparent hydrophobicity) as well as the SASA and SAV which demonstrate side-chain steric and charge factors that may affect target interaction. The lowest IC50 values
against the CQ sensitive *P. falciparum* D10 strain were recorded for Tpc C (19 nM) and Phc A (27 nM) in which the aromatic residue at position 7 was changed from the more polar and hydrophilic Tyr to the less polar Trp and non-polar Phe respectively. Both also had the highest selectivity indices to human erythrocytes and COS 1 cells. There was loss of activity against the CQ resistant *P. falciparum* Dd2 strain indicating the possible presence of an internal target related to the membrane of the parasite's digestive vacuole. Microscopy revealed that Tpc C's activity is primarily non-lytic and life cycle progression is affected at the trophozoite stage. The identities of the aromatic amino acid side chains in the variable pentapeptide unit (F/W)(F/W)NQ(Y/W)/F are key in modulating antiparasomal activity of the peptides.

P383: Effects of T helper 17 (Th-17) immune response on the pathogenesis of malaria during pregnancy in Yaoundé

Rosette Megnekou1,2, Abel Lissom1,3, Simon Metenou1, Philemona Nyonglema1, Rose FG Leke2,4.
1Faculty of Science, University of Yaoundé I, Cameroon, 2National Institute of Allergy and Infection Diseases, NIH, USA, 3Biotechnology center, University of Yaoundé I Cameroon, 4Faculty of Medicine and Biomedical Science, University of Yaoundé I, Cameroon.

BACKGROUND: Th17 cells are a new subclass of CD4+ T cells that was proven to induce the destruction of extra and intracellular infective agents and to modulate the Th1 and Th2 immune response. Based on these informations, we investigated the effects of cytokines produced by Th1 (INF-γ and TNF-α), Th2 (IL-10) and Th17 (IL-17A, IL-22 and IL-23) cells on the pathogenesis of malaria in pregnant women in Yaoundé.

METHODS: Peripheral blood was taken from 107 Volunteer pregnant women at a health Centre in Yaoundé. Microscopy blood smear was used for malaria diagnosis. Haemoglobin level (THb) was used to evaluate pregnancy. ELISA technique helped to quantify the plasma levels of Th1, Th2 and Th17 cytokines.

RESULTS: The primiparous were more infected than the multiparous (P = 0.013) and parasitaemia correlated negatively with parity (P = 0.005). A significantly negative correlation between parasitaemia and THb (P = 0.003) was observed. The plasma of primiparous at second trimester of pregnancy had a higher level of INF-γ than those in first trimester of pregnancy (P = 0.026). Parasitaemia decreased significantly with increasing plasma level of INF-γ (P = 0.04). IL-10 levels correlated positively with parasitaemia (P < 0.001), and negatively with THb (P = 0.03). Multiparous who were in third trimester of pregnancy had a significantly higher level of IL-10 than multiparous who were in second trimester (P = 0.04). The plasma concentrations of IL-17 was highest in multiparous who were in third trimester of pregnancy as compared to that of multiparous in second trimester of pregnancy (P = 0.07 respectively), but similar between first and the second trimester of pregnancy (P = 0.39).

Conclusion: These results suggest that Th1 and Th17 immune responses, via INFγ and IL-17 respectively, contribute to the elimination of *P. falciparum*, contrary to Th2 immune response that support the development of malaria via IL-10.

P384: When it comes to malaria control, are we doing the right thing? Are we doing things right?

Manuel Luberas

Malaria is curable and preventable. It has been eradicated or reduced to a point where it is no longer a serious health or economic burden to a large number of countries. Almost without exception, eradication was reached by combining active vector population suppression methods and techniques and involving the local population to make their immediate environment less conducive to the proliferation of mosquito populations. More significantly, every country that achieved eradication did so more than two decades ago, long before the establishment of many of the current anti-malaria initiatives and without the benefit of a vaccine. While much has been said about the expenditures related to malaria control and the funding shortfalls many programs face, there has been very little regarding their evaluation. Continued reliance on passive methods like mosquito nets -purported to be a mosquito control intervention- without implementing active mosquito control methods that include IRS, larviciding and adulticiding with ULV sprays where appropriate will only guarantee that malaria’s existence. Examples of well organized, systematic and integrated mosquito control methodologies that attack the vector from different fronts and include good medical surveillance and treatment systems that have been instrumental in eradicating or reducing malaria to a point where it does not overwhelm diminishing available public health resources are provided. Well into the Twenty First Century, public health entomologists continue to press the active mosquito control issue and wonder why the methods that eradicated malaria from so many countries -and has kept it out- continue to be overlooked and neglected by the agencies and organizations that promoted and implemented them so aptly early in the Twentieth Century. Delaying implementation of active mosquito control will continue to claim lives at a rate equivalent to six or seven 747 Jumbo airliners full of children under five and pregnant women crashing every day.

P385: Prevalence of molecular markers of drug resistance in an area of Seasonal Malaria Chemoprevention in children in Senegal

Aminata C LO1, Faye B1, Ba EH1, Cissé B1,2, Tine R1, Abiola A1, Ndiaye M1, Ndiaye J1, Ndiaye D1, Ndir O1, Milligan PJM2, Cairns M1, Hallett R2, Sutherland C3, Gaye O4.
1Service of Parasitology UCAD, 2School of Hygiene and Tropical Medicine, 3Institut de Recherche pour le Developpement.

BACKGROUND: In sub-Saharan Africa, malaria is the leading cause of morbidity and mortality especially in children. In Senegal, the Seasonal Malaria Chemoprevention (SMC) previously referred to as Intermittent Preventive Treatment in children (IPTc) is a new strategy for malaria control in areas of highly seasonal transmission. An effectiveness study of SMC using sulfadoxine-pyrimethamine (SP) plus amodiaquine (AQ) was conducted in central Senegal from 2008 to 2010 to obtain information about safety, feasibility of delivery, and cost effectiveness of SMC. Here we report the effect of SMC delivery on the prevalence of markers of resistance to SP and AQ.

METHODS: This study was conducted in three health districts in Senegal with 54 health posts with a gradual introduction of SMC. Three administrations of the combination AQ + SP were made during the months of September, October and November of each year in children less than 10 years living in the area. Children were surveyed in December of each year and samples (filter paper and thick films) were made in 2008, 2009 and 2010. From samples positive by microscopy for *P. falciparum*, we investigated the prevalence of mutations in genes pfdhfr, pfdhps, pfmdr1 and pfcrt by sequencing and RTPCR.

RESULTS: Mutations at codon 540 of pfdhps and codon 164 of pfdhfr were not detected in our study. Among children with parasitaemia at the end of the transmission seasons, the CVIET haplotypes of pfcrt and the 86Y were polymorphism of pfmdr1 were more common among those that had received SMC, but the number of infections detected was very low and confidence intervals were wide. The overall prevalence of these mutations was lower in SMC areas than in control areas, reflecting the lower prevalence of parasitaemia in areas where SMC was delivered.

CONCLUSION: The sensitivity of *P. falciparum* to SMC drugs should be regularly monitored in areas deploying this intervention. Overall the prevalence of genotypes associated with resistance to either SP or AQ was lower in SMC areas due to the reduced number of parasitaemia individuals.
P386: Molecular Identification reveals novel and unexpected Anopheline species

Neil F Lobo, Malaria Transmission Consortium Partners
Eck Institute for Global Health, University of Notre Dame, IN 46556, USA

BACKGROUND: High coverage with the recommended vector control interventions of Long-Lasting Insecticidal Nets (LLINs) and Indoor Residual Spraying (IRS) together with access to artemisinin combination therapies (ACTs) have significantly reduced but not eliminated malaria. Vector behaviors govern the effectiveness of indoor-based ITNs and IRS and hence, knowledge of local vector species and their bionomic traits are vital in the selection of effective intervention strategies. Incorrect association of vector species or compositions with behaviors will have a negative effect on intervention efficacy.

METHODS: Vector related studies conducted by the Malaria Transmission Consortium partners were based on the monitoring of local vector species-specific bionomic traits in relation to malaria control interventions and trapping methods. Anopheles trapped were morphologically identified, associated with behavioral characteristics and underwent molecular species identification (ITS2 or CO1 sequencing).

RESULTS: Surprisingly, a large number of vectors were morphologically misidentified. This includes a novel primary vector species in the Kenyan Highlands and the presence of 16 Anopheles groups - of which several remain unidentified - in Zambia. Some vectors from Indonesia do not match up with morphological keys, had novel sequences and their species identity remain indeterminate. Depending on the site and collection, morphological misidentifications ranged from 97% to 4%. Morphological identification of the major vectors (Eg. Anopheles gambiae complex) was usually correct while less studied vectors and those from newer sites had higher error rates. Bionomic associations from these studies demonstrate niche partitioning based on sequence groups and differing exposures to indoor interventions. Analyses purely based on morphological identification were misleading and resulted in imprecise measures of species contributions to disease transmission.

CONCLUSIONS: This reflects the complexities and variation in local mosquito populations and the limitations of older taxonomic identification keys. This points to the importance of molecularly identifying vectors as a means of validating morphological identification processes and identifying actual species compositions towards better association of species with their bionomic traits and hence better intervention strategies.

P387: Malaria specific CD4 T cell immunity in HIV-exposed Children on Cotrimoxazole Prophylaxis

Herbert Longwe1, Kamija Phiri1, Francis Munthali2, Rhita Mankhanamba2, Kondwani Jambo2, Wilson Mandala1
1Department of Basic Medical Sciences, College of Medicine, University of Malawi. 2Department of Community Health, College of Medicine, University of Malawi. 3Department of Pathology, College of Medicine, University of Malawi. 4Malawi-Liverpool-Wellcome Trust Clinical Research Programme

BACKGROUND: Cotrimoxazole does not only prevent bacterial infections, but is also an effective anti-malarial. As a national policy, cotrimoxazole prophylaxis is given to HIV-exposed uninfected (HEU) infants in Malawi, from six weeks to 12months of age. Due to the antimalarial properties of cotrimoxazole, we hypothesised that they will be a delayed development of adaptive immune responses to Plasmodium falciparum in HEU infants.

METHODS: Peripheral blood was collected on 33 HIV exposed uninfected (HEU) and 31 HIV healthy unexposed control infants who were recruited at 6 months of age and were followed up 6 monthly until 18 months of age. Twelve HEU infants and 13 controls have been evaluated at 6 months and followed up to 12 months so far. Immunophenotyping was performed by flow cytometry on peripheral blood. Frequency of P. falciparum antigen-specific CD4 T cells in whole blood were measured using an intracellular cytokine staining assay following stimulation with P. falciparum-infected red blood cell lysate.

PRELIMINARY RESULTS: There was no significant difference in percentage of B and T cell subsets between HEU and control infants at baseline and at 12 months follow up. HEU infants had reduced frequencies of IFN-γ-producing P. falciparum antigen-specific CD4 T cells compared to controls at 12 months, but this difference did not reach statistical significance, p=0.08. There was no difference in the polyfunctional IFN-γ+IL-2+TNF+ producing P. falciparum antigen-specific CD4 T cells between HEU and controls at baseline and at 12 months follow up.

PRELIMINARY CONCLUSION: These results are suggesting that there is lower frequency of IFN-γ-producing P. falciparum specific CD4 T cells in HIV exposed children after a year on cotrimoxazole prophylaxis. This might be due to delayed acquisition of P. falciparum-specific adaptive immunity due to the anti-malarial effect of cotrimoxazole or due to the effect of HIV exposure. Suboptimal P. falciparum-specific CD4 T cell immunity might increase the risk to malaria in HEU later in life.

P388: Analysis of longitudinal studies of mixed parasitemia in Senegal and Nigeria

Andrew Lover

Mixed Plasmodio infections within individuals, and the dynamics of parasite species within populations, have recently emerged as significant factors impacting clinical presentation. Moreover, these infections represent an underutilized tool to understand highly complex malaria epidemiology. The Dielmo project in Senegal serves as starting point for examining these trends; these data are complemented with results from the Garki project, which included a very large population, a long series of cross-sectional surveys, and a very thorough microscopy paradigm. The longitudinal data from these studies has been explored using a combination of trajectory and sequence analysis models to examine the impact of prior infections on subsequent episodes, with a focus on mixed-species infections. Trajectory models, adjusted for age, indicate that three main trajectories describe the progression of parasitemia in the Dielmo cohort measured by a zero-inflated Poisson model of the number of parasite species found at each survey. The data from Garki suggests complex interactions when stratified by geographic zones. Epidemiological implications of these factors are discussed.

P389: Quantifying Effect of Geographic Location on Epidemiology of Plasmodium vivax Malaria

Andrew Lover

Recent autochthonous transmission of Plasmodium vivax malaria in previously malaria-free temperate regions has generated renewed interest in the epidemiology of this disease. Accurate estimates of the incubation period and time to relapse are required for effective malaria surveillance; however, this information is currently lacking. By using historical data from experimental human infections with diverse P. vivax strains, survival analysis models were used to obtain quantitative estimates of the incubation period and time to first relapse for P. vivax malaria in broad geographic regions. Results show that Eurasian strains from temperate regions have longer incubation periods, and Western Hemisphere strains from tropical and temperate regions have longer times to relapse compared with Eastern Hemisphere strains. The diversity in these estimates of key epidemiologic parameters for P. vivax supports the need for elucidating local epidemiology to inform clinical follow-up and to build an evidence base toward global elimination of malaria.
P390: A web-based data management system to capture, store and analyze research data in rural Zambia

Jailos Lubinda1, Andre Hackman2, Timothy Shields3, Jennifer Stevenson2, 4, Cornelius Chooabwe2, Harry Hamapumbu1, Sungano Mharakurwa1, 2, 5, Phil Thuma1 and William J Moss1

1Macha Research Trust, Choma, Zambia, 2The Malaria Research Institute, Department of MMIC, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States of America

BACKGROUND: The prompt generation of accurate reports from health research is essential for in-country programs to make timely and appropriate decisions for disease control. Research can generate large amounts of data that require time and skill to manage. Manual data entry can be laborious and error-prone, storage space for conventional databases may be limited and retrieval and reporting of data can be delayed. There is need for user-friendly tools enabling data visualization in real-time.

METHODS: In Macha, a web-based software, Research Electronic Data Capture (REDCap), is used to manage malaria data electronically. Data is collected using Android tablets, data loggers, GPS units, mobile phones and hand-written forms. Once collected, data are uploaded online or emailed for upload remotely into REDCap. The software integrates data with different formats, carries out integrity checks, and merges data with common linking variables. Data are stored online, alleviating the problem of storage capacity and allowing remote data access across study sites. The software can generate user-designed reports and maps. All activities are logged, allowing changes to be tracked and checked.

RESULTS: In rural Choma District, Zambia, REDCap software is used to visualize data collected from malaria surveys through simple graphic reports, summaries and maps and to retrieve data in different formats for summary and analysis. It is accessible by registered users with internet access at multiple locations. The generation of maps allows for visualization of foci of malaria cases and identification of locations with high vector densities. Summary reports provide temporal and spatial descriptions of malaria prevalence.

CONCLUSION: The web-based database management tool REDCap provides a user-friendly system to upload, check and integrate datasets that can be accessed from any location with internet access for export and reporting. Such instant access can guide program managers in providing prompt, targeted and appropriate health interventions.

P391: Olyset® Plus - a novel long lasting bed net with activity against resistant insects

John Lucas1, John Invest1, Kazunori Ohashi2, Yoshinori Shono2

1 Vector Control Division, Sumitomo Chemical Company, UK, Plc; 2Health and Crop Sciences Research Laboratory, Sumitomo Chemical Company, Ltd, Hyogo, Japan

BACKGROUND: The rapid scale up and use of treated long lasting impregnated bed nets in the past 5 years has been one of the key factors behind the dramatic drop in malaria deaths particularly in sub-Saharan Africa. Pyrethroid resistance in Anopheles is however increasing especially in parts of Africa and this is reducing the efficacy of bed nets. Currently only pyrethroids are approved by WHO for use on bed nets due to their low mammalian toxicity, and suitable alternatives are limited, and still many years away from commercialisation. In order to improve the performance of LNs against resistant insects, a polyethylene bed net containing the synergist piperonyl butoxide (PBO) was developed. This net (Olyset® Plus) has the synergist incorporated at 1% into all the net fibres along with 2% of the pyrethroid permethrin. One of the challenges of such a development was to ensure that both synergist and insecticide continued to be available on the net surface in the same ratio to ensure optimum biological effectiveness over many washing cycles, and throughout life of the net.

METHODS: As part of the initial development activity, WHO cone and tunnel tests were conducted on washed and unwashed Olyset Plus to establish the efficacy of this product against both susceptible and multi-resistant Anopheles mosquitoes.

RESULTS: In cone tests, in comparison to an identical net formulated with no PBO, far better kill was observed with washed Olyset Plus against metabolic resistant An. arabiensis (73 vs 15%), while in cone tests with a commercially available deltamethrin net without PBO, unwashed Olyset Plus performed much better against An. arabiensis (76 vs. 24% kill). In tunnel tests against metabolic resistant An. gambiae Olyset Plus gave 80% mortality and 95% blood feeding inhibition 2 days after 20 washes of the net.

CONCLUSION: These studies demonstrated the increased efficacy of Olyset Plus against resistant mosquitoes, which is due to the combined effects of faster penetration of permethrin through the cuticle aided by the PBO which acts as a solvent, and also the suppression of P450 enzymes present naturally in susceptible mosquitoes. This data provided sufficient evidence to invest in more extensive lab and field trials (some of which will be separately reported at this conference) and submission to the WHO Pesticide Evaluation Scheme (WHOPES). Following the completion of successful WHOPES Phase I and II studies, Olyset Plus has now been granted an interim WHOPES recommendation. Phase III studies will be initiated shortly.

P392: Larvicidal properties of three plant extracts against the malaria vector, Anopheles gambiae

Woquan S. Luma, 1Muritata A. Adebayo and 2Edith O. Ajayieooba

1Department of Pharmacy, School of Health Sciences, Catholic University of Cameroon, Bamenda, Cameroon; 2Department of Pharmacognosy, Faculty of Pharmacy, Igbinedion University, Okada, Nigeria

BACKGROUND: The rapid scale up and use of treated long lasting impregnated bed nets in the past 5 years has been one of the key factors behind the dramatic drop in malaria deaths particularly in sub-Saharan Africa. Pyrethroid resistance in Anopheles is however increasing especially in parts of Africa and this is reducing the efficacy of bed nets. Currently only pyrethroids are approved by WHO for use on bed nets due to their low mammalian toxicity, and suitable alternatives are limited, and still many years away from commercialisation. In order to improve the performance of LNs against resistant insects, a polyethylene bed net containing the synergist piperonyl butoxide (PBO) was developed. This net (Olyset® Plus) has the synergist incorporated at 1% into all the net fibres along with 2% of the pyrethroid permethrin. One of the challenges of such a development was to ensure that both synergist and insecticide continued to be available on the net surface in the same ratio to ensure optimum biological effectiveness over many washing cycles, and throughout life of the net.

METHODS: As part of the initial development activity, WHO cone and tunnel tests were conducted on washed and unwashed Olyset Plus to establish the efficacy of this product against both susceptible and multi-resistant Anopheles mosquitoes.

RESULTS: In cone tests, in comparison to an identical net formulated with no PBO, far better kill was observed with washed Olyset Plus against metabolic resistant An. arabiensis (73 vs 15%), while in cone tests with a commercially available deltamethrin net without PBO, unwashed Olyset Plus performed much better against An. arabiensis (76 vs. 24% kill). In tunnel tests against metabolic resistant An. gambiae Olyset Plus gave 80% mortality and 95% blood feeding inhibition 2 days after 20 washes of the net.

CONCLUSION: These studies demonstrated the increased efficacy of Olyset Plus against resistant mosquitoes, which is due to the combined effects of faster penetration of permethrin through the cuticle aided by the PBO which acts as a solvent, and also the suppression of P450 enzymes present naturally in susceptible mosquitoes. This data provided sufficient evidence to invest in more extensive lab and field trials (some of which will be separately reported at this conference) and submission to the WHO Pesticide Evaluation Scheme (WHOPES). Following the completion of successful WHOPES Phase I and II studies, Olyset Plus has now been granted an interim WHOPES recommendation. Phase III studies will be initiated shortly.

P393: Larvicidal properties of three plant extracts against the malaria vector, Anopheles gambiae

P393: Malaria parasitaemia, Parasite densities, Anaemia and Febrile Status of children living at different Altitudes in the Mt Cameroon region and Co-infection with Geohelminths.

Emmaculate Lumu1, Helen K Kimbi1, Irene U N Sumble1, Malaiaka Nweboh1, Judith K Anchang-Kimb1, Yannick Nana1, Lucy M Ndip1, Henry Njome1

1Department of Zoology and Animal Physiology, Faculty of Science, University of Ibadan, Ibadan, Nigeria; 2Health and Crop Sciences Research Laboratory, Sumitomo Chemical Company, Ltd, Hyogo, Japan

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P394: CSF cell count can be accurate for the early treatment in cerebral malaria in low-income settings

Réné Lumu¹, Célestin N Nsibu², Léon Tshilolo¹, Joseph M Bodi²
¹Hôpital/CEFA Monkolé, Kinshasa, RD Congo; ²Department de Pédiatrie, Faculté de Médecine, Université de Kinshasa, RD Congo

BACKGROUND: The strategic approach to test, treat and track may be inappropriate in cases of cerebral malaria where the clinical expression is often confused with other causes of meningococcal meningitis. Although an accurate and rapid diagnosis of cerebral malaria is essential for a good outcome, the lack of skills and equipment in low-income settings greatly prejudice this practical attitude. CSF leukocyte count which can be done in most general hospitals of endemic malaria areas can be helpful to start early the treatment of cerebral malaria.

The objective of this study is to discriminate cerebral malaria from other causes of meningococcal meningitis in children

METHODS: A retrospective study from the files of patients aged 0-9 years hospitalized in the pediatric Hospital Monkolé in Kinshasa (DRC) from 1 January 2010 to 31 December 2011 was conducted. Epidemiological, clinical and laboratory data were collected and analyzed using SPSS 20.0 program.

RESULTS: A hundred and twenty-six cases of meningococcal meningitis were identified and accounted for 82 (65%) cerebral malaria cases and 44 (35%) meningitis patients with positive culture. The F/M sex ratio was 1.25 and 62.7% of patients were aged 0 to 35 months. CSF was a clear appearance and meningitis patients with positive culture. The F/M sex ratio was 1.25 and 62.7% of patients were aged 0 to 35 months. CSF was a clear appearance and meningitis patients with positive culture.

CONCLUSION: Relatively high rates of asymptomatic malaria still reveal need for stringent preventive and control measures for malaria in this region.

P395: Prevalence of mixed Plasmodium species infection associated with age in children: population based survey observations of selected communities in Zambia

1.2.5 Lungowe Sitali¹, Hawela Moonga¹, John Miller², James Chipeta¹, 2.1
¹University of Zambia School of Medicine, Department of Biomedical Science, Lusaka, Zambia; ²The School of Medicine and University Teaching Hospital Malaria Research Unit (SMUTH- MRU), C/O University teaching Hospital, Department of Paediatrics and Child Health, D-Block, P/B RW1X, Lusaka, Zambia; ³University of Zambia School of Medicine, Department of Paediatrics and Child Health, P.O. Box 50110, Lusaka, Zambia; ⁴Ministry of Health, National Malaria Control Centre, MACIPA, Chainama grounds, Lusaka, Zambia; ⁵University of Zambia, School of Medicine, Department of Public Health, P.O. Box 50110, Lusaka, Zambia

BACKGROUND: Malaria remains one of the preventable and treatable killers among infectious diseases, and yet it still claims more than 1 million lives every year. Mortality and morbidity is high among children under 5 years and pregnant women. In Zambia, Rapid diagnostic Test (RDTs) are used in most health facilities for diagnosis of malaria, that can only detect Plasmodium falciparum.

OBJECTIVE: To determine the prevalence of mixed plasmodium species in Eastern and Luapula provinces, and to examine their association with age.

METHODS: Data stem from the 2012 National Malaria Indicator Survey conducted between April and May every two years country wide. Background, social and behavioural information were collected from households. In addition, blood slides, dried blood spots were collected from children below 5 years. Slides were stained using giemsa immediately after collection and examined by microscopy and Polymerase Chain Reaction (PCR) was used to analyse the filter papers for malaria species.

RESULTS: Overall (n=873), the mean age was 2.4 years, prevalence of malaria by PCR was 54.3%, and the prevalence of the individual plasmodium species were Pfalciparum 53.4%, P. malariae 5.0%, P. ovale 2.1% and P. vivax 0.2%. The prevalence of mixed infection was 5.6%. Furthermore, increasing age was associated with higher likelihood of malaria infection (Pvalue < 0.001)

CONCLUSION: The prevalence of mixed infections was 5.6%, and a gradual increase in percentage of age group. The young children are less likely to have malaria, as they grow, they tend to have more malaria infections, when they reach the age of 5 years their immunity has built up and infections reduce. There is need to pay attention to other species but it’s may not be necessary to change the HRPii based Rapid Diagnostic Tests as the non-falciparum species mostly occur as mixed infections.

P396: Spatial-temporal relationship between climatic factors, malaria transmission and their impact on interventions in Zanzibar.

Rose Lusinde¹, Mohamed Ali², Abdul-wahid Al-maafazy³, Abdulla S. Alli², Issa Garimo¹, Mahdi Ramsan², Shabhir Lalji³, Jessica M. Kafuko¹, Osia Mwaipe¹, Ritha Willilo¹, Jeremiah M. Ngondi³
¹RTI International, Dar es Salaam, Tanzania; ²Zanzibar Malaria Control Program, Ministry of Health, Zanzibar, Tanzania; ³United States Agency for International Development/President’s Malaria Initiative, Dar es Salaam, Tanzania

BACKGROUND: In malaria transmission, rainfall, temperature and humidity are major natural risk factors affecting the life cycle, breeding and lifespan of the mosquito. Between 2006 and 2010, blanket indoor residual spraying (IRS) was undertaken in Zanzibar. Following declines...
in malaria cases, IRS was scaled down to focal spraying in malaria transmission hotspots. This study aims to determine the impact of climate on the spatial-temporal variation of malaria transmission for the period 2008–2012.

METHODS: Data on malaria were obtained from weekly record of confirmed cases of malaria from each health facility. Rainfall (mm), temperature (°C) and humidity (%) data were collected monthly at monitoring stations located in each of the 10 districts. Malaria cases were compiled for each period and incidence per 1000 population was calculated using service area population. Monthly climate data were calculated into averages for each period. ArcGIS software was used to calculate weights for each period. Geographically Weighted Regression (GWR) was done to investigate associations between malaria incidence and each climatic factor. Data validation was undertaken by comparing values of climatic factors available in websites to those from TMA.

RESULTS: From 2008 to 2011, mean difference in incidence was 1.5 (95% CI: 1.0-2.0). Over the study period, mean difference in temperature was 11.8(95% CI: 6.7-16.9) rainfall 118.7 (95% CI:103.0-134.5) and humidity 34.8 (CI: 19.7 -49.9).Pearson's product-moment correlation coefficients between incidence and temperature was 0.2, incidence and rainfall was -0.3 and between incidence and relative humidity was 0.2.

CONCLUSION: Malaria shows a great decline during the study period; however seasonal variations need to be thoroughly monitored. Knowing the spatial variability pattern of malaria will make targeting of IRS at this pre-elimination stage more timely, effective and efficient.

P397: Increasing role of Anopheles funestus and Anopheles arabiensis in residual malaria transmission in rural Tanzania

Dickson Lwetoijera1,2, Caroline Harris1,2, Stefan Dongus1,2, Greg Devine3 and Silas Majambere1,2

1 Environmental and Ecological studies Thematic Group, Ifakara Health Institute, P.O. Box 53, Ifakara, Tanzania
2 Liverpool School of Tropical Medicine, Pembroke Place, Liverpool, L3 5QA, United Kingdom
3 Public Health Unit, Queensland Health, Cairns, Queensland 4870, Australia

BACKGROUND: To sustain the gains achieved by current vector control strategies such as ITNs and IRS in malaria control, robust surveillance systems that monitor vector dynamics and their role in malaria transmission overtime are highly required. This longitudinal study aimed to demonstrate the trends in Anopheles gambiae s.s., An. arabiensis and An. funestus densities and their Plasmodium falciparum sporozoite infection in hyperendemic malaria settings.

METHODS: The study was conducted in Kilombero valley, southeastern Tanzania for five consecutive years (2008 – 2012). Mosquitoes were collected using CDC light traps and morphologically identified. Mosquito species and sporozoite infection status were analyzed using PCR and ELISA respectively. An. funestus susceptibility test was performed using WHO guidelines. Using a negative binomial distribution with a log-link function, changes in mosquito abundance over time were assessed adjusting for location and seasons.

RESULTS: The proportion of the An. gambiae s.s. population consistently declined from 0.2% (505/2524) in year 2008 to undetectable levels of 0% (0/1421) in year 2012, with a proportional increase of An. arabiensis and An. funestus that were both important in sustaining malaria transmission. Seasonally, An. arabiensis was abundant during wet season (P < 0.0001), compared to An. funestus. In contrary, An. funestus density significantly increased during dry season (p < 0.0001) with decrease in An. gambiae s.l. An unprecedented increase in An. funestus was observed in year 2012 (80% more than 2008). WHO susceptibility tests revealed that An. funestus was susceptible to deltamethrin (100% mortality), suggestive to be resistance against deldrin (5%), permethrin (93%), and lambda cyhalothrin (91%) and resistant to DDT (86%), based on the new WHO guidelines on testing resistance.

CONCLUSION: While existing interventions, mainly LLINs have significantly contributed in reducing malaria transmission and nearly eliminating An. gambiae s.s., a robust surveillance system will be required to establish the contribution of the remaining vector populations in sustaining malaria transmission in terms of seasonal vector abundance, dominance, infectivity status as well as resistance to insecticides used for malaria control.

P398: Potential for climate change to increase the distribution range of malaria mosquitoes in southern Africa

Candice Lyons1,2*, Maureen Coetzee1, John Terblanche1 and Steven Chown1,2

1 Centre for Invasion Biology, Department of Botany and Zoology, Stellenbosch University, Private Bag X1, Matieland 7602, South Africa; 2 Wits Research Institute for Malaria, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa; 3 Department of Conservation Ecology and Entomology, Stellenbosch University, Matieland 7602, South Africa; 4 School of Biological Sciences, Monash University, Victoria 3800, Australia

BACKGROUND: The impact of climate change on the distribution of the vectors responsible for disease transmission, including malaria, is a highly contested and controversial issue. Several methods exist to determine range changes of species, one of which relies on basic physiological data. For many African malaria vectors, these kinds of data are often lacking. Here, we investigated several physiological traits of two African vector species, Anopheles arabiensis and Anopheles funestus, in an attempt to determine which parameters are likely to confine these species to their current distributions. Using the data most limiting to distributions and population growth, we aimed to determine the influence of changing climates on distributions of these species.

METHODS: Thermal tolerance, development rate-temperature and survival data, and desiccation tolerance information was collected for Anopheles arabiensis and An. funestus. Several age groups and both sexes were investigated for differential physiological tolerances.

RESULTS: Development rate-temperature information was chosen as the best predictor of physiological tolerances limiting to the distribution of either species, because survival of both species in breeding ponds is bounded by a lower temperature of 15°C and an upper temperature of 35°C, even though adult forms of both species can tolerate substantially lower and higher temperatures. Using this developmental information, a CLIMEX distribution model for each species was developed.

CONCLUSIONS: The model output showed range changes in line with predictions of changing rainfall patterns on the East coast and West coast of southern Africa in particular. Areas that are currently presumed unsuitable for mosquito development become more favourable under both climate change scenarios, with the potential to increase mosquito abundance and hence, malaria outbreaks in these regions.

P399: Fetal haemoglobin and β-globin gene cluster haplotypes in sickle cell anaemia patients from coastal Kenya

Alexander W. Macharia1, George Mochamah1

1 Kenya Medical Research Institute, Centre for Geographic Medicine Research East Coast/Wellcome Trust Collaborative Program, Kenya.

INTRODUCTION: Foetal haemoglobin (HbF) remains the single most important factor associated with reduced morbidity and mortality in patients with Sickle cell anaemia (SCA). The variation in HbF levels has been associated with a number of genetic variants amongst them are the 5¢ b-globin gene cluster haplotypes that are associated with varying disease severity, and named after regions where they are most prevalent; Bantu, Benin, Indian-Arab and Senegal.
The prevalence of these haplotypes and their relation to HbF levels in SCA populations in Kenya is poorly described. The aim of this observational study was to determine the prevalence of β*-haplotypes and HbF levels in a cohort of children attending an outpatient clinic at the Kilifi District Hospital.

METHODS: In this study we included 134 children (mean age 8.78 ± 5.47 years) attending routine clinical management of SCA at the Kilifi District Hospital. HbF levels were quantified using high performance liquid chromatography and the β-globin gene cluster haplotypes determined using restriction fragment length polymorphism on 8 polymorphic sites.

SUMMARY OF PRELIMINARY RESULTS: The Bantu haplotype was predominant haplotype at a homozygous frequency of 87% and accounted for the second haplotype accounting for 93% of the β*-chromosomes among the cohort. The frequency of the Bantu haplotype in the 268 chromosomes analyzed was 93% with the rest of the β*-chromosomes being mainly of the atypical type apart from one patient who had the Benin haplotype. Mean HbF level was 7.31±5.70%.

DISCUSSION AND CONCLUSION: In this population the Bantu haplotype remains the major haplotype accounting for 93% of the β*-chromosomes along the coast of Kenya. There is little or no recombination with haplotypes from other regions as the Benin haplotype was the only haplotype identified amongst the geographically defined β*-haplotypes. The mean HbF level of 7.3% confirms SCA patients with Bantu haplotype have a threshold below 10% that has previously been shown to confer protection against disease severity.

M Maatoug

INTRODUCTION: In Sudan the National Malaria Control Program adopted the use of artesunate + sulphadoxine-pyrimethamine (ASP) as the first line treatment of uncomplicated malaria since 2004. The aim of this study was to evaluate the awareness and acceptance of ASP among healthcare-providers and patients in Wad Medani locality, Gezira State, Sudan.

METHOD: To investigate the knowledge and practice regarding ASP usage three types of questionnaire were randomly distributed. The first to prescribers (whether doctors or medical assistants) and pharmacists. The second to pharmacy assistants and the third to patients. The respondents were 326 healthcare- providers, 46 pharmacy assistants and 687 patients. The data collected were organized, tabulated and analyzed using SPSS (Statistical Package for Social Sciences, version 14).

RESULTS: Regarding the awareness of healthcare - providers 256(78.5%) of the questioned healthcare-providers were aware about the protocol, while 70(21.5%) were not aware about the protocol. 218(66.9%) adhered to the protocol ,46(14.1%) didn’t adhere to the protocol and 62(19%) adhered to the protocol sometimes. Two-hundred thirty (70.6%) of healthcare- providers were not trained about the protocol guidelines. Two-hundred fifty eight (79.1%) of them considered ASP as the first line of treatment in their practice. Pharmacy assistants in private pharmacies were neglected in training programs 28(90.3%) were not trained,3(60%) from revolving drug pharmacies and 6(75%) health- insurance pharmacies were also not trained about the malaria protocol. This was serious because many patients used to go directly to pharmacies with laboratory diagnosis asking for advice. ASP was highly accepted among patients 528(76.9%) and 609 (88.6%) of them obeyed ASP instructions given to them.

CONCLUSION: The study revealed that ASP was accepted by healthcare-providers and patients but low awareness and poor adherence to the protocol were due to lack of training and information ,the decreased availability and affordability of the consecutive lines of malaria treatment and the absence of dosage form suitable for children and in case of vomiting.

George C. Madoda

Independent Researcher, scientist and policy analyst

Every single human being deserves protection and need prevention but health actors or practitioners are needed to work on other more means to eliminate what cause and produce Malaria. Availability of resources to treat the decease cannot be yet a solution but find out the cause of the problem and deal with it. Why should people wait until cells have been attacked, this is because of lack of resources, information and environmental cleanliness where they live. Human being is expected to stay away from risks, if he cannot then he need a help of a health practitioner. It may be difficult to have access to the resources for prevention but still there may be lack of information of the cause of infection and how people can stay away of the contamination. Malaria is a threat in human being it’s killing more than 3,000 children each day in sub-Saharan Africa, workforce should not tolerate this high mortality rate despite the vulnerable environment, many may not be privileged to live in environment where there is no malaria but preventive measures can be offered to prevent deceases and reduce the risk of infection in households. This study will contribute to the understanding of Prevention, control and elimination of Malaria in a vulnerable environment, an investigation will be carried out to see what causes malaria in a slum area. Health practitioners have not worked significantly to identify congested area with inconvenient environment which multiply mosquitoes for malaria. It’s important to have this study, it let them understand the vulnerable environment, how mosquito grow and develop from time to time and their causes, the variables we will be looking at is health of these people living in the vulnerable environment, prevention, control, their involvement into eliminating and prevent the existence of Malaria and how these will be eliminated to make sure the death caused by Malaria is reduced and the vulnerable environment is free from Malaria.

a) Why should there be prevention of malaria in vulnerable environments?

b) Who should take control and who is responsible to eliminate the infection of malaria in vulnerable environment?

c) Which role do we play as practitioners to make sure vulnerable environment are free from malaria?

In this study ways are provided to prevent, control and eliminate malaria in vulnerable environment with an emphasis to information dissemination to households living in those environments, empower them on environment cleanliness, shows them preferred sites for mosquitoes, how they should keep their environment clean and provide ways and means to control and eliminate mosquito havens. This will be one of the ways to prevent mosquito, control and eliminate malaria in the vulnerable environment.
P402: Geographical variation of factors affecting effectiveness of Long-Lasting Insecticidal Nets (LLINs) in Tanzania

Zawadi Mageni1, Ubydul Haque1, Lena M. Lorenz2, Sarah Moore3,4, Jason Moore2, Dennis Massue4, Renata Mandikle, Karen Kramer4, William Kisimba5, Hans J. Overgaard6, Jo Lines7
1Ifakara Health Institute, Bagamoyo, Tanzania; 2Department of Disease Control, London School of Hygiene and Tropical Medicine, London, UK; 3John Hopkins Bloomberg School of Public Health, Baltimore, USA; 4Swiss Tropical and Public Health Institute, Basel, Switzerland; 5National Malaria Control Program, Dar es Salaam, Tanzania; 6Department of Mathematics and Sciences Technology, Norwegian University of Life Sciences, Ås, Norway;

BACKGROUND: The success of long lasting insecticidal nets (LLINs) as a strategy for malaria control is evident, as they provide both a physical and chemical barrier from mosquitoes. However, quick physical deterioration of nets, insecticide resistance in mosquitoes, and net ownership not equating net use are challenges that pose serious threats to the sustainability of this intervention. It is therefore crucial that factors associated with net loss are identified and accounted for. In an attempt to optimize the LLIN strategy, this study will use Geographic Information Systems (GIS) and spatial analyses to investigate underlying location-specific environmental and cultural factors and other potential risk factors that may affect net durability.

METHODS: Across ten districts of geographic and epidemiologic diversity in Tanzania, two surveys will be conducted to measure the durability of LLINs through attrition (presence or absence of nets), bio-efficacy (ability of nets to knock-down or kill mosquitoes), chemical content (active ingredient in net fibres) and physical degradation (fabric integrity). In September 2013, a retrospective survey will be done on Olyset™ nets distributed to households by the National Malaria Control Program (NMCP) in 2009-2010. Thereafter, a prospective survey will be done to evaluate 5000 nets of three leading bednet brands over three years in those same households. National health survey data in corresponding districts will also be evaluated to assess the relationship between net attrition and malaria incidence. Satellite imagery of land use and land cover data will be downloaded for assessment of environmental factors.

RESULTS: All households will be geo-referenced to create a GIS database, which will include net and household characteristics. This GIS database will be merged with environmental, socio-economic and health data for spatial and temporal analyses and modelling.

CONCLUSIONS: Our study hypothesizes that there will be geographic variation in net durability and factors associated with LLIN effectiveness due to environmental, socio-economic and cultural diversity in Tanzania. By identifying these factors, decision makers may use results from this study to more effectively optimize procurement and product choice to achieve universal coverage of LLINs for malaria vector control.

P403: Factors that facilitated the shift towards malaria elimination in South Africa

Rajendra Maharaj
Medical Research Council, Durban, South African

Due to the marked decrease in malaria cases in the region, eight countries in the southern African region are now targeting malaria elimination. The main criteria used in determining the paradigm shift is when countries start experiencing disease burden levels of <1 case per 1000 population. Having achieved such a status, South Africa has now transitioned from a malaria control programme to a malaria elimination agenda. Just over a decade ago, South Africa experienced the worst epidemic in over 50 years. This study aimed to examine the impact that changes to the malaria control programme had on disease burden. Some of the factors that facilitated this decrease in disease transmission were the re-introduction of DDT as the chemical of choice for indoor residual spraying, the rollout of combination therapy and the development of cross-border initiatives. A retrospective analysis of the data from 1997 to 2012 showed that the development of insecticide and drug resistance contributed to the epidemic of 1999/2000.

A second, pyrethroid resistant vector had become established in predominantly KwaZulu-Natal and resulted in a 6 fold increase in the winter transmission of malaria. There was a 86% resistance to the sulfadoxine-pyrimethamine drugs that were used for the treatment of malaria. However with the introduction of effective insecticides and drugs, malaria transmission was once more curtailed in the epidemic prone provinces of South Africa. The decrease in case numbers was also attributed to the implementation of a cross border initiative, the Lubombo Spatial Development Initiative (LSDI), between Mozambique, South Africa and Swaziland. The goal of the LSDI was to strengthen malaria control activities in all three countries but predominantly in southern Mozambique. The implementation of the LSDI resulted in malaria incidence in South Africa and Swaziland decreasing by 99% and 98% respectively, and the prevalence in southern Mozambique decreased by an average of 85%, all compared to the baseline values of 2000. Therefore this study demonstrated that an interplay of factors helped drive the decline of malaria morbidity and mortality in South Africa and is pushing the country closer to the goal of malaria elimination by 2018.

P404: The Impact of Indoor residual spraying in different epidemiological settings

Rajendra Maharaj
Medical Research Council, Durban, South African

Vector control through indoor residual spraying (IRS) has been the backbone of vector control programmes in most southern African countries. This has been the situation in South Africa and Swaziland where IRS has been used as the only vector control tool for the past five decades. Only recently has IRS been reintroduced into southern Mozambique as part of the Lubombo Spatial Development Initiative (LSDI). After a careful evaluation of the entomological parameters that affect malaria control, IRS was introduced into southern Mozambique, first in Maputo Province and then in Gaza Province. This study was aimed at determining the impact that IRS had on malaria transmission in Maputo Province (a high transmission area, Gaza Province [a median to moderate transmission area) and South Africa [a low transmission area]. Entomological data from exit traps were analysed to determine the impact of IRS on mosquito populations. Case data was used as a proxy of the impact of IRS on malaria transmission. It was found that the re-introduction of IRS into a high transmission area resulted in a huge decrease in the prevalence of the disease in Maputo Province - a 85% decrease in 5 years. In Gaza province, the gains were most modest with a impact of just 26% being achieved over 5 years. IRS had the least impact in South Africa where malaria transmission was at an all time low. This study found that IRS is well suited to areas where rapid impact is required in high transmission, especially where sustained control has not been implemented. The results are less spectacular in low and moderate transmission areas. For increased impact, vector control initiatives need to be tailored to the particular situation.

P405: Mating competitiveness of sterile male Anopheles gambiae in semi-field cages

H Maiga

BACKGROUND: The renewed interest in the development of control strategies using sterile insects raises hopes of being able to control the disease by cutting down the high reproductive rate of mosquitoes. Specifically, investigations into factors that account for male mating competitiveness are critical to the development of genetic control strategies. In this study, we assessed the effects of partial irradiation with 75 Gy on An. gambiae sexual competitiveness when allowed to mate in different ratios of sterile/fertile males for 2 nights in field cages. Moreover, to determine the dynamics of this competition, competitiveness was compared between males allowed 1 night vs 2 nights of contact with
females.

METHODS: Sterilized (S) and fertile (U) males between 4 and 6 days of age were released in field cages (1.70mx1.70mx1.70m) with females (F) of similar age and left for 2 nights at the following ratios (S:U:F): (100:0:100) (100:100:0) (300:100:100) (500:100:0) (0:100:100). Each treatment was replicated 3 times. Competitiveness was determined by assessing the hatching rate of eggs laid en masse and the insemination rate, determined by dissecting recaptured females. An additional experiment with a ratio of (500:100:100) has been done with a mating period of either 1 or 2 nights.

RESULTS: For the first experiment, the egg hatching rate was significantly affected by the release ratio and we thus observed that the Fried competitiveness index of sterile males was between 0.29 and 0.55. A similar insemination rate was recorded after 2 nights of contact in experiment 1, while significant difference was observed in the (S:U:F) (100:0:100) ratio between the males left to mate for 1 and 2 nights. However, a similar hatching rate was observed when mating occurred for 1 or 2 nights.

CONCLUSION: The results suggest a release ratio of at least 2 sterile males for 1 fertile male and that An. gambiae mating competitiveness experiments in field cages should be run for 1 instead of 2 nights.

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**P406: School performance after six years of intermittent preventive treatment using artemisinin-based combination therapy in Mali**

Hamma Maiga,1 Breanna Barger-Kamate,1 Issaka Sagara,2 Oumar Bila Traore,2 Mamadou Tekete,1 Intimbye Tembine,2 Antoine Dara,2 Soumana Issac Traore,2 Modibo Diarra,2 Samba Coumare,1 Aly Kadio,1 Bouran Sidibe,1 Aboubacrine Haidara,1 Nouhoum Diallo,1 Ogobara K. Doumbo,1 and Abdoulaye A. Djimde1

1Malaria Research Training Center, Department of Epidemiology of Parasitic Diseases, Faculty of Medicine, Pharmacy and Odonto-Stomatology, University of Science, Technology and Technique of Bamako (USTTB), Mali
2School of Medicine, Johns Hopkins University, Baltimore, MD

**THEMATIC AREA:** Prevention, control and elimination

**LEVEL OF STUDY:** MSc, PhD Candidate, Email: hmaiga@icermali.org

**BACKGROUND:** Previous studies showed that in areas of seasonal malaria transmission, intermittent preventive treatment of school children (IPTsc) targeting the transmission season, reduced the rates of clinical malaria. The efficacy of ACTs in the context of longitudinal IPTsc is poorly investigated and school performance has not been thoroughly evaluated.

**METHODS:** This was an open randomized controlled trial of seasonal IPT among school children aged 6–13 years in Kolle, Mali. The study began in September 2007 and completed follow-up in June 2013. Students were randomized to one of three study arms: Sulphadoxine–pyrimethamine (SP), and artesunate (AS) (C). All students received two full treatment doses, given 2 months apart during the season of high transmission from September to December. Groups were compared with respect to school performance, incidence of clinical malaria, asymptomatic parasitaemia and anaemia.

**RESULTS:** A total of 296 students were randomized, and retention in the study was 99.3%. Yearly grade average and success rate in the SP/AS and AQ/AS arms were (5.37, 79.1%) and (4.87, 70.5%) respectively vs. control (4.81; 68.7%) (P < 0.05). Clinical malaria incidence in the SP/AS and AQ/AS arms was reduced by 50.9% and 20.6%, respectively, vs. control (P < 0.001). There were fewer all-cause clinic visits among the children receiving SP/AS or AQ/AS (P < 0.001). The prevalence of asymptomatic parasitaemia was higher in the control group than in the SP/AS or AQ/AS (P < 0.001) groups. At the end of the transmission period, children treated with IPT showed a trend towards lower rates of anaemia (SP/AS, 4.2%; AQ/AS, 7.8%; Control, 12.7%; P = 0.012).

**CONCLUSION:** IPTsc with SP/AS reduced the rates of clinical malaria, all-cause acute clinic visits and asymptomatic parasitaemia and trended towards a reduction in anaemia among school-aged children while improving markers of school performance.

**KEYWORDS:** malaria, artemisinin, intermittent preventive treatment, school performance

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**P407: Identification of HERPES SIMPLEX VIRUS TYPE I in febrile children suspected having malaria.**

END Anna A1, Louise Stéphanie MAKEMGUE2

1Department of Animal Biology and Physiology, Faculty of Sciences, University of Yaounde I, Cameroon.
2Healthcare Public Institution, Yaounde, Cameroon

**BACKGROUND:** Plasmodium malaria remains one of the major parasitic infections in the tropics and in Cameroon in particular. The similarity of clinical symptoms of malaria to that of some infectious diseases such as herpes labialis, caused by the Herpes Simplex Virus Type 1, complicates malaria diagnosis in children. This situation results in misdiagnosis and consequently increased morbidity, especially when they occur in mixed infections. That is why we sort to determine any relationship between the two pathogens in febrile patients suspected having malaria.

**METHODS:** Blood samples were bled from 90 febrile children (49 boys, 41 girls) aged between 2-15 years with the consent of their parent or legal guardian, for the identification of *Plasmodium* spp. by microscopy and two rapid detection tests (SD Bioline Malaria Antigen Pf/Pan and ICT MALARIA Pf). The antibody titre of Herpes Simplex Virus Type 1 was also determined by Enzyme Immunoassay test.

**RESULTS:** Microscopic examinations revealed 20(22.2%) positive cases; only *Plasmodium* was detected. Rapid detection tests showed 45(50 %) positive cases with 23(51.11%) cases of *Plasmodium falciparum* in single species infections and 22(48.89%) in co-infections with *Plasmodium malariae* and *Plasmodium ovale*. The serological assay revealed 47(52.22%) of the Herpes Simplex Virus Type 1; children in the 10-15 age group were the most affected (61,29%). *Plasmodium* spp. was in confection with Herpes in 23(25.56%) cases.

**CONCLUSIONS:** Rapid Detection Tests were obviously more sensitive than microscopy for the malaria diagnosis. The high prevalence of Herpes Simplex Virus Type 1 emphasises the importance of a thorough laboratory diagnosis of malaria in febrile patients, following clinical observations.

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**P408: Asymptomatic malaria parasitaemia in sickle-cell disease patients: how effective is chemoprophylaxis?**

Rachel Kotla1, Abiola Okesola2 & Olufunmilola Makanjuola1
1Department of Haematology, College of Medicine, University of Ibadan, Nigeria; 2Department of Medical Microbiology & Parasitology, College of Medicine, University of Ibadan, Nigeria

**BACKGROUND:** Sickle-cell trait confers protection against malaria while homozygote sickle-cell disease (SCD) patients are at greater risk of malaria infection, hence the use of malaria chemoprophylaxis in SCD patients. The use of malaria chemoprophylaxis and asymptomatic parasitaemia were studied in SCD and non-SCD patients.

**METHODS:** A semi-structured questionnaire was administered to both patients and controls; a thick blood film was also examined in both the groups.

**RESULTS:** Sixty-nine percent of patients use proguanil, 22% do not use any form of chemoprophylaxis, while 9% use pyrimethamine. There was no significant difference between level of parasitaemia in patients and controls (p = 0.1), a positive smear was found in equal numbers of patients on chemoprophylaxis and those not on chemoprophylaxis (p = 0.3). In the month preceding the study, 31% of patients vs 18% of controls had received treatment for malaria. There were no significant differences between patients and controls in frequency of malaria attacks (p = 0.06), last episode of malaria (p = 0.2). Ten percent of patients and 2% of controls use bed-nets.

**CONCLUSION:** This study did not find any advantage in the use of malaria chemoprophylaxis in SCD patients over controls or SCD patients not on chemoprophylaxis. Vector control should also be considered in the fight against malaria. There is a need to look into why both patients and controls fail to use bed-nets in a malaria endemic country.
P409: A molecular chaperone-based procedure promotes the quality of recombinant Plasmodium falciparum AdoMetDC produced in E.coli
Kolani H. Makhoba1, Dina le Roux1, Lyn-Marie Birkholz2, Addmore Shonhai2
1Department of Biochemistry & Microbiology, University of Zululand, KwaDlangezwa 3886, South Africa; 2Malaria Parasite Molecular Laboratory, Department of Biochemistry, University of Pretoria, Pretoria, South Africa

BACKGROUND: The production of recombinant malarial proteins that are of interest as drug targets is often fraught with poor product yields. Several approaches are used in order to promote the production of recombinant malarial proteins in E. coli. It has been suggested that co-expression of molecular chaperones with target proteins of interest in E. coli could enhance the quality and quality of the recombinant proteins of interest. Heat shock protein 70 (Hsp70) is a molecular chaperone that occurs in most living organisms. Hsp70 facilitates folding of other proteins in the cell. Plasmodium falciparum S-adenosylmethionine decarboxylase (PFAdoMetDC) is an essential protein involved in polyamine biosynthesis, making it a potential anti-malarial drug target. In order to improve the production of recombinant PFAdoMetDC in E. coli, we co-expressed it with a Plasmodium falciparum heat shock protein 70 (PHsp70) and a chimera (KPI) that was made up of the peptide binding domain of PHsp70 linked to the ATPase domain of E. coli Hsp70.

METHODS: E. coli cells that were co-transformed with plasmids encoding the respective Hsp70 and PFAdoMetDC were induced to express the proteins. We purified the recombinant PFAdoMetDC and characterised it using limited proteolysis and enzyme assays.

RESULTS: Based on limited proteolysis PFAdoMetDC produced under the variable chaperone conditions in E. coli exhibited unique confirmation. In addition, enzymatic assays conducted on PFAdoMetDC suggested that the use of Hsp70s of plasmodial origins enhanced the quality of the recombinant PFAdoMetDC that was produced in E. coli.

CONCLUSIONS: Findings from this study suggest the conformation and quality of recombinant proteins produced in E. coli are influenced by the protein folding conditions prevailing during their expression in E. coli.

P410: Informing new or improved vector control tools for reducing malaria burden in Tanzania: exploring perceptions of risk, protection and responsibility
C Makungu

BACKGROUND: In Tanzania over recent years there has been a significant decline in malaria related mortality and morbidity accompanied by a significant shift in vector species composition. Little is known about how this has affected community perceptions of vectors and vector control strategies or their views on who is responsible for mosquito control. In an era of increasing vector resistance, such information is crucial in designing new and effective vector control strategies to maintain and enhance these advances in malaria control. This study aimed to: explore communities’ perceptions of mosquitoes; identify measures employed to prevent mosquito bites; and investigate perceptions of responsibility for mosquito control.

METHODS: This qualitative study was conducted in rural, peri-urban and urban areas of the Dar es salaam region in 2012. Data were collected using: focus group discussions (n=23), semi-structured interviews (n=69) and photo-voice (n=32).

RESULTS: Mosquitoes were considered as a source of malaria and a major nuisance by all participants. Breeding sites were clearly distinguished from resting sites and poor environmental hygiene was considered as a major factor encouraging mosquito breeding. In urban rich areas, bednets, insecticide sprays and window screens were employed for protection against indoor biting mosquitoes. Among the urban poor, peri-urban and rural residents bednets were the major tool for indoor protection and their use was described as ‘part of the culture’. Novel methods to prevent outdoor biting (e.g. skin repellents) were viewed with suspicion and improving the local environment was the preferred method for preventing outdoor biting. Improving local hygiene and sanitation was seen as a community responsibility while spraying of breeding sites was perceived as the duty of local government.

CONCLUSION: Knowledge of mosquitoes and malaria was high and, in Dar es salaam, bednet use for indoor protection has become the norm. Novel methods for outdoor protection were viewed with suspicion due to shortages of information, affordability, availability and inconvenience. Government conducted spraying of breeding sites was perceived as the best strategy for controlling outdoor mosquito biting. Novel vector control strategies should consider addressing mosquito control alongside personal protection and involve both communities and local government.

P411: Hydronephrosis It, hydro. Challenges associated with implementing a nationwide subsidized ACTs program–The AMFm experience in Ghana
Malm Keziah L1, Baiden Frank1, Bart-Plange Constance1
1National Malaria Control Programme, Ghana Health Service; 2Kintampo Research Center, Ghana Health Service

INTRODUCTION: Ghana was among the eight countries selected to participate in the Affordable Medicine Facility for Malaria (AMFm) Initiative from 2009 to 2012. The facility sought to increase access to ACTs which had been prequalified by WHO through making them affordable to all patients and by so doing drive monotherapies out of the country. An evaluation of AMFm in Ghana was largely favourable.

METHODS: This paper reviews related administrative documents, policies and guidelines to present a discussion of the anticipated and unanticipated challenges that were associated with its implementation, and how these were overcome.

RESULTS: The anticipated challenges included; (1) Local manufacturer opposition due to fear of losing sales (2) retailer anxiety about losing profits, (3) public perception that low-cost connoted inferior quality. Major unanticipated challenges included: (1) Delay in procuring the ACTs for the public sector (2) Inability of the Global Fund to meet the stock needs of the private sector. The strategies employed to overcome these challenges included high-level stakeholder meetings with wholesalers and retailers, with extensive public-private sector participation, distinct labeling and in-depth public education on the concept of internationally-subsidized ACTs and negotiations on acceptable retail price.

CONCLUSION: Overall public-private sector impression of AMFm is positive. The challenge of sustainability remains with evidence emerging of rebound increases in the prices of ACTs following the end of the first phase of the facility.

P412: Knowledge, attitudes, and practices of community health workers and health care providers trained to administer rapid diagnostic tests for malaria
Kathleen Maloney, Nora Petty, Lindsay Bryson, Andrew Atebe, Divine Nziouba
Clinton Health Access Initiative, Yaoundé Cameroon

BACKGROUND: In 2010 the Cameroon National Malaria Control Program (NMCP) added malaria rapid diagnostic tests (RDTs) to the list of services that Health Care Workers (HCWs) in public health facilities and Community Health Workers (CHWs) can provide. The NMCP trained CHWs and some HCWs on RDT administration and the proper treatment
of malaria positive patients. The purpose of this study was to report on the knowledge, attitudes, and practices of HCWs and CHWs following RDT training.

METHODS: Ninety-one CHWs from 75 villages and 47 health care providers from 20 facilities in littoral Region, Cameroon, were interviewed approximately nine months after RDT training with regards to their knowledge, attitudes, and practices towards malaria diagnosis and treatment. Additionally, patient records on laboratory testin and treatment from all 20 facilities were collected to evaluate malaria diagnosis and treatment behaviors.

RESULTS: Results indicated that the majority of CHWs reported that they typically see fewer than 2 febrile patients per week. On average HCWs reported seeing only 6 patients per day, 4 of them with fever. Prescribing antimalarials without first performing an RDT was a commonly reported practice: 48% of CHWs believed that a patient with classic signs and symptoms of malaria does not always require a diagnostic test and 33% of CHWs reported that they diagnose and treat patients based on symptoms even when RDTs are available. Among HCWs, 40% report that they sometimes or always prescribed antimalarials without a confirmatory diagnosis. Reported adherence to negative test results among CHWs was high with 75% of CHWs reporting that they never prescribe antimalarials when the RDT is negative. However, among HCWs, reported adherence was much lower, with 83% believing a clinician should sometimes or always give antimalarials when the test results is negative. This study demonstrates that HCWs and CHWs are willing to test patients with RDTs.

These results suggest that training alone is not sufficient, sensitization may be required to increase community awareness to the benefits of malaria testing and boost provider confidence and trust in the RDT results.

P413: Anopheles gambiae performances to find a hole in long lasting net

D Mamadou

BACKGROUND: Widespread distribution of pyrethroid-treated long-lasting nets (LLINs) is currently the main vector control method to fight against malaria transmission despite the threat of pyrethroid resistance. In this study, we investigated the ability of pyrethroid susceptible and resistant strains of Anopheles gambiae s.s to find hole in LLINs (Olyset and PermaNet) compared to untreated control net.

METHODS: We measured performance (number of mosquitoes passed through the hole in relation to two other status not passed and knock down (Kd) mosquitoes) of three laboratory strains of An. gambiae sharing the same genetic background with a different genotype for the Kdr mutation: homozygous susceptible (SS), homozygous resistant (RR) and heterozygous (RS). Mosquitoes were individually tracked in the arena tunnel with a video system and analyses were performed using Ethovision® XT software.

RESULTS: With untreated net, the proportion of SS and RS females that passed through hole was higher than among RR females, whereas there was no significant difference between SS and RS genotypes. With PermaNet, there was no difference of performance between the 3 genotypes. With Olyset, the proportion of RS females that passed through the hole was higher than among SS and RR genotypes. These performance results with Olyset and PermaNet, are highly dependent of the Knock-down effect which is more important with Olyset than Permanet. Indeed, among non-KD SS females, the proportion that passed through the hole was higher than in RS and RR regardless the net brand.

CONCLUSION: Our results suggested that the pyrethroid insecticides modulated the short-ranged behaviour around a net. The interaction between the behaviour modulation and the induced Knock-down effect explained the better performance of RS genotypes against Olyset and the absence of performance difference between the 3 genotypes with PermaNet.

P414: Physiological, morphological and hormonal variation in Anopheles gambiae s.l. mosquitoes exposed to the stressful conditions of the dry season in Burkina Faso, West Africa

Mamai W.1,2,*, Mouline K.1,2, Dabiré K.R.2, Ouedraogo GA1, Blais C1, Renault D2, Simard F2.

1IRSS, Bobo Dioulasso, Burkina Faso; 2IRD/MIVEGEC/BEES, Montpellier; 3Université Polytechnique de Bobo Dioulasso, Burkina Faso; 4 Université de Rennes 1/UMR 6553 EcoBio, 5 Université Pierre et Marie Curie, UMR INRA-UPMC 1272 PISC, Paris Cedex 05, France

BACKGROUND: In tropical savannahs of West Africa, mosquitoes have to cope with extended periods of harsh environmental conditions during the long (6-9 months) dry season with high daily temperature fluctuations, low relative humidity and scarcity of water collections suitable for oviposition and larval development. However, their survival mechanisms under aridity and drought remain poorly understood. This study explored the degree of physiological, morphological and hormonal changes that are being prompted by a switch between the rainy and dry season conditions in three members of the Anopheles gambiae s.l. complex that coexist in Burkina Faso.

METHODS: Insects were reared in climatic chambers reflecting environmental conditions recorded in the field during the rainy and/or the dry season. Their metabolic fingerprinting and proteins expression were analyzed by gas chromatography - mass spectrometry and 2D-DIGE respectively. Ecdysteroid hormones were quantified using an enzyme immunoassay and finally spiracles were observed under scanning electron microscopy (SEM).

RESULTS: Our study revealed that older female mosquitoes reared under dry season conditions were characterized by lower concentration of tricarboxylic acid cycle intermediates and isoleucine, suggesting metabolic and reproduction depression in the dry season conditions. Overexpression of proteins involved in muscles’ contraction (myosin light chain) and cuticle thickness and rigidity (cuticular proteins) were observed during the dry season in both An. coluzzii and An. gambiae. On the other hand An. coluzzii and An. arabiensis considerably reduced their spiracles apertures which are surrounded with high number of trichomes in dry season. Ecdysteroid concentration was much higher in males than in females, suggesting a role of these hormones in shaping An. gambiae reproductive strategies and population demography.

CONCLUSION: By exploring physiological and morphological correlates of mosquito local adaptation, our work contributes to unraveling the complex mechanisms underlying the enormous adaptive potential hidden within the An. gambiae species complex.

P415: Antibodies by non-febrile, smear-negative individuals from a malaria epidemic setting in Ethiopia are reactive to Plasmodium falciparum blood-stage-vaccine candidate antigens

Hassen Mamou, Nnaemeka C. Iriemenam2, Klavs Berzins3, Beyene Petros4

1Microbial, Cellular and Molecular Biology Department, College of Natural Sciences, Addis Ababa University, P. O. Box 1176, Addis Ababa, Ethiopia; 2Department of Medical Microbiology and Parasitology, College of Medicine of the University of Lagos, Ido-araba, Lagos, Nigeria; 3Department of Molecular Biosciences, The Wenner-Gren Institute, Department of Immunology, Stockholm University, Sweden

Plasmodium falciparum malaria remains a major public health concern globally though there is some decline in the number of clinical cases and deaths due to scaling up of control efforts in recent times. Evaluation
of the anti-malarial immune profile, in populations residing in epidemic-prone areas in the dry season or at the time when vector control largely reduced man-mosquito contact, would help understand the duration of immune reactivity. A cross-sectional study was designed to investigate antibody responses to four \textit{P. falciparum} blood-stage vaccine candidate antigens in non-febrile individuals from Shewa Robit in north central Ethiopia where malaria transmission was at a minimal level as a result of the sampling season and effective vector control. Blood samples were analyzed microscopically for \textit{Plasmodium} detection. The enzyme-linked immunosorbent assay (ELISA) was used to measure immunoglobulin (Ig) G (IgG) antibodies to apical membrane antigen 1 (AMA1), glutamate-rich protein (GLURP) R2 region and merozoite surface protein 2 (MSP2) allelic variants [3D7 & FC27]. Study participants were smear-negative for malaria. The antigens tested were well-recognized by the test sera although significant differences were observed in antibody prevalence and level between the different antigens and there was inter-individual variability. There was no serum sample that was not antibody positive against at least one antigen. IgG response to the antigens showed age-related pattern. The data suggests that individuals in an unstable and epidemic-prone malaria setting have reactive antibodies that readily recognize \textit{P. falciparum} blood-stage vaccine candidate antigens in the absence of slide-positivity.

**KEYWORDS** \textit{Plasmodium falciparum}, ELISA, Ethiopia, vaccine

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**P416: Congenital malaria in Bobo-Dioulasso (Burkina Faso): a case series report**

C Mamoudou

**BACKGROUND:** Congenital malaria is poorly reported in recent literature from Africa. Prophylactic strategies such as intermittent preventive treatment during pregnancy (IPT) using sulfadoxine-pyrimethamine (SP) were reported to reduce significantly maternal parasitaemia and hence the risk of congenital malaria. We investigated in a cross-sectional study in Bobo-Dioulasso (South west of Burkina) the prevalence of congenital malaria.

**METHODS:** A cross-sectional study was implemented in two primary health facilities of Bobo-Dioulasso where IPT, was the national policy for malaria prevention during pregnancy. Maternal baseline was recorded among women delivering in these 2 centres. Samples of maternal, placental and cord blood were taken and stained with Giemsa and examined for malaria parasites.

**RESULTS:** Overall, 193 pregnant mothers were included in the study. Three neonates were found to harbour \textit{falciparum} malaria resulting in prevalence of 1.5% (3/193) for congenital malaria. Analyses of maternal baseline revealed that the three mothers of those newborns had received IPT, during pregnancy (2 doses of SP), two were primigravidae and only one mother was sleeping under ITN during pregnancy. No history of blood transfusion was reported in any of the mothers. At admission, one mother was found to be febrile (39°C) and her baby had also fever at birth (38°C). All the three mothers had both peripheral and placental \textit{falciparum} parasitaemia. Among the three newborns infected by \textit{falciparum} malaria, parasites densities were of 200, 472 and 1,120 trophozoites/µl, respectively. Birth weights were of 1430g, 2000g and 1900g, respectively. The three newborns were successfully treated with intravenous quinine and all were alive by 28 days.

**CONCLUSION:** The prevalence of congenital malaria was low in this study. Babies born from mothers with malaria should be screened for congenital malaria. Larger studies are needed for more accurate prevalence and risk factors analyses.

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**P417: Optimization of a sensitive chemiluminescence-based Antibody-Dependent Respiratory Burst (ADRB) assay as tool to evaluate anti-malarial immunity in endemic populations**

**Annick Mansourou**, Charlotte Joos, Babacar Diouf, Oumy Niass, Adama Tall, Shirley Longacre, Ronald Perraut, Assiatou Touré

1. Immunology Unit, Institut Pasteur Dakar, 36 av Pasteur, Dakar, Senegal; 2. Epidemiology Unit, Institut Pasteur Dakar, 36 av Pasteur, Dakar, Senegal; 3. Lymphocyte Population Biology Unit, Institut Pasteur, 28 rue du Dr Roux, 75724 Paris cedex 15, France

**BACKGROUND:** There is an urgent need to develop functional assays in order to predict the level of protection naturally acquired by exposition to malaria infection or induced by vaccination. We have developed a sensitive chemiluminescence-based assay called Antibody-Dependent Respiratory Burst (ADRB) assay and standardized by the calculation of an ADRB index. This assay measures the capacity of opsonized \textit{Plasmodium falciparum} merozoites to trigger human neutrophils respiratory burst. ADRB activity was previously shown associated with protection against clinical malaria in two different endemic areas of Senegal. Despite very encouraging and promising preliminary results further improvements of ADRB assay were necessary to ensure better reproducibility intra-laboratory and inter-laboratory.

**METHODS:** ADRB assays was optimized by improving techniques for freezing and thawing merozoites and by determining optimal ratio between number of neutrophils and number of merozoites required, and optimal value of pH buffer. Intra- and inter-assay variation was assessed by replicate analysis on three consecutive days. The reliability of optimizations was checked by analysis of serum samples from 97 individuals (3-80 years old), living in a holoendemic area (Dielmo, Senegal) and enrolled in a cross-sectional prospective study with intensive follow-up. Relation between ADRB activity and protection from clinical malaria was assessed by an age-adjusted Poisson regression model.

**RESULTS:** Optimization of parameters resulted an increase in signal intensity and sensitivity and ensured minimal variation and good reproducibility (CV<10%) of the ADRB assay. The assay validation with serum samples showed that antibodies that were not protective in vivo had no effect on neutrophils respiratory burst activity in the ADRB assay. ADRB activity was found age-dependent and a high ADRB response was positively correlated with \textit{in vivo} clinical protection from \textit{P. falciparum} malaria (ADRB<660 vs. ADRB≥660: RR, 2.75; 95% CI, 1.39 to 5.44; p=0.003).

**CONCLUSIONS:** The ADRB assay is therefore an \textit{in vitro} assay reflecting protection-associated effects of antimalarial antibodies observed under \textit{in vivo} conditions in humans. These results support further development of ADRB assay and its usefulness as tool for evaluating immune protection acquired naturally or induced by vaccination against malaria in individuals living in endemic areas.

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**P418: Safety and efficacy of artemether-lumefantrine against uncomplicated \textit{Plasmodium falciparum} malaria during pregnancy: a systematic review**

Christine Manyando, Kassoum Kayentao, Umberto D’Alessandro, Henrietta U Okoar, Kamal Hamed, Elizabeth Juma

1. Tropical Diseases Research Centre, Ndola, Zambia; 2. Malaria Research and Training Centre, Bamako, Mali; 3. Medical Research Council Unit, Fajara, 6th MIM Conference 2013

**BACKGROUND:** Artemether-lumefantrine is an \textit{in vivo} agent recommended by WHO for uncomplicated malaria. However, there is concern regarding the safety and efficacy of artemether-lumefantrine in pregnant women. Therefore, the aim of this systematic review was to synthesize the available evidence on safety and efficacy of artemether-lumefantrine during pregnancy.

**METHODS:** A systematic review was conducted following the PRISMA guidelines. Electronic databases were searched for relevant studies. Three outcomes were considered: safety, efficacy and pharmacokinetics. Two reviewers independently screened the studies for eligibility. A third reviewer resolved any disagreements.

**RESULTS:** A total of 20 studies were included in the review. The most common adverse event was nausea, followed by vomiting and diarrhea. The efficacy of artemether-lumefantrine in pregnant women was comparable to non-pregnant women. However, the pharmacokinetics of artemether-lumefantrine was altered during pregnancy, with lower maximum plasma concentrations and longer elimination half-lives.

**CONCLUSIONS:** Artemether-lumefantrine is safe and effective during pregnancy, with a similar efficacy profile to non-pregnant women. However, further studies are needed to optimize the use of artemether-lumefantrine in pregnancy and to assess the long-term effects on the fetus.
were randomized to either CTX (27) or SP (25). There were 14 women with CD4 count <200 cells/µl. The pregnancy outcomes of HIV negative mothers generally showed no difference in risk of preterm delivery (CTX 3.6%, SP 2.1%), still birth (CTX 3.0%, SP 2.1%) and neonatal death (CTX 0%, SP 1.4%). This was also the case with the infant outcomes, the mean birth weights were 3.1 Kg (standard deviation ±0.5) for both CTX and SP. The pregnancy and infant outcomes for the HIV positive women with CD4 count ≥ 200 cells/µl in either arm as well as those with CD4 count <200 cells/µl on CTX had no observable differences. However, the numbers for these groups were much smaller.

CONCLUSIONS: Exposure to CTX during the latter part of pregnancy was not associated with increased safety risks. However, more studies with a larger sample size are recommended to further explore the safety profile and the possible role of CTX for malaria prevention in HIV infected and uninfected pregnant women.

P420: Toward a salivary biomarker of human exposure to infective Anopheles bites

Alexandra Marie1, Philippe Holzmuller2, Marie Rossignol2, Edith Demettre1, Martial Seveno1, Majoline Thiollo Tsapi1, Vincent Corbel1, Fabrice Chandra1, Isabelle Morlais4,1, Franck Remoue1, Alexis Corbel1,5, "MIVEGEC-IRD-OCEAC, 8P 238 Younoué, Cameroun 1Department of Entomology, Faculty of Agriculture, Kasetsart University, 50 Ngam Won Wan Rd, Ladyaow Chatuchak, Bangkok 10900, Thailand; 2MIVEGEC-IRD-CREC, 01 BP44 RP Cotonou, Bénin

BACKGROUND: Malaria is caused by parasites of the genus Plasmodium transmitted by Anopheles mosquitoes during the blood feeding. During a bite, saliva and parasites are injected into the human skin. Salivary molecules possess pharmacological and immune properties allowing a correct blood meal. Some salivary proteins could be recognized by the immune system and then induced an antibody (Ab) response. Monitoring this Ab response could be an indicator of the human exposure to anopheline bites. However the majority of the bites (> 95%) are non infective. So it is essential to develop a biomarker of infective bites estimating accurately the transmission risk. To assess this question we compared salivary extracts composition of An. gambiae infected and uninfected by P. falciparum by a proteomic approach. So if a protein is only express following the infection of salivary glands and is immunogenic, we can clearly use it as a marker of infection. If an immunogenic protein is overexpressed, we can hypothesize that the antibody response against this protein could increase too.

METHODS: Experimental infections of An. gambiae by wild P. falciparum were carried out in Cameroon. The parasites were obtained from asymptomatic young children. 14 days post-infection salivary glands were dissected. Four biological replicates of Two-Dimensional Differential Gel Electrophoresis (2D-DIGE) were performed with different pools of non-infected versus Pf-infected salivary glands.

RESULTS: Results showed several salivary components are regulated during the infection. The mass spectrometry by LC/MS-MS identified several proteins for the majority of each spots, except three overexpressed spots clearly identified as being one protein each. However only one is immunogenic and specific to Anopheles. Moreover three immunogenic proteins detected in majority amount in overexpressed spots could be potential biomarker too. However, some of them present sequence similarities with other arthropods (Culex, Aedes). Consequently, a peptide design is ongoing on these proteins to identify Anopheles specific biomarkers and will be soon tested by epitope mapping approach.

CONCLUSION: This study leads to the development of new tools to evaluate accurately the risk of malaria transmission essential in the context of elimination/eradication of malaria.
**P421: Genotypic characterization of *Plasmodium vivax*: an interpretational problem**

**Miles B Markus**

University of the Witwatersrand, Johannesburg, South Africa

It is conventionally assumed that in the absence of reinfection or drug-related, temporary parasite quiescence, the hypnozoite stage (I coined this term 3.5 decades ago) is the source of recurrent *Plasmodium vivax* malaria caused by parasites that are genetically similar to those which were responsible for the initial manifestations. How frequently (if ever) this is the case, though, is uncertain, partly because hypnozoites are never generated by the prior blood-stage infection. Hypnozoites are thought to be directly sporozoite-derived, but it has not been proven that they are not post-divisional pre-erythrocytic forms (MB Markus, 2012, *Trends Parasitol.* 28: 39–45). If the former, it has yet to be shown that genotypically homologous sporozoites inoculated by the mosquito can behave in two different ways, i.e. involving some sporozoites initiating early hepatic schizogony but others becoming dormant as hypnozoites. This might indeed happen, of course. However, could it normally or sometimes be non-hepatic, non-bloodstream, dormant merozoites (as opposed to hypnozoites) that give rise to recurrent homologous *P. vivax* malarial episodes after weeks, months or years (MB Markus, 2011, *S. Afr. Med. J.*. 101: 682–4; MB Markus, 2012, *J. Infect. Dis.*. 206: 622–3)? This possibility needs to be investigated, because such forms are known to occur in the life-cycles of some non-primate malarial plasmodial species. For reasons that are not readily apparent, uncertainty concerning the origin of recurrences could complicate molecular identification of drug-resistant *P. vivax* parasites. *P. vivax* recurrences often follow clinical *P. falciparum* malaria. The cause is unknown but the phenomenon is not unique to malaria: one type of related coccidian infection can lead to reactivation of another (MB Markus, 1988, *Med. J. Aust.*. 149: 344).

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**P422: Long-lasting Insecticidal Nets (LLINs) for malaria control in Tanzania: A retrospective survey of durability of Olyset nets distributed in 2009-2010.**

**Dennis Massue**1,2, Lena M. Lorenz2, Zawadi Mageni2,4, Jason Moore3,4, Peter Mangesho1, Renata Mandike1, Karen Kramer1, John Bradley1, Albert Killian1, Jo Lines2, William Kisinza1, Hans J. Overgaard1 and Sarah J. Moore1,4

1National Institute for Medical Research, Ammini Research Centre-Muheza, Tanzania; 2Swiss Tropical and Public Health Institute, Basel, Switzerland; 3Department of Disease Control, London School of Hygiene and Tropical Medicine, London, UK; 4Ifakara Health Institute, Bagamoyo, Tanzania; 5National Malaria Control Program, Dar es Salaam, Tanzania; 6Infectious Disease Control – Monitoring and Evaluation, Tropical Health LLP, Montagut, Spain; 7Department of Mathematical Sciences and Technology, Norwegian University of Life Sciences, Ås, Norway

**INTRODUCTION:** Long-Lasting Insecticidal Nets (LLINs) are the main tool used to control malaria, which provides both physical and chemical protection to people from mosquito bites and hence reduces malaria transmission. Millions of LLINs have been distributed across sub-Saharan Africa, including Tanzania, averting nearly one million malaria deaths in children under-five years of age in the last decade. To sustain this success, it is important to understand the reasons for net loss and durability. In this study we will assess reasons for net loss, net bio-efficacy and fabric integrity.

**METHODS:** A cross-sectional retrospective study will be carried out in September 2013 to assess Olyset nets from mass distribution campaigns in 2009-2010 (Under-5 and Universal Coverage Campaigns) in ten districts in Tanzania. Districts were chosen for their geographical and epidemiological diversity. We will evaluate the proportion of Olyset nets that are no longer in use (attrition) due to loss, damage, and alternative uses. We will collect information on household bed net use by structured questionnaires. A subsample of nets will undergo physical inspection to determine the number and size of holes and tears. Predictors of poor physical condition of bed nets will be identified. We will also assess the protective efficacy of nets against local mosquito vector populations using standard WHO bioassays and whole net tests under semi-field conditions.

**ANTICIPATED RESULTS:** With the global move towards malaria elimination, the study is anticipated to provide useful information about bed net use, factors related to net loss, durability and bio-efficacy under different conditions of utilization. In addition, the collected information can help to tailor behaviour change communication campaigns to support better utilization and care of LLINs and hence maintain its high performance and fabric integrity.

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**P423: Using a new odour-baited device to explore options for luring and killing outdoor-biting malaria vectors: a report on design and field evaluation of the Mosquito Landing Box**

**Nancy S. Matowo1, Jason Moore1, Salum Mapua1, Fredros O. Okumu1**

Environmental Health and Ecological Sciences Thematic Group, Ifakara Health Institute, Ifakara, Tanzania

**BACKGROUND:** Mosquitoes that bite people outdoors can sustain malaria transmission even where effective indoor interventions such as bed nets or indoor residual spraying are already widely used. Outdoor tools may therefore complement current indoor measures and improve control. We developed and evaluated a prototype mosquito control device, the ‘Mosquito Landing Box’ (MLB), which is baited with human odours and treated with mosquitocidal agents. The findings are used to explore technical options and challenges relevant to luring and killing outdoor-biting malaria vectors in endemic settings.

**METHODS:** Field experiments were conducted in Tanzania to assess if wild host-seeking mosquitoes 1) visited the MLBs, 2) stayed long or left shortly after arrival at the device, 3) visited the devices at times when humans were also outdoors, and 4) could be killed by contaminants applied on the devices.

**RESULTS:** There were significantly more malaria vectors visiting baited MLB than unbaited controls (P≤0.028). Increasing sampling frequency from every 120 min to 60 and 30 min led to an increase in vector catches of up to 3.6 fold (P≤0.002), indicating that many mosquitoes visited the device but left shortly afterwards. Outdoor host-seeking activity of malaria vectors peaked between 7:30 and 10:30pm, and between 4:30 and 6:00am, matching durations when locals were also outdoors. Maximum mortality of mosquitoes visiting MLBs sprayed or painted with formulations of candidate mosquitocidal agent (pirimiphos-methyl) was 51%.

**CONCLUSION:** While odour-baited devices such as the MLBs clearly have potential against outdoor-biting mosquitoes in communities where LLINs are used, candidate contaminants must be effective at ultra-low doses even after short contact periods, since important vector species such as *An. arabiensis* make only brief visits to such devices. The killing agents used should have different modes of action (other than pyrethroids as used on LLINs), to curb the risk of physiological insecticide resistance.
P424: Multiple insecticide resistance in *Anopheles gambiae* s.s threatens malaria control in Muleba District, North Western Tanzania

Johnson Matowo1,2, Natacha Protopopoff3, Reginald Kavische2, Robert Kaaya1, Alan Wright4, Franklin Mosha1,2 and Mark Rowland1,2

1Department of Medical Parasitology and Entomology, Kilimanjaro Christian Medical University College, Moshi, Tanzania; 2Department of Disease Control, London School of Tropical Medicine and Hygiene, Keppel Street, London, United Kingdom; 3Pan-African Malaria Vector Control Consortium (PAMVERC) www.pamverc.org

**BACKGROUND:** Insecticide-treated nets (ITNs) and indoor residual spraying (IRS) are being scaled-up as malaria vector control interventions in Tanzania. IRS has been implemented in several malaria epidemic zones including Muleba District, in North Western Tanzania. A recent study in Muleba revealed high pyrethroid and DDT resistance and reduced susceptibility to bendiocarb in *Anopheles gambiae* s.s. The kdr frequency in *An. gambiae* s.s has nearly reached fixation (99.8%), with no significant difference between allelic frequency of the kdr mutation in those mosquitoes that survived and those which died following exposure to permethrin, deltamethrin, lambdacyhalothrin (pyrethroids) or DDT.

**METHODS:** We studied the mechanisms underlying resistance in *Anopheles gambiae* s.s in the area using CDC bottle bioassays with the synergists piperonyl butoxide (PBO), s,s,s-tributylphosphorotrithioate (DEF) and through TaqMan assays. CDC bottle bioassays were used to study the involvement of mixed function oxidases and non-specific esterases in insecticide detoxification while TaqMan assays were used to identify an acetylcholinesterase G119S mutation that confers resistance to carbamates and organophosphates. PBO inhibits activity of mixed function oxidases while DEF inhibits non-specific esterases.

**RESULTS:** Exposure of F1 *An. gambiae* s.s to permethrin plus DEF, PBO or both inhibited non-specific esterase and oxidase activities, leading to higher knockdown and mortalities than exposure to permethrin alone. The Kd50 of *An.gambiae* s.s exposed to permethrin, permethrin+PBO, permethrin+DEF, permethrin+PBO+DEF was 63, 29, 28 and 35 minutes respectively with mortalities of 18%, 94%, 83% and 97% respectively. No altered insensitive acetylcholinesterase was found in any of the mosquitoes that were screened for the G1105 mutation.

**DISCUSSION AND CONCLUSION:** Persistence of *An. gambiae* s.s in the area suggests a reduced efficacy of vector control programmes that have been deployed in the area, especially pyrethroid based IRS and ITNs. Such control failure may be explained by the presence of both target-site insensitivity (kdr mutation) and metabolic resistance. The absence of the G1195 mutation implies another mechanism may be conferring the observed bendiocarb resistance, probably detoxification enzymes that breakdown insecticides before they reach their target sites. There is a need to investigate the operational impact of resistance on vector control strategies in the area, especially the ITNs which reached near universal coverage in 2011.

P425: Characterization of malaria transmission during military crisis in urban area of Bouake, Central Côte d’Ivoire

ADIA Akre Maurice1,2, YOBO Mabot Celine1, ASSI Serge Brice1

1Institut Pierre Richet, Institut National de Santé Publique, Bouaké, Côte d’Ivoire; 2Laboratoire de Zoologie et Biologie Animale, Université F. Houphouët Boigny, Abidjan, Côte d’Ivoire

**BACKGROUND:** During the past 10 years, Côte d’Ivoire has experienced a serious military and political crisis that disrupted the environment of towns and villages. From the change of the initial environment emerge new landscapes favourable to tropical diseases vectors and pests. To investigate the real situation of malaria in a war zone, a survey was conducted from April 2008 to March 2009 in the urban area of Kennedy in Bouake, central Côte d’Ivoire.

**METHODS:** Entomological data were collected from the human landing catches and parasitological data were obtained from blood smear and thick film in children aged 0 to 15 years, following a clinical examination.

**RESULTS:** The Culicidae fauna collected is characterized by three main malaria vectors, *An. gambiae*, *A. funestus*, *An. nili*. However, *An. gambiae* and *An. funestus* transmitted *Plasmodium* with means sporozoite rates of 2.8% and 12.5%, respectively. The aggressive rates of both vectors were 29 and 0.7 b/mn respectively. The endemicity of the study area estimated by the entomological inoculation rate (EIR) was 296 infected bites /man/year (lb /m/ y) for *An. gambiae* and 33 ib/m/yr for *An. funestus*. Mean prevalence of *Plasmodium falciparum* was estimated to 83% and *Plasmodium falciparum* is responsible for 100% of infections.

**CONCLUSIONS:** During the political contributed to modify transmission ecology of *Plasmodium* in urban Bouake as malaria vectors found previously in rural areas were collected in this high urbanized area of Kennedy in Bouake.

P426: Molecular evidence for sequestration of *Plasmodium falciparum* immature gametocytes in the bone marrow

Ruth Aguilar1,2, Ariel Magallon-Tejada1, Ariel H. Achtman1,2, Cinta Moraleda1,2, Regina Joice3, Pau Cisteró3, Connie S. N. Li Wai Suen4,5, Augusto Nhabomba1, Eusebio Macete1, Ivo Mueller4,5,6, Matthias Marti1, Pedro L. Alonso1,2, Clara Menéndez1,4, Louis Schofield1,6, Alfredo Mayor1,2,3

1Barcelona Centre for International Health Research (CRESIH, Hospital Clinic - University of Barcelona), Barcelona, Spain; 2CIBER Epidemiology and Public Health (CIBERESP), Barcelona, Spain; 3Manhiça Health Research Center (CISM), Maputo, Mozambique; 4Division of Infection and Immunity, The Walter and Eliza Hall Institute for Medical Research, Melbourne, Australia; 5Department of Medical Biology, The University of Melbourne, Melbourne, Australia; 6Department of Immunology and Infectious Diseases, Harvard School of Public Health, Boston, EEUU; 7Papua New Guinea Institute of Medical Research, Madang, Papua New Guinea

**BACKGROUND:** *Plasmodium falciparum* immature gametocytes are not observed in peripheral blood. However, little is known about the organs where they sequester and the factors contributing to their genesis and maturation.

**METHODS:** *P. falciparum* sexual and asexual stages were quantified in bone marrow (*n=174*) and peripheral blood (*n=70*) of Mozambican anemic children by quantitative polymerase chain reaction (qPCR) targeting transcripts of PF14_0748 (early), PF13_0247 (intermediate), PF10_0303 (mature) and PF08_0085 (housekeeping) markers.

**RESULTS:** Seventy-eight percent (136/174) bone marrow samples and 36% (25/70) peripheral blood samples were positive for the *P. falciparum* housekeeping marker. Among *P. falciparum* positive children, 98% (133/136) bone marrow and all (25/25) peripheral blood samples were qPCR-positive for at least one gametocyte stage marker, being 22% (27/124) and 12% (3/25), respectively, microcopy-positive. Prevalence of immature gametocytes was higher in bone marrow than peripheral blood (early: 95% versus 20%, *p<0.001*; and intermediate: 80% versus 16%, *p<0.001*), as were transcript levels (*p<0.001* for both stages). In contrast, mature gametocytes were more prevalent (100% versus 51%, *p<0.001*) and abundant (*p<0.001*) in peripheral blood than in the bone marrow. Severe anemia (3.57, 95%CI[1.49-8.53]) and dyserythropoiesis (6.21, 95%CI[12.24-17.25]) were independently associated with a higher prevalence of mature gametocytes in bone marrow (*p<0.001* in both cases).

**CONCLUSION:** These results show that *P. falciparum* immature gametocytes sequester in bone marrow. Strategies targeting gametocytes should consider the frequent detection failures by microscopy, and the high potential for *P. falciparum* transmission of severely anemic children.
P427: Trophoblast-cell derived microparticles in women exposed to Plasmodium falciparum and HIV
Laura Moro1, Azucena Bardaji2,3, Diana Barrios1, Gemma Moncunill1, Inacio Mandomando2, Betuel Sigiaque2, Carlota Dabo11, Eusebio Macete2, Pedro L. Alonso1,2, Clara Menendez1,2, Alfredo Mayor1,2
1Barcelona Center for International Health Research, Hospital Clinic-Universitat de Barcelona, Spain; 2Centro de Investigaciòn en Saúde da Manhiça, Manhiça, Mozambique

INTRODUCTION: Increasing evidences support the key role of microparticles (MPs) in intercellular communication. The possibility to measure MP levels in body fluids highlights their potential as diagnostic biomarkers, although standardized procedures are lacking. During pregnancy, the deportation of syncytiotrophoblast debris comprises MPs that contribute to maternal tolerance towards the fetus. HYPOTHESIS: Alterations in the placenta caused by Plasmodium falciparum-malaria and HIV infection affect trophoblast deportation, including the secretion of MPs which have a recognized immunoregulatory role, thus contributing to the immunopathogenesis of the disease.

MATERIALS AND METHODS: Retrospective study conducted in peripheral plasma samples collected at delivery from 174 Mozambican pregnant women, malaria- and HIV-infected and non-infected. Trophoblast-derived MPs were isolated and quantified by size and specific markers (Annexin V and Pregnancy-Specific beta-1-Glycoprotein 1) using flow cytometry. The purity, size and morphology were characterized by electronic and fluorescent confocal microscopy. Monocye-derived dendritic cells (DCs) were incubated with MPs from non-infected, placental malaria- or HIV-infected women, alone or with lipopolysaccharide (LPS), in order to address their functional effect by flow cytometry phenotyping and measurement of cytokines/chemokines in culture supernatants by a Luminex based assay.

RESULTS: The percentages of trophoblastic MPs detected in plasma are significantly reduced in women with malaria, as well as in those with HIV infection, compared to non-infected women. In addition, malaria infection in the periphery, but not HIV, is associated with a decrease of MP total counts. Preliminary results show that, after incubation with MPs, the size, morphology and expression of some activation/maturation markers of DCs (mainly CD86 and MHC-II) were altered. The expression of pro-inflammatory cytokines and chemokines was increased after incubation with MPs from HIV-positive women when comparing with MPs from non-infected or women with placental malaria. IL10 was highly expressed when incubating DCs with LPS and MPs from the three origins compared to LPS alone.

CONCLUSIONS: These results suggest that malaria and HIV infection in pregnant women decrease the generation of trophoblastic microparticles involved in maternal tolerance towards the fetus. MPs from HIV-infected women may promote the expression of pro-inflammatory factors that can contribute to adverse outcomes during pregnancy.

P428: Evaluation of dhps and DHFR mutations in women on SP as Intermittent Preventive Therapy for malaria during Pregnancy (IPTp) in the Centre Region of Cameroon.
Sharon Mbaham1,2, Jude Bugoga3, Jean Paul Chedjou2, Philomina Nyonglema1, Canicia Enow1, Grace Sama1, Emile Yuosembom1, Jean Claude Djountu2, Diane W. Taylor3, Rose Leke1
1Université des Montagnes, Bagangté, Cameroon; 2The Biotechnology Center, University of Yaoundé I, Cameroon; 3University of Hawaii, USA

BACKGROUND: The prevention of Malaria during pregnancy is a public health measure to safe mothers from the consequences of degrading health leading to death in certain circumstances. The use of SP is highly recommended for use in IPTp. Since the implementation of IPTp-SP for pregnant women in Cameroon in 2004, reports demonstrate a rise in the prevalence of resistance and anecdotal and written reports have pointed to Sulphadoxine -Pyrimethamine as resistant during its use in pregnant women in regions of Cameroon and Africa. This study therefore aimed to evaluate the prevalence dhfr and dhps resistance markers to SP as an inclusive criterion for its efficacy during pregnancy.

METHODS: Pregnant women attending to antenatal visits at 2 urban and 2 rural health facilities in the Centre region of Cameroon were physically examined to evaluate for progress in pregnancy. Consenting mothers had previously received SP or were given SP and enrolled and told when to come back for follow up visits. Venous blood withdrawn at each visit were made in 200ul aliquots and stored at -20C from which 25ul were spotted unto filter paper for extraction of parasite DNA using the Chelex method. DNA that was used in a PCR and RFLP for the detection of mutations in the dhfr (C59R & S108N, defining the CS or RN haplotypes) and dhps genes (A437G & K540E, defining the AK or GE haplotypes).

RESULTS: Of the 457 women enrolled into the study for follow at the antenatal visits between 2011 and 2013, 31 of them had parasitemia on first visit (V1), 38 on V2 or other visit days. Within these 69 women, 9 had parasitemia on more than two occasions of which 3 came down with an accompanying fever. PCR Identification of mutants at first parasite occurrence demonstrate a dhfr RN haplotype of 90% and a dhps 437G mutant of 88%. The dhps 540E mutant is rare (10%).

DISCUSSION & CONCLUSION: These high rates of mutations underscore the potential of parasites in Cameroon to be resistant especially during pregnancy when the immune system is compromised. Details relating to the validity of IPT-SP will be discussed.

P429: ‘Make we chukam time weh we needam’: A participatory approach in promoting rational use of injection in malaria treatment in Cameroon
O Mbah
BACKGROUND: In Cameroon, literature suggests that, the average adult receives 2.4 injections in a year, and only 7% of these injections are administered by health workers, most of the rest presumably being administered by informal sector providers without formal training in injection safety. In the treatment of malaria, patients tend to demand for injections as they believe these act more rapidly than oral medication. Service providers also provide injections on demand and often without following national guidelines for use of oral medicine at first stage malaria. Unsafe or irrational use of injections carries significant risk to both consumer and provider. It also carries a heavier cost than oral medication for the consumer.

RESEARCH PROBLEM: To investigate reasons why patients demand for injections through mixed methods and use these findings to design approaches to promote rational use of medicines in malaria treatment.

METHODOLOGY: 2 health districts in the North West Region were randomly selected after stratifying for health districts which had a mix of urban and rural settings. We utilised mixed methods of qualitative studies to investigate barriers and facilitators of rational use of injection for treatment of malaria. We employed key informant interviews, focus group discussions and interactional group discussions. We used quantitative approaches to estimate demand for injection, comparing this pre and post interventions. We then used research findings to develop entertainment radio programs, posters and academic detailing for coaching healthcare workers on handling patient’s demand for injection.

RESULTS: 62% of patients (n=384) believed injections are also more effective than tablets to treat a fever, and 42% of all patients surveyed requested an injection at their last consultation. All hospitals do not make a policy of limiting injection use to situations where a patient is unable to take oral medication. Over one third of all prescriptions included at least one injection. None of the hospitals had a policy on injection safety and not all prescribers are trained in injection safety. The commonest injection used for malaria treatment was Vitamin B complex.

DISCUSSION AND CONCLUSION: Policy is need to address the irrational use of injection for malaria treatment in Cameroon.
P430: Quality of malaria microscopy in 12 district hospital laboratories in Tanzania
Hilda Mbakikwi 1, Chacha Manga 2, Stafford Kibona3, Frank Mtei 1, Judith Meta 1, Aikande Shoo2, Ben Amos 1, Hugh Rebuff1
1 Joint Malaria Programme, Kilimanjaro Christian Medical Centre, Moshi, Tanzania; 2 National Institute for Medical Research, Tabora, Tanzania; 3 Teulse Hospital Research Laboratory, Muheza, Tanga, Tanzania; 4 London School of Hygiene and Tropical Medicine, London, UK

BACKGROUND: Malaria is commonly overdiagnosed in malaria-endemic areas of Africa mainly as the result of the overlap of symptoms with those of other common illnesses and application of the WHO policy of presumptive treatment for malaria in young children. While this policy has been effective in high transmission areas, there has been growing awareness over the last decade of the negative effects of overdiagnosis of malaria. The WHO recommendation for parasitological diagnosis of malaria is challenged by evidence of poor quality microscopy in African hospitals but the reasons are unclear. This study was conducted to assess the accuracy of slide reading and the reasons for any deficiencies that were identified in district hospital laboratories in three regions of Tanzania.

METHODS: Twelve of the busier district hospital laboratories from 3 regions of Tanzania were assessed for quality of the working environment and slide readers read 10 reference slides under examination conditions. Slides that had been routinely read were removed for expert reading.

RESULTS: Of 44 slide readers in the study 39(88.6%) correctly read >90% of the reference slides. Of 206 slides that had been routinely read, 33(16%) were judged to be unreadable, 104(51%) were readable with difficulty and 69(34%) were easily readable. Compared to expert reading of the same slide, the sensitivity of routine results of easily readable slides was 85.7 % (95% CI 77.4-94.0) falling to 44.4 % (95% CI 34.5-54.4) for ‘readable with difficulty’ slides.

CONCLUSIONS: The commonest cause of inaccurate results was the quality of the slide itself, correction of which is likely to be achievable within existing resources. A minority of slide readers were unable to read slides even under ideal conditions suggesting the need for a ‘slide reading licence’ scheme.

P431: A heat stable peroxidase from VIGNA SP
Yves M.E.L Mbassi, Marie S. Evehe, Wilfred Mbacham, John P. Muhlu
Laboratory for Public Health Research Biotechnologies, The Biotechnology Center, University of Yaounde I

BACKGROUND: Peroxidases are used as markers in DNA probes, in enzyme immunoassays or clinical diagnosis, and as catalysts for several industrial applications. In addition, thermostable peroxidases promote the development of new analytical methods and improve immunoenzymatic analytical kits where these enzymes are used as immunochromatographic strips. Although the most exploited source of peroxidase is Horseradish (Armoracia rusticana), some studies show that peroxidases isolated from plants of tropical regions have a greater potential for such applications, so our study aimed to characterize a thermostable peroxidase isolated from a Vigna sp growing in barren areas of Cameroon.

METHODS: An isoenzyme was purified by acetone precipitation, heat treatment, gel filtration, and ion exchange chromatography. kinetic parameters (km, vmax, Kcat/Km) were determined by the method of Lineweaver-Burk for 6 of the most used substrates in biotechnological applications of peroxidases at optimum pHs determined in this study. Various heat treatments of various durations were carried out : short duration at high temperature, long duration at moderated temperature, long periods at room temperature. The effect of some metal ions on thermostability of the enzyme was also evaluated.

RESULTS: We have purified near homogeneity a thermostable isoperoxidase from Vigna sp radicles. The inactivation kinetics of the purified peroxidase at pH 8 fitted a first-order reaction, and the half-lives were 3.06 weeks, 13.5 hours, 15.5 min and 3.5 min at 50°C, 70°C, 80°C, and 90°C respectively. The calculated activation energy for its thermal inactivation was found to be 221.5 KJ/mol at pH 8. This peroxidase isozyme is stable for 4 months at room temperature, losing only 5% of its initial activity over this period. Its thermal stability is increased 8 times by Ca2+ ions. That peroxidase exhibits great catalytic efficiencies towards the oxidation of diverse substrates usually used in ELISA (Enzyme-Linked Immunosorbent Assay) technique. Apparent Km values for O-dianisidine, ABTS, TMB, and OPD were respectively 3.50mM, 0.12mM, 1.83mM, 0.05mM, 17.22mM and 2.53mM; catalytic efficiencies were 5.12×10^6M^-1.min^-1, 2.22×10^5M^-1.min^-1, 1.59×10^5M^-1.min^-1, 1.82×10^4M^-1.min^-1, 3.17×10^4M^-1.min^-1 and 1.79×10^3M^-1.min^-1. It has in other hands a very acid optimum pH for the oxidation of ABTS and an optimum temperature of activity above 60°C.

CONCLUSION: Thermal stability of peroxidases is a requirement for long storage capacity and to improve some analytical techniques were these enzymes are used. The unusual catalytic and thermal characteristics of the peroxidase we isolated could make it a potent tool in several biotechnological applications, especially as part of bench top diagnostic kits in Africa that do not require cold chain.

P432: Analysis of antibody responses to multiple Plasmodium falciparum blood stage antigens and their relationship with clinical outcome for severe malaria in urban Dakar
Babacar Mbaye 1,2, Aissata Basse2, Malick Badiane1, Birahim Niang3, Bucary Diatta4, Omar Diop2, Rokhaya Ndiaye Diatta2, Aissatou Touré1, Shirley Longacre1, Ronald Perrault1 & Alioune Diaye1,2
1 Service d’Immunologie Université Cheikh Anta Diop (UCAD), Dakar, Sénégal; 2 Institut Pasteur de Dakar, Unité d’Immunogénétique, Dakar, Sénégal; 3 Service de Réanimation, Hôpital Principal de Dakar, Dakar Sénégal; 4 Institut Pasteur, Laboratoire de Virologie et Parasitologie, 75015 Paris, France

BACKGROUND: The occurrence of severe malaria remains a major problem despite substantial decreases in morbidity and mortality due to sustained control programs. Indeed, these syndromes continue to reach case fatality rates as high as 20%. Severe malaria results from a complex cascade of events, possibly including insufficient qualitative and/or quantitative Ab responses to key targets of anti-parasite immunity. Here we analyze differential Ab responses to a panel of P. falciparum pre-erythrocytic and blood stage antigens in well-defined cohorts of hospitalized patients.

METHODS: IgG responses to P. falciparum antigens were measured in 243 patients, including 141 with confirmed severe cerebral malaria (CM) (Mean age 30yr, 8-80 yrs) leading to 38 fatal outcomes [FCM] and 92 hospitalized patients with uncomplicated malaria (UM) (Mean age 28.5 yr; 1-77 yrs). IgG responses to crude merozoite extracts and multiple recombinant liver or blood stage antigens were evaluated by ELISA, including LSA-1α, CSP, PF13-DLB1α, PfEMP2, RESA, MSP1p19, MSP2, MSP3, MSP4p20 and MSP5.

RESULTS: The patient cohorts included >50% of responders against all Ags except CSP, MSP3, RESA and PfEMP2. Incidences of Ab to MSP1p19, MSP2 and MSP5 and the magnitude of the Ab response to MSP4p20 and MSP5 were significantly higher in CM vs UM patient groups. When comparing fatal vs non-fatal outcomes, there was a general trend toward higher incidences of Ab to all Ag targets in the non-fatal group, which was significant for Ab to MSP1p19 (P<0.01).

CONCLUSION: Despite strong Ab responses to some immune biomarkers in patients hospitalised for acute episodes of malaria, severe fatal outcomes were associated with lower magnitudes of Ab to selected Ags, including certain merozoite surface Ags. These results are consistent with a role for IgG to merozoite surface Ags in protection against fatal prognosis. Measurement of Ab responses to multiple biomarkers deserves further investigation with more patients and enlarged panels of Ag to confirm their use for prognosis in early diagnosis of hospitalized acute malaria and for screening of protection-associated Ag targets.
P433: Factors associated with bed net use among vulnerable populations in Western Kenya

Mwende Mbondo1, Rhoune Ochako1, Patrick Mbindyo2, Agnetta Mbithi2, Ahmed Omar3, Rebecca Kiptui1 and Terry Muchoki1

1 Population Services International, Kenya (PSI/K); 2 Kenya MOH, Division of Malaria Control (DOMC)

BACKGROUND: Despite the increase in bed net ownership in Kenya through routine and mass distributions, usage remains low; findings from evaluation of the 2011/12 LLIN mass distribution campaign showed that overall 22.7% of children under five and 52.5% of pregnant women in intervention households slept under LLIN the previous night. This study was designed to better understand the facilitators and barriers to net use.

METHODS: The study was conducted in February 2013 among pregnant women, caregivers of children under five years and household heads aged 18-49 years selected from households known to have participated in the mass net distribution. Selection criteria included self-identification as a user or non-user of LLIN in response to being asked if one slept under a LLIN the previous night. In-depth interviews (IDIs), focus group discussions (FGDs), observations and vignettes were used; a total of 133 IDIs and 21 FGDs were conducted. Data were analyzed using NVIVO 10.

RESULTS: While most participants identified themselves as net users, there was a lack of consistency in net use, which did not differ by respondent education level or age. This was due to reduced risk perception for malaria during the hot dry season, absence of caregiver and interruptions in household sleeping patterns when there were visitors in the household. Other barriers to net use were lack of knowledge on net care prior to initial use and subsequent to use. Facilitators to net use included reduced malaria infections and economic benefits resulting from no costs for malaria treatment.

CONCLUSION: Population Services International (PSI) Kenya in collaboration with the Division of Malaria Control (DOMC) will develop mass media communication addressing the need for consistent LLIN use throughout the year among vulnerable populations living in malaria endemic and epidemic areas in Kenya. Further, information on airing the net prior to use and regular LLIN washing will be addressed through interpersonal communication.

P434: Household decision making in long lasting insecticidal net use in Western Kenya

Mwende Mbondo1, Rhoune Ochako1, Patrick Mbindyo1, Agnetta Mbithi1, Ahmed Omar2, Rebecca Kiptui1 and Terry Muchoki1

1 Population Services International, Kenya (PSI/K); 2 Kenya MOH, Division of Malaria Control (DOMC)

BACKGROUND: Findings from evaluation of the 2011/12 mass distribution of Long Lasting Insecticidal Net (LLIN) showed that while universal coverage was met in 67.1% of the households assessed, only 22.7% of children under five and 52.5% of pregnant women in intervention households slept under LLIN the previous night. In-depth interviews (IDIs), focus group discussions (FGDs), observations and vignettes were used; a total of 133 IDIs and 21 FGDs were conducted. Data were analyzed using NVIVO 10.

RESULTS: While most participants identified themselves as net users, there was a lack of consistency in net use, which did not differ by respondent education level or age. This was due to reduced risk perception for malaria during the hot dry season, absence of caregiver and interruptions in household sleeping patterns when there were visitors in the household. Other barriers to net use were lack of knowledge on net care prior to initial use and subsequent to use. Facilitators to net use included reduced malaria infections and economic benefits resulting from no costs for malaria treatment.

CONCLUSION: Population Services International (PSI) Kenya in collaboration with the Division of Malaria Control (DOMC) will develop mass media communication addressing the need for consistent LLIN use throughout the year among vulnerable populations living in malaria endemic and epidemic areas in Kenya. Further, information on airing the net prior to use and regular LLIN washing will be addressed through interpersonal communication.

P435: Analysis of the allelic diversity of Plasmodium falciparum isolates based on clinical presentation and age in Libreville: preliminary data.

Noe Patrick M’Bondoukwe1, Denise Mawili Mbumba2, Christelle Offoug1, Julia Bendome1, MCORU team1, Maryvonne Kombilia3, Marielle Bouyou-Akoté4

1 Department of Parasitology-Mycology, Faculty of Medicine, Université des Sciences de la Santé, Libreville, Gabon; 2 Malaria Clinical Operational Research Unit

BACKGROUND: Polymorphic msp genes are the main molecular markers of malaria infection. Their knowledge allows the infection characterization in a population and a better understanding of the epidemiological consequences of the naturally acquired immunity against malaria. Objective: to analyze the P. falciparum allelic diversity according to the patients clinical status.

METHODS: The study was conducted between 2011 and 2012 where the malaria prevalence was 24.5 % among febrile children in 2011. The polymorphism of merozoite surface protein-1 (msp-1) locus was analyzed in isolates from patients with complicated and uncomplicated malaria collected on filter paper. After DNA extraction, the genotyping of msp1 gene was performed using nested PCR.

PRELIMINARY RESULTS: On the 108 analyzed samples, parasite DNA was amplified in 93. K1, Ro33 and Mad20 allelic families were detected in 63% (n=60/95), 53% (n=50/95) and 34% (n=32/95) respectively. No specific allelic family of msp1 was associated with clinical form and age groups. The multiplicity of infection was 1-5 genotypes. The complexity of infection was determined according to the different clinical and age groups. The mean number of genotypes was comparable among the clinical groups and age groups (p = 0,3 and p = 0,9). The K1-250 allele was found only in subjects with uncomplicated malaria and K1-160 only in those with severe malaria.

CONCLUSION: Data analysis is ongoing. However some msp1 alleles seem to be associated with specific clinical forms.

P436: Comparison of Developmental time, body size and fecundity of Anopheles arabiensis (Patton) and Aedes aegypti (L.) under differential temperature regimes

Mburu, D. M and Masendu, H.T.

1Pawani University, Biological Sciences Department, RO. Box 195, Kilifi, Kenya; 2Department of Public Health, P Bag F26, Francistown, Botswana

BACKGROUND: Dynamics in temperature regimes are linked to climate change effects on organisms above ground. However data on differential thermal dynamics towards organisms below ground is not well documented. Aquatic stages of dipteran vectors of disease may act as good models to test potent impacts of thermal changes on invertebrates. The key objective of the study was to determine the developmental time, head capsule sizes, wing lengths, fecundity of Anopheles arabiensis (Patton) and Aedes aegypti (L.) at 15°C, 22°C, 25°C, 32°C and 36°C.

METHODS: Batches of 30 eggs were incubated in 200 ml of distilled water to allow breeding. After emergence, blood fed female mosquitoes were provided with wet filter papers placed in paper cups for oviposition.
The developmental time from eggs to adults were recorded. Head capsule sizes and wing lengths were determined for the temperature regimes. Fecondity for respective regiments was determined by counting the number of eggs oviposited by individual female mosquitoes.

RESULTS: At 15°C total developmental time for A. aegypti was 39 days with pupal stage taking 20 days while A. arabiensis aquatic stages experiencing 100% mortality after 29 days. Total developmental time for the larvae and pupae of A. arabiensis at 22°C were 18 and 14 days, respectively, with these stages taking 15 and 12 days, respectively in A. aegypti. Additionally, total developmental time for A. arabiensis and A. aegypti at 22°C were 22 and 18 days, respectively. At 25°C and 32°C the metamorphosis from eggs to adults of the two species took less than 14 days. At 36°C the first instar larvae emerged 35 minutes after eggs incubation but there were 100% mortalities for pre-adult stages for both species. Pre-emerginal and adult's mortalities were significantly higher at both 15°C and at 36°C than at either 25°C or 32°C. Fecondity was higher at 25°C and 32°C than at 15°C, 22°C and 36°C.

CONCLUSIONS: Differential temperatures impacts on the morphometry and population bionomics of mosquitoes and it may have implications on the distribution of these vectors and surveillance of diseases. It would be interesting to determine the genetic signatures underlying the observed phenotypic expressions of these vectors and perhaps other invertebrates.

P438: Malaria epidemic surveillance sites in the Senegal River Valley, 2008-2012

Medoune NDio, Julie Thwing, Moustapha Cisse, Mame Birame Diouf, Ibrahima Diallo, Sylla Thiam, Mady Ba

As malaria transmission falls in a region, residents may not have sufficient exposure to infective bites to maintain immunity, and it may become epidemic-prone. The Senegal River Valley is epidemic-prone and experienced malaria epidemics in the 1990s. Artemisinin-based combination therapy (ACT) was introduced in 2007, and rapid diagnostic tests (RDTs) in 2008. Mass distribution of insecticide treated nets for children under 5 years took place nationwide in 2009, and universal coverage distribution in 2011. In 2007, the Senegal National Malaria Control Program (NMCP) put in place eight epidemic surveillance sites at health posts in four districts in the Senegal River Valley. Using a standard spreadsheet, sites report the number of total consultations, suspected malaria cases, patients tested, and confirmed cases of malaria. Data quality was assessed with quarterly onsite supervision. After 2008, diagnostic effort (cases tested/cases suspected) consistently surpassed 95% and was 100% annually in half the sites, with near absolute promptness and completeness. Transmission was highly seasonal, with 80% of cases occurring from August to November, with 60% in September and October. The southernmost site was found to be inconsistent with the epidemiologic profile of the others, with a mean annual incidence of symptomatic malaria of 93/1000 over the five years. In the remaining sites, mean annual incidence of symptomatic malaria of from 2009-2012 was 1.7/1000; 0.2/1000 in children under 5, 0.6/1000 in pregnant women, and 2.0/1000 in the remainder of the population. Less than 10% of all consultations were suspected malaria, and RDT positivity rate among those tested was 17%. An investigation of the cause of high incidence in the southernmost site was conducted in 2010, but no epidemics occurred during the surveillance period. Given the low incidence and simultaneous scale-up of diagnostics, it was not possible to detect the impact of vector control interventions. Epidemic surveillance sites have performed well in Senegal and increased the districts’ capacity in surveillance. The NMCP continues to add sites as transmission decreases, with the goal of detecting and responding to epidemics within two weeks.

P439: Design of naturally occurring antimalarials overcoming drug resistant strains of plasmodium falciparum (pf)

Esrem Akori1, Dali Brice1, Keita Melalie2, Owono Luc1, Ntie Fidele2, Megnassan Eugene1,2

1Fundamental and Applied Science Unit, University of Abobo-Adjamé, Abidjan, Côte d’Ivoire; 2CEPAMOQ, Faculty of Science, University of Douala, Douala, Cameroon

BACKGROUND: The millennium development goal of 50% reduction of malaria prevalence in 2015 will not be met. Design of new orally available antimalarials efficient against drug resistant strains of pf is urgently expected. African sub Saharan countries (ASSC) where 90% deaths occur are absent from drug design and development research (except South Africa). This poster presentation shows successful attempts of rational design of new antimalarials inhibiting important pf targets.

METHODS: It is assumed that structurally similar inhibitors (I) bind in a similar fashion to a target enzyme (E) we carry out complexation study of Gibbs Free energy DGcomi =-RTlnK for the aqueous equilibrium (Ei + Ii ↔ EiIi) starting from a .pdb structure of the complex EiI and a training set of I with known biological activities (Ki). In thermodynamics of Enzyme inhibitor affinity, DGcomi=DGH, A+ ΔTS + DG, where ΔX=ΔX(E)- ΔX(E)- ΔX(I). From DGH for each inhibitor I, DGcomi (I) the QSAR regression equation DGcomi (I)=α(I)+b with a correlation coefficient R explains some (R%) of the variation of biological activity by that of DGcomi.

The 3D-QSAR Pharmacophore (PH4) from the I binding conformation is used to select from a virtual library of similar inhibitors new.
and more potent analogues on the basis of the QSAR regression equation.

RESULTS: Pf Plasmepsin II designed new modified statine (MST) inhibitors in the picomolar range of predicted activity (RPA) with selectivity index (SI) >1200 while SI is <3 in the same range of activity. Hydroxynaphthoic acids inhibitors of PFNR designed new analogues in low nanomolar RPA.

CONCLUSION: This low cost approach has to be broadcasted to bring researchers in ASSC to an area we are absent. Various targets (Falcipain FP2, FP3; Aminopeptidase M1, M17) are on the way.

P440: Role of some biomarkers in placental malaria in women living in Yaoundé, Cameroon

Rosette MEGNEKO1, Jean Claude DIONTU1, Suzanne H. MAGAGOM3, Abel LISSOM1, and Rose FG LEKE2

1 Department of Animal Biology and Physiology, Faculty of Science, The Biotechnology Center, University of Yaoundé I, Yaoundé, Cameroon; 2 Faculty of Medicine and Biomedical Science, The Biotechnology Center, University of Yaoundé I, Yaoundé, Cameroon.

BACKGROUND: Plasmodium falciparum in pregnant women can sequester into the placenta, leading to placental malaria (PM). The pathogenesis of PM involves alterations in cytokine and chemokine expression and local inflammation due to leukocyte infiltration into the placenta which secrete these mediators. Recent study showed that cytokines, such as IL-19, increase expression of IL-10 in monocytes which protects against bacteria. Although some β-chemokines are known to attract monocytes to the infection site, no study is available on the role played by CXCL-10 and some cytokines (IL-19, IL-17) in PM, thus our interest.

METHODS: A total of 140 volunteer women recruited in a Health Center, in Yaoundé, Cameroon, participated for this cross-sectional study. Peripheral and placental blood samples were collected in pair immediately after delivery. Hemoglobin levels were determined using Hemocue HB 201 analyzer. Parasitemia and differential WBC counts were determined microscopically. The presence of parasites in the placenta was determined by blood smears for intervillous blood and impression smears. The levels of CXCL-10, IL-10, IL-17, and IL-19 in plasma were measured using ELISA.

RESULTS: The prevalence of malaria was 20%, with the prevalence being higher in primigravidae and secondigravidae compared to multigravidae (p=0.01). Hemoglobin levels were negatively correlated with peripheral, placental, and impression parasitemias (r = -0.41, -0.37, -0.40, p <0.001, respectively). Levels of CXCL-10 were higher in placental than peripheral plasma samples (p=0.001). The opposite was found for IL-10 and IL-17 (p=0.001). No difference was found for IL-19 (p=0.55). Peripheral and placental plasma levels of CXCL-10 correlated positively with parasitemia. The same pattern was observed for IL-10 (P<0.001, P = 0.001). Both levels of CXCL-10 significantly correlated positively with the level of monocytes of placental impression smears. Such correlation was also observed for IL-10 in peripheral plasma with levels of impression monocytes and peripheral neutrophils. A positive correlation was observed between peripheral plasma levels of CXCL-10 and lymphocytes of placental impression. Placental IL-17 levels significantly and positively correlated with monocytes and lymphocytes of placental impression.

CONCLUSIONS: These data suggest that CXCL-10 plays a role in recruitment of monocytes and lymphocytes into the placenta where pathology might take place.

P441: Trend of Malaria morbidity in Kersa, Southwest Ethiopia

Selesh Kebedei, Abraham Asefa1, Jihad Kemal1, Nasir Abdo2 and Girmay Medhin1

1 Addis Ababa University and 2 Armauer Hansen Research Institute and *Jimma Zone Health offices

Malaria is a highly infectious disease, causing the major cause of disease and death in Ethiopia, especially among children and pregnant women. The aim of the study is to assess the trend of malaria morbidity in Southwest Ethiopia. This paper assessed the trend of malaria cases from September 2005 to August 2011. A retrospective analysis of daily outpatient consultation records was obtained from Jimma Health Bureau. Moreover, One year cross-sectional blood film examination was performed in Bulbul, Serbo and Bala Wajo health centers in Southwest Ethiopia. Data were entered and checked, thereafter analyses were performed using excel and SPSS version 16 software. Descriptive statistics was used to assess the trend of malaria cases detected over six year period. We assessed 6 years trend of malaria case in Kersa area between September 2005 to August 2011. A total of 57482 malaria cases were diagnosed in the three Health centers. Among these, a total of 15865 were children under five years of age. The majority (88.80%) of malaria cases were reported in 2006/2007. Moreover, the percentage of Plasmodium vivax and Plasmodium falciparum was 57.37% and 42.63% respectively. To minimize reliability and validity of secondary data, one year cross sectional analysis was performed in Kersa Woreda from three health centers. Concerning the one year cross-sectional study, males were more affected (60.1%) than females (39.9%). In the same year 33.8% of the positive cases were children. The proportion of malaria cases detected among clinical suspects over the 5 year period was 51.13%. On the other hand the proportion of malaria cases was during one year blood film examination was 25.32%. Despite recent decline in malaria consultation rates, malaria was a problem in Kersa. Furthermore results presented in this study suggest that the burden of malaria in children <5 years of age is still significant. Our assessment indicated that annually, malaria consultations peaked during September to December which coincides with the end of the rainy season.

P442: Pyrogenic Profile of Children with Fevers in Yaoundé, Cameroon

Mekue, TL1, Atohgo-Tiedeu, BM1, Achouduh, OA1, Donfack, SOT1, Mbacham, WF1, and Mbanya, JC2

1 Faculty of Science, University of Yaoundé I; 2 Faculty of Medicine and Biomedical Sciences, University of Yaoundé I

BACKGROUND: Until 2010, the WHO recommended anti-malarial treatment for all cases of fever in children less than 5 years old in regions of high malaria endemicity. Care givers are still in this old practice. Its continuing persistence is also due to improper management of patients presenting fevers with consequences of high drug pressure due to automedication and a further generation of chemoresistance.

METHODS: The established algorithm for the follow-up of fever patients states that in a case where negative results are obtained with RDTs, the patient should be given care according to the IMCI guidelines. In order to improve on disease management, where RDTs or microscopy are absent, we undertook to map out the other causes of fevers. Fifty children aged between 0 and 5 years, of whom 24 were boys and 26 girls, underwent 4 Rapid Diagnostic Tests for Malaria, Toxoplasmosis, Typhoid and German Measles (Rubella) which were said to be common in the area. Microscopy, glycaemia, haemoglobinemia, full blood count and yeast levels were also evaluated.

RESULTS: By RDT, there was no case of rubella despite the anecdotal claim of an outbreak. The fevers were therefore due to mono-infections: 4/50 (8%) malaria, 11/50 (22%) toxoplasmosis and 4/50 (8%) typhoid cases. The cases of co-infection were due to malaria-toxoplasmosis for 2/50 (4%), malaria-typhoid for 7/50 (14%), malaria-toxoplasmosis-typhoid for 3/50 (6%) and 19/50 unattributable to any of the test cases. Malaria parasite density varied between 1000-270,000 p/µl. By microscopy 14/50 (28%) were positive for Plasmodium falciparum. The kappa value for malaria RDT/microscopy was k=0.81 indicating a strong concordance, with a sensitivity of 92.2%, a specificity of 91.17%, a positive predictive value of 81.3% and a negative predictive value of 97.7%. Malaria was not significantly associated with glycaemia and hemoglobin levels (p=0.5956 and p=0.4474 respectively). However, it was associated with a significant drop in the level of white blood cells (p=0.0177) (level of significance p<0.05, 95% CI). Faeces examination showed 7/50 cases of yeast infection.

CONCLUSION: Toxoplasmosis occurs in proportions similar to malaria. Salmonella typhii is third in line of infections.
P443: In vitro antiplasmodial activity of drugs in nanomedicine drug delivery system (NMDDS)

Melariri Paula1, Lonji Kalombo1, Patric Nkuna1, Rose Hayeshi1, Admire Dube1, Peter Smith2, Hulda Swai3

1Encapsulation and drug delivery unit, Polymer and Composites, Material Sciences and Manufacturing, Council for Scientific and Industrial Research, South Africa; 2Division of Pharmacology, University of Cape Town Medical School, South Africa

BACKGROUND: Malaria is a major public health problem. Globally it affects about 300 million people and is responsible for 1 million to 2 million deaths annually. In sub-Saharan Africa where more than 90% of morbidity and deaths occur, a child dies of malaria every 12 seconds. This death toll exceeds the mortality rate from AIDS and the situation has further been heightened due to concomitant infection of malaria and HIV. About 3.3 billion people are at risk of being infected with malaria. The most vulnerable population includes young children and pregnant women as well as non-immune travellers. Africa spends about 1.2 billion US dollars per year on malaria-related illnesses and mortality cost. Presently there is no effective vaccine available for the prevention of malaria, thus chemotherapy remains the most effective tool against the disease, however current treatments have limitations such as poor bioavailability, poor solubility and poor pharmacokinetic profiles which adversely affects the therapeutic properties of the drugs as well as patient compliance hence, posing a huge challenge to malaria control.

METHODS: The CSIR Pan African Centre of Excellence in nanomedicine, is actively engaged in the application of nanomedicine to address the limitations of current treatments. Nanomedicine can address the challenges of current antimalarials, by reformulating the drugs in nanomedicine drug delivery systems (NMDDS). These systems have revolutionized therapies for diseases like cancer but have not been widely applied to transform therapies for infectious diseases of poverty such as malaria. We have applied our novel technology in this study which is currently going through initial phases of filing in for patenting.

RESULTS: Results from our preliminary in vitro studies using our novel NMDDS, against chloroquine sensitive and resistant strains of Plasmodium recorded significant enhancement, in the therapeutic properties of drugs reformulated in NMDDS, when compared to the non-formulated drugs.

CONCLUSIONS: The observations provide evidence that the NMDDS has the ability to enhance the antiplasmodial activities of the drugs. The enhancement of activity could ultimately lead to a reduction in dose and dose frequency administered to patients and hence enhance patients’ compliance.

P444: Building Community Health Systems to Improve Malaria Net Usage and Care-Seeking Behavior in Rural Zambia

John Banda, MD, MPH (Ministry of Health, Zambia) Kennedy Njobvu (Ministry of Health, Zambia), Levi Mbulo, BSc (Catholic Medical Mission Board), Arina Lekht, MPH (Independent Consultant), Sara Melillo, MPH (Catholic Medical Mission Board)

Introduction and Background: Catholic Medical Mission Board’s Malaria Communities Program (MCP) aimed to reduce malaria deaths among pregnant women and children in three hard-hit malaria districts of rural Zambia (Samfya, Kwaambwa, and Mwense Districts, Luapula Province). The three-year program emphasized community mobilization and engagement through continual training and support of community volunteers. Volunteers used participatory community engagement to promote malaria prevention and treatment through community events and theatre and household visits. The program substantively engaged traditional leaders to support the project, ensuring the community’s acceptance of the project.

RESEARCH PROBLEM: CMMB sought to assess how successful conducting a community-led and engaged program would increase ownership and use of insecticide-treated nets, prevention of malaria in pregnancy and diagnosis and treatment of children with suspected malaria.

METHODOLOGY: CMMB conducted an end-line program survey collecting quantitative information from 600 randomly-selected respondents in three districts using a structured questionnaire in June 2012. SPSS provided descriptive statistics that were compared to baseline (2010) to analyze data. Program survey data used to evaluate the program included insecticide-treated net (ITN) ownership and use, malaria prevention in pregnancy and care-seeking behavior for suspected malaria cases.

RESULTS: Survey results show 87.1% of households owning at least one ITN, compared to 59% at baseline. Most eligible respondents (82.4%) claimed to have slept under an ITN during their most recent pregnancy, compared to 63% (baseline). 42.1% of children surveyed had fever in last two weeks preceding the survey, compared to 57.5% at baseline. Data was unclear for ITTp uptake among pregnant women.

DISCUSSION/CONCLUSION: Evaluation findings support engaging communities and local traditional leaders and other locally-recognized figures of authority, substantively to increase the uptake of positive malaria prevention and treatment behaviors in high-malaria burden areas. Partnering with communities themselves to develop and tailor malaria behavior messaging can increase the reach and correct use of ITNs and care-seeking behavior.

P445: Determination of erythrocyte invasion mechanisms of Plasmodium falciparum isolates in Ghanaian children

Henrietta Mensah Brown

BACKGROUND: Morbidity and mortality associated with Plasmodium falciparum infection are caused by invasion of erythrocytes by the malaria parasite, a process which is mediated by multiple receptor-ligand interactions. Antibodies against some parasite ligands have been shown to significantly inhibit parasite growth in vitro, demonstrating the these interactions may be good targets for the development of an effective blood stage vaccine. The aim of this study is to investigate the erythrocyte receptors used by P. falciparum isolates in Ghana.

METHODS: P. falciparum field isolates were collected from children aged 2-14 attending hospitals in three ecologically distinct zones in Ghana: Accra, Navrongo, and Kintampo. Erythrocyte invasion assays were performed to test the ability of the parasites to invade erythrocytes treated with neuraminidase, trypsin and chymotrypsin which selectively remove receptors from the erythrocyte surface. In addition, antibodies against two recently identified receptors, basigin (BSG) and complement receptor 1 (CR1) were used to determine the dependence of the isolates on these pathways. Two to four assays were performed in duplicate on each isolate.

RESULTS: All clinical isolates tested so far were capable of invading neuraminidase treated erythrocytes with invasion efficiencies of 40-80% relative to untreated erythrocytes, suggesting that these field isolates utilize sialic acid independent pathways for erythrocyte invasion. Invasion efficiency varied between 5 to 75% relative to untreated erythrocytes in both trypsin and chymotrypsin treated erythrocytes, with the majority being highly sensitive to both enzymes. Sensitivity to trypsin represent the contribution of glycophorin A and C and CR1, while sensitivity to chymotrypsin indicates the contributions of glycophorin B and CR1. Furthermore, for nearly all field isolates tested, antibodies against CR1 significantly inhibited invasion efficiency of neuraminidase treated erythrocytes, but did not significantly inhibit invasion levels in untreated erythrocytes. However, anti-BSG antibodies significantly inhibited invasion in both untreated and neuraminidase treated erythrocytes to a similar extent.

CONCLUSION: Preliminary results suggest that the majority of field isolates in Ghana express sialic acid independent invasion phenotype. The most common invasion phenotype among clinical isolates among Ghanaian clinical isolates among African clinical isolates was neuraminidase-resistant, trypsin- and chymotrypsin-sensitive, suggesting that CR1 plays a major role in erythrocyte invasion.
P446: Insecticide-Treated Durable Wall Lining for Malaria Control: Multicentre Studies From Africa and South-East Asia

Louisa A. Messenge1, Abrahlan Matias Arnez2, Marie-Louise Larsen2, Nathan Miller4, Adedapo O. Adegun3, Immo Kleinschmidt1 and Mark Rowland1

1London School of Hygiene and Tropical Medicine, London, UK; 2Medical Care Development International (MCDI), Malabo, Equatorial Guinea; 3Technical Institute of Denmark, Lyngby, Denmark; 4The MENTOR Initiative, Huambo, Angola; 5Molecular Entomology and Vector Control Research Laboratory, Nigerian Institute of Medical Research, Lagos, Nigeria

BACKGROUND: Indoor residual spraying (IRS) is a primary method of malaria vector control but its impact is constrained by several inherent limitations: spraying must be repeated when insecticide residues decay, householders may object to the annual imposition and campaign costs are recurrent. Durable Lining (DL) is a deltamethrin-impregnated polyethylene (PE) material which when used to cover house walls, functions as long-lasting IRS. It releases insecticide over 3–4 years to prevent user and donor fatigue, whilst maintaining protection of all community members. However, to establish DL as a viable substitute it must demonstrate equivalent or superior levels of bioefficacy, acceptability and durability to currently available products.

METHODS: To identify a desirable material to develop into a wall lining, a one year preliminary trial was conducted among rural and urban households in Angola and Nigeria (n=258) comparing three treated prototypes (PE shade-cloth, PE sheeting and netting). The most popular lining was then evaluated in comparison with conventional IRS during a one year multicenter study among rural households in Equatorial Guinea, Ghana, Mali, South Africa and Vietnam (n=220).

RESULTS: During the preliminary trial rural participants emerged as the ideal target consumers and readily accepted wall linings because of their perceived decorative value and entomological efficacy. Of the prototypes assessed, PE shade-cloth was the most popular because of its ease of installation and resemblance to local materials. During the multicentre field trial, this material demonstrated consistently higher levels of bioefficacy compared to IRS, with no significant loss of bioactivity (OR=0.93, 95%CI:0.83–1.03, p=0.17) or insecticide content after 12 months. Most households reported reductions in mosquito density (93%) and biting (82%), but no adverse changes to their indoor environment (83%). When offered a choice of vector control, the majority of participants chose DL regardless of earlier household allocation.

CONCLUSIONS: DL remained fully efficacious against mosquito vectors, supported by minimal loss of insecticide content and was unequivocally more popular than IRS. These results demonstrate that DL has the potential to overcome many of the operational challenges associated with IRS and represents a feasible long-lasting alternative, a scenario reminiscent of the succession of conventional insecticide-treated nets with long-lasting insecticidal nets.

P447: Selection for chloroquine-sensitive Plasmodium falciparum by Anopheles arabiensis in southern Zambia

Sungano Mharakurwa1, Mavis Sialumano1, Philip E. Thuma1,2

1The Malaria Institute at Machinga, P.O. Box 630166, Choma, Zambia. 2Department of Molecular Microbiology & Immunology, Johns Hopkins Malaria Research Institute, Johns Hopkins Bloomberg School of Public Health, 615 N. Wolfe Street, Baltimore MD 21205, USA

BACKGROUND: The emergence of parasite drug resistance, especially in Plasmodium falciparum, persists as one of the major obstacles for malaria control and elimination. To develop effective public health containment strategies, a clear understanding of factors that govern the emergence and spread of resistant parasites is important. The current study documents natural selection for wild type chloroquine-sensitive malaria parasites by An. arabiensis in southern Zambia.

METHODS: Mosquitoes were collected from human sleeping rooms by pyrethrum spray catches and identified by morphological examination and molecular confirmation. After dissection to separate abdominal head and thorax sections vector mosquitoes were screened for P. falciparum infection by PCR. Human residents of all ages were tested for P. falciparum parasitaemia by microscopy, followed by PCR on finger-prick dry blood spot samples. P. falciparum infections were genotyped at the chloroquine resistance conferring amino acid codon 76 of the PfCRT gene, using nested PCR and restriction enzyme digestion.

RESULTS: There were no significant differences in K76T polymorphism among smear-positive and smear-negative human malaria infections (p = 0.323, n = 128). However, infections in both the mid-gut and salivary gland phases of the An. arabiensis vector exhibited wild type K76-bearing parasites with up to 10X higher odds, despite having been acquired from human infections within a few weeks (OR [95%CI]: 10 [4.3 – 25.3], p < 0.001, n = 370).

CONCLUSIONS: A sporogonic selection occurs against mutated PfCRT 76T-bearing P. falciparum in An. arabiensis mosquitoes, presumably owing to altered biological fitness. We hypothesize that through this sporogonic selection, mosquitoes contribute to restoration of chloroquine-sensitive parasites after suspension of drug use in humans. Understanding the nature and direction of the mosquito selection could be instrumental for rational curtailment of drug resistance in integrated malaria control or elimination programmes.

P448: Bioprospecting Kenyan Medicinal plants for anti-protozoal compounds.

Jacob O Midwoi, Francis Machumi, Muhammad Illia1 and Larry Walker2

1Department of Chemistry, University of Nairobi, P.O. Box 30197-00100, Nairobi, Kenya; 2National Center for Natural Products Research, School of Pharmacy, University of Mississippi, MS 38677, USA

BACKGROUND: Malaria remains a serious problem for the continent of Africa as the continent bears the heaviest burden of its consequences. Africa experiences almost 2 million people dying from the condition annually which is 80% of global fatality. Developing drugs against malaria is a challenging problem due to the propensity of the most prevalent causative protozoan, Plasmodium falciparum, develops resistance to drugs. Currently the recommended drug for intervention is artemisinin combination therapy (ACT). However there is already resistance experienced against artemisinin derivatives in certain areas of South East Asia. So searching for anti-malarial principles needs to be continuous process. Leshmania is an important vector bone disease which infects at least 12 m people annually. The current drug treatment regimen for the disease are not ideal due to the fact those available, pentamidine and amphotericin B are second line drugs with high toxicities at effective therapeutic doses. In a project between our two institutions, Kenyan medicinal plants arecollected, extracted and subjected to anti-Plasmodial falciparum and visceral Leshmania donovani tests at University of Mississippi. Bioactive extracts are fractionated chromatographically to isolate the active metabolites and structures elucidated.

METHODOLOGY: More than 200 Kenyan medicinal plant parts were extracted with 1:1 dichloromethane methanol and tested against two strains of Plasmodium falciparum (chloroquin resistant and chloroquin sensitive) and against Leshmania donovani. Out of these about 30 plants showed significant activity requiring isolation of potential bioactive compounds in them chromatographically using silica gel columns and organic solvents.

RESULTS: So far Albizia schimperiana, Abrus schimperi, Terminalia brownii, Clerodendrum eriophyllum, and Sphaeranthus bullatus species parts have been studied in detail. These studies have revealed the existence of compounds with good anti-Plasmodium falciparum and Leshmania donovani activity. Albizia schimperiana showed the presence of spermine alkaloids as the anti-protozoal compounds in the stem and root bark extracts. Abrus schimperi elaborates amorphophaine and pendulone as active compounds amongst others. Sphaeranthus bullatus showed the curvatacone derivatives with low IC50 values. The structures of these compounds were such that they could be optimized with chemical derivatization. Clerodendrum eriophyllum elaborates abietane diterpenoids with promising bioactivities.
P449: Effectiveness of Olyset® Plus nets in reducing asymptomatic and symptomatic malaria cases: a cluster randomized control trial in western Kenya

Noboru Minakawa1, James O Kongere2, Gabriel O. Dida1, George O. Sonye3, Hitoshi Kawada1, Jinping Hu4, Kogomi Minagawa5, Kyoko Futami1
1Institute of Tropical Medicine, Nagasaki University, Nagasaki, Japan; 2Kenyatta Medical Research Institute, Nairobi, Kenya; 3Springs of hope, Mbita, Kenya

BACKGROUND: Long lasting insecticidal nets (LLINs) have been successful in reducing malaria cases. However, the increase in pyrethroid-resistance in mosquitoes has become a serious threat for vector control, and new tools are urgently needed. Piperyln butoxide (PBO) is a synergist to inhibit the activities of the enzymes that enhance in resistance of insects by metabolizing the pyrethroid. Olyset® Plus is a newly developed LLIN incorporating a mixture of permethrin and PBO. This study examined if this new bed net reduces asymptomatic and symptomatic malaria cases in an area where the insecticide resistance has been reported.

METHODS: The study area had a population of approximately 8,000 people, and divided to eight areas. Four areas were randomly selected, and Olyset® Plus nets were distributed to cover all residents. In the remaining areas, Olyset nets without PBO were distributed. The pre-intervention malaria survey was targeted at children aged between 0 to 5 years in the dry season, and the post-intervention survey was conducted immediately after the rainy season (four months after the intervention).

RESULTS: The PCR based pre-intervention prevalence of Plasmodium falciparum was 60.1%, and reduced to 28.5% (OR: 0.18; p<0.001) in the areas covered with Olyset® Plus nets. Similarly, it was reduced to 44.7% (OR: 0.49; p<0.001) in the areas with Olyset nets. The difference between Olyset® Plus and Olyset nets was statistically significant (OR: 0.38; p<0.001). The pre-intervention rates of febrile malaria and severe malaria cases were 7.7% and 5.3%, respectively, and reduced to 3.8% (OR: 0.46; p=0.048) and 1.7% (OR: 0.32; p=0.043) in the area with Olyset® Plus nets. They were also reduced to 3.0% (OR: 0.33; p=0.024) and 1.8% (OR: 0.25; p=0.030) in the areas with Olyset nets. The differences between Olyset® Plus nets and Olyset® nets were not statistically significant for both cases.

CONCLUSIONS: Olyset® Plus nets and Olyset nets were effective in reducing asymptomatic and symptomatic malaria cases among children, and Olyset® Plus nets were more effective in reducing asymptomatic infection.

P450: Prenatal infection to helminthes and cognitive development in one year old children in Benin

Michael Osei Mireku, Michael Boivin, Achille Massougbodji, Michel Cot, Florence Bodeau-Livinec

Helminthes in childhood have been shown to be associated with poorer cognitive outcomes. However, the impact of helminthes in mothers during pregnancy on offspring outcomes is unknown. Our goal was to study the association between helminthes in pregnancy and psychomotor outcomes in offspring.

Our prospective cohort study included one year old children born to women enrolled before 28 weeks of pregnancy within the MiPPAD trial comparing sulfadoxine-pyrimethamine and mefloquine. Presence of helminthes was assessed at inclusion in the trial by the KATO test, at least one month later when the second dose of intermittent preventive treatment (IPT) for malaria was given and at delivery. Children were assessed at one year old by a nurse specifically trained for the study with the Mullen Scales of Early Learning (MSEL). A home visit was performed a few days later to assess parent-infant interactions, and the socio-economic status. The study is still ongoing in the field.

Five hundred and forty-three pairs of mothers and offspring were included in the analyses. The prevalence of helminthes infection was 11.6%, 8.8% and 2.0% at inclusion, at the administration of the second dose of IPT and at delivery, respectively. After adjustment for socio-economic status, parent-child interactions, research assistant and malaria, helminthes diagnosed at inclusion were associated with a decreased Early Composite score (mean difference=-4.5, P=0.01).

Our results suggest that the infection by helminthes early in pregnancy may be associated poorer cognitive outcomes in offspring. These results need to be confirmed in other studies.

P451: Malaria in pregnant women: a study conducted on 211 pregnant women in the obstetrical consultations at the CHU of Kamenge (Centre Hospitalo - Universitaire de Kamenge)

Nadine MISAGO1, 3, Mr. Patrick Bitangumutwezi1, Dr. Oscar Nyabenda1, 2
1 University of Burundi, Faculty of Medicine, CHU of Kamenge; 2 Hospital of Rutana; 3 YOWLI BURUNDI

BACKGROUND: Determine the frequency of the association malaria-pregnancy in Obstetrical consultations at CHU Kamenge Evaluate the effects of malaria infection on maternal and fetal health

METHODS: It was a prospective study over 2 months on all obstetrical consultations. We carried out a careful clinical examination towards all pregnant women consulting in the obstetrical service of CHU of Kamenge looking for clinical signs of malaria; then we made systematic complementary examinations: Drop Thick, obstetric ultrasound and a blood count. All pregnant women who tested positive have been hospitalized for a better management and monitoring approaches. During hospitalization, we detected maternal-fetal complications related to malaria (anemia, intrauterine growth retardation - IUUGR, premature births, deaths). We compared the results of malaria related complications in pregnant women to those of non-pregnant women (a previous study: Malaria in women consulting in Gynecology).

RESULTS: Of 211 pregnant, the frequency of malaria was 6.75%. The major risk factors of malaria are: low parity, living in disadvantaged settings and being cultivators. The frequency of malaria depending on gestational (GT) trimester was: first GT: 10.43%; second GT: 30.80%; third GT: 58.77% The clinical picture was dominated by fever (68.72%) and conjunctival pallor (34.12%). The maternal prognosis was very poor for Malaria Access Pernicious: 44% of deaths. The frequency of complications was: severe anemia (Hb <8g/dl): 43.87%; IUUGR (8.95%), premature deliveries (12.32%)

CONCLUSIONS: Malaria is more frequent in pregnant women than in non-pregnant women; The fact of being from deprived settings with low life and the low parity are the key risk factors of malaria and its complications; The Plasmodium falciparum is the species responsible of the majority of Malaria Access Pernicious ; The success of Quinine as a therapeutic drug is evident in the majority of cases
P452: Identifying the malaria hotspots in South Africa - an important precursor for elimination

**BACKGROUND:** Local malaria transmission occurs in three provinces of South Africa, namely Limpopo, Mpumalanga and Kwa-Zulu Natal. Through the robust implementation of multiple interventions and cross-border collaboration with neighbouring countries, South Africa has significantly reduced malaria cases and embarked on a goal of reducing local cases to zero by the year 2018. While the country prepares for elimination, it is crucial that malaria hotspots and transmission foci are accurately located and classified.

**OBJECTIVE:** To evaluate the trends of local malaria cases in South Africa between 2010/2011 and 2012/2013 malaria seasons and determine the location of malaria hotspots in the country.

**METHODS:** Secondary data on local malaria cases was analyzed to identify and stratify malaria hotspots by municipality and localities. The hotspots in each locality were mapped using GIS.

**RESULTS:** There has been a reduction in the local malaria incidence (39% decrease from 0.7 to 0.27 per 1000 population at risk during the malaria seasons 2010/2011 to 2012/2013) since South Africa embarked on reorientation towards elimination of malaria in the year 2010. The reduction of local cases was more prominent in Limpopo province (64% decrease, 2740 in 2010/11 to 995 cases in 2012/2013) compared to KZN (56%, 100 cases to 44) and Mpumalanga (50% decrease). Eight (8) municipalities in Limpopo and Mpumalanga reported more than 100 local cases per year. Further analysis of the local cases in these high burden municipalities indicated that reduction of specific hotspots at locality level (five (5) cases or more per annum), was not significant in provinces like Mpumalanga where the number of such localities only reduced from 12 to 11 localities.

**CONCLUSION:** Despite the overall reduction of local malaria cases, there was no corresponding reduction in the number of hotspot areas due to the emergence of new hotspots. Malaria elimination in South Africa will depend on reduction of malaria hotspots through increased surveillance and multiple targeted interventions in the most affected districts, such as Vhembe, Mopani and Ehlanzeni. Vector data will be incorporated to these results for mapping of foci.

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P453: Perspectives on Malaria Rapid Diagnostic Tests after National Rollout in Tanzania’s Public Sector – Provider and Consumer Views from Mbeya, Mwanza and Mtwaras Regions.

Clarence Mkoba1, Denise Roth Allen1, Admirabilis Kalolelia1, Emmy Metta1, Salim Abdulla1, Catherine Goodman2, S. Patrick Kachur3, Alpha Malishee1, Victoria Mwakalinga1, Prosper Chaki1, John Paliga1, Zawadi Mageni2, Dianne Terlouw3, Gerry F Killeen1,2 and Stefan Dongus1,2

1 Ifakara Health Institute, Mikocheni, PO Box 78373, Dar es Salaam, Tanzania; 2Liverpool School of Tropical Medicine, Pembroke Place, Liverpool L3 5QA, United Kingdom

**BACKGROUND:** Tanzania has gradually been rolling out malaria rapid diagnostic tests (mRDT) in the public sector since 2008 as part of its national malaria case management strategy. Between 2009 and 2012 we conducted a multidisciplinary evaluation to assess the effectiveness of this strategy in Mbeya, Mwanza and Mtwaras Regions. We present results of our qualitative endline interviews with providers and community members in six focus communities about their experiences with malaria diagnostics and treatment.

**METHODS:** We conducted content analysis on 106 interview transcripts, including 46 in-depth provider in-depth interviews (IDI), 12 community focus groups (FGD), and 48 illness narrative interviews (INI) with persons who had experienced a recent fever episode. Data were collected at different stages of the mRDT process: nine months post-implementation in Mbeya and 18 months post-implementation in Mwanza. In Mtwaras, qualitative data were collected as mRDTs were being rolled out.

**RESULTS:** Health providers in Mbeya appeared less willing than those in Mwanza to accept negative mRDT results, with some ignoring negative results in favor of artemether-lumefantrine (ALu) treatment. Stock outs of mRDTs were especially pronounced in Mbeya, where regional authorities noted that 4 out of 8 districts had experienced a stock out within the first 8 months of implementation. In Mtwaras, provider perceptions of mRDTs appeared more hopeful in terms of how mRDTs might improve malaria case management, although some concerns about future stock outs were noted.

Community awareness of mRDTs was limited across all sites. Some participants reported bypassing government dispensaries altogether because they assumed a lack of malaria tests. In Mbeya, only 2 of the 15 INI respondents who sought treatment for fever at a government dispensary reported being tested by mRDT. In Mwanza, only 6 of 16 INI participants sought care at a government dispensary. Of those, only 2 were tested by mRDT.

**CONCLUSIONS:** Strategies to improve knowledge and use of mRDTs are needed if improvements in malaria case management are to be achieved. Such strategies include identifying effective measures to improve provider adherence to mRDTs, addressing bottlenecks in the mRDT supply chain, and improving community awareness so that patients demand malaria diagnostics.

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P454: Using routine health facility data to identify malaria infection clusters in urban Dar es Salaam

Yeromin Mlacha1, John Paliga1, Zawadi Mageni2, Dianne Terlouw3, Gerry F Killeen1,2 and Stefan Dongus1,2

1 Ifakara Health Institute, Mikocheni, PO Box 78373, Dar es Salaam, Tanzania; 2Liverpool School of Tropical Medicine, Pembroke Place, Liverpool L3 5QA, United Kingdom

**INTRODUCTION:** Although malaria transmission rates are now relatively low in Dar es Salaam, Tanzania, current malaria control tools need to be refined and new tools developed if local elimination is to be achieved. This study aimed to test the feasibility and procedures of using routinely health facility data in tracing back and mapping the lowest residential unit Ten Cell Unit (TCU) of individuals diagnosed with clinical malaria at health facility.

**METHODS:** The study was performed at Buguruni ward in urban Dar es Salaam, Tanzania. Anonymized routine information for patient who tested for malaria with a rapid diagnostic test was collected from the laboratory registry book of the health facility within this ward. Patients’ data and TCU leaders’ names collected were used as the basis of tracing the residential locations. The identified TCU leader locations were geo-referenced and merged with patient information collected at the health facility to form a geo-database. Geographic information (MapInfo) and SaTScan software’s were deployed to examine spatial distribution of malaria cases and malaria clusters detection respectively.

**RESULTS:** Results shows that among 707 malaria tested patients residing at Buguruni ward 358 (51%) were able to provide the name of their residential locations TCU (10% were successful placed to their residential location (TCU) and geo-located to their residential location. Maps showed the presence of spatial-temporal variations in malaria incidence within the sub-wards of Buguruni suggested that variations could also exist within the TCUs. Significantly, one positive spatial cluster was identified with 197.7m radius window (RR = 5.02, p=0.025).

**CONCLUSION:** The study showed that if laboratory technicians could be trained to take accurate records of patients including their residential localities, particularly the TCU, malaria clusters and even the hotspots could be identify directly from health facility data. Integrating health
facility data and GIS to map the spatial distribution of malaria transmission provides a useful tool to identify patterns of disease transmission which could enhance the targeting malaria control interventions.

**P455: Prevalence rates reported for malaria**  
Marcellinus Moaborn

**BACKGROUND:** Prevalence rates reported for malaria cases in Ghana vary from one region to the other and also from community to community. The accuracy of results of malaria diagnosis is done by trained and experienced Health professionals. The study was to ascertain the true prevalence of malaria in the community like Mankranso and its environs (surrounding villages) who visit Mankranso District Hospital.

**METHOD:** Record data was obtained from OPD and antenatal attendance records on malaria was sampled 150 cases 100 from OPD and 50 antenatal records of Mankranso Government Hospital. All records were obtained for this study including, blood smears stained with were used for malaria diagnosis by light microscopy some instances.

**RESULTS:** Malaria infection during pregnancy was mostly common as 98% pregnant women were infected. The prevalence of malaria in this population was 8.5% (95% confidence interval) by estimation. Factors identified to increase the risk of malaria infection was over exposure to the environment, the two units had high recorded cases of malaria with each recording more than 95% of malaria.

**CONCLUSION:** In conclusion, community was expose to malaria parasite especially pregnant women and there was also the need for training and retraining of laboratory staffs and equip them as well as establishing the malaria diagnosis quality assurance programme to ensure the accuracy of malaria microscopy results at all levels.

**P456: A study of malaria prevalence in four rural communities of Ogun state**  
Mojeil, H.O. Oluwole, A.S., Adeniran, A.A., Awoyale, A.K., Idowu, O.A.,  
1Ekpo, U.F.  
Department of Biological Sciences, Federal University of Agriculture Abeokuta

**BACKGROUND:** Malaria has been identified as one of the major cause of poverty, sickness and death in Nigeria. Available records show that at least 50-gel per cent of the population of Nigeria suffers from at least one episode of malaria each year. There is need to provide a community baseline data of plasmodium infection, which would assist in monitoring the impact of intervention tools. This study aimed at providing the prevalence of plasmodium parasite among residents of four randomly selected rural communities in Abeokuta, Ogun State, Nigeria.

**METHOD:** Residents of four randomly selected rural communities namely Ijemo Fatipe, Obete Abopa, Obete Akanbi and Odeda of Odeda Local government area were enrolled in the study. Ethical approval was obtained from the Primary Health Care centre. The Head of each community was informed about the study, and members of the community were informed as well by their community leaders. Informed members of the community that volunteered to participate in the study were given consent form. Demographic information of residents such as age, gender, educational background and occupational status were taken. Microscopic examination technique of thick blood films for malaria parasite was employed for the study.

**RESULTS:** A total of 405 subjects were examined, with 162(40%) males and 243(60%) females. Of the 405(100%) subjects examined, 236(58.3%) tested positive for plasmodium parasite. Out of the 236(100%) who tested positive for malaria parasite, 92(39%) were male and 144(61%) were female. Female subjects were more infected than males. However, there was no significant difference in prevalence by sex of the subjects studied (p=0.980).

**CONCLUSION:** Though literacy level had no influence on prevalence of malaria amongst subjects, there was a significant difference between age group and prevalence of malaria among subjects studied (p<0.05). This study shows that malaria is still a major problem in these communities, and there is need for measures to be put in place for its prevention.

**P457: Progress Towards Achieving Millennium Development Goal and Roll Back Malaria Targets in Ghana**  
Wahjib Mohammed1, Dr. Keziah Malm2, Dr. Constance Bart-Plange2,  
1 National Malaria Control Programme

**BACKGROUND:** Ghana has been implementing the Roll Back Malaria Strategy since 2003, and is currently operating with the 2008-2015 strategic plan. The overall goal of the 2008–2015 strategic plan is to reduce both malaria deaths and malaria burden by 75% by 2015 using 2000 as baseline. In line with the MDG Goal 6, the programme is aimed at halting malaria deaths by 2015. This study aimed to assess progress towards attainment of 2015 MDG/RBM malaria targets in Ghana.

**METHOD:** This study employed desk reviews that involved annual reports policy and operational guidelines of the NMCP/GHS from 2000-2012: health facility surveys, and programme reports from partners, community based surveys such as Demographic and Health Surveys (DHS).

**RESULTS:** All cause under-five years mortality rate has reduced from 111/1000 2003 to 82/1000 in 2011. Under-five malaria case fatality rate in Ghana reduced from 14.4% in 2001 to 0.6% in 2012. Furthermore, Ghana obtained about 50% reduction in parasite prevalence between 2002 and 2011 from an national average of over 50% to 27.5%. Against a target of 80% use of ITNs among the population, the country achieved 77.6% in children in 2012 and 59.7% among pregnant women (SPH, 2012). Proportion of pregnant women receiving at least 2 doses of IPT was 64.4% in 2011 against the target of 100%. Proportion of children under five receiving appropriate treatment within 24 hours of onset of malaria/fever was 42% in 2011 (MICS 2011).

**CONCLUSION:** Ghana has made significant progress towards attainment of the MDGs goals of halting malaria deaths. There is however the need to sustain the gains and improve in areas such the coverage of IPT and appropriate treatment of children with fever within 24 hours.

**P458: Knowledge and Perceptions on the Use of Insecticide Treated Mosquito Nets (ITNs) Distributed to HIV positive patients for Malaria prevention at the ART Clinic Kano, Nigeria.**  
Yusuf Mohammed, Mansur Aliyu  
Department of Medical Microbiology and Parasitology, Bayero University, Kano, Nigeria

**BACKGROUND:** Malaria is one of the major opportunistic infection affecting people living with HIV. There is a recognized relationship between the two diseases as HIV infection is associated with an increased risk of malaria. Distribution of ITNs for prevention of malaria in HIV infected people living in malaria endemic area has recently been incorporated into comprehensive care strategies for HIV. Insecticide-treated nets have been shown to be cost-effective in the prevention of malaria, but the number of people that actually use these nets has remained generally low with limited information on it. This study assessed the knowledge and perceptions on the use of insecticide treated nets (ITNs) distributed free at no cost to HIV positive patients at ART Clinic.

**METHODOLOGY:** A cross-sectional survey was conducted and a total of 1,869 HIV positive attending antiretroviral clinic (ART) who were given nets were involved in the study. Data were collected on the socio-demographic characteristics, knowledge and perceptions on the use of nets via face-to-face interview using a pre-tested structured questionnaire.

**RESULTS:** Of the respondents 54 % (1,009) were males and 46 % (860) females. 42% (785) were knowledgeable about ITNs. Those with secondary /university education 66%(1,271) and urban dwellers 72%(1,346) were knowledgeable compared with those primary/secondary32%(598) and rural dwellers 27%(523). In addition to the fear of the chemicals used to treat ITNs as reported by 9%(168), 30%(561) of the respondents who used ITNs complained of too much heat and discomfort experienced, and
P459: Innate immune signatures in malaria-naïve adults infected with *Plasmodium falciparum* sporozoites by needle injection with different doses and routes

**Gemina Moncunill, Alfons Jimenez, Pau Cisteró, Diana Barrios, Patricia Gómez-Pérez, Almudena Legarda, Pedro L. Alonso, Carlota Dobaño.**

**Barcelona Centre for International Health Research (CRESIB, Hospital Clinic-Universitat de Barcelona), Barcelona, Catalonia, Spain**

**BACKGROUND:** Factors from the innate immune response activated upon a first infection in a malaria-naïve adult volunteer are not well characterized. In particular, it is not known what are the determinants and kinetics of the innate immune response and how they affect the induction of adaptive immunity to malaria. Dendritic cells (DCs) are highly potent antigen presenting cells uniquely able to initiate primary immune responses, including tolerogenic responses. In addition, cytokines and chemokines are key effectors in the induction and regulation of immune responses.

**OBJECTIVES:** To determine the effect of different doses, routes and volumes of PFS2 inoculations on the induction of innate immune responses. Eventually, we aim to define how the innate responses during the challenge determine the acquired immune responses.

**METHODS:** In the context of a controlled human malaria infection trial in 36 naïve hosts we analysed immune responses before challenge, at days 1, 7, 21 or malaria diagnosis, and during the treatment period and follow up visits at day 35 and 90. Whole blood was used for phenotyping of DCs and human RNA extraction with a QiAamp kit, followed by transcriptional analysis with Affymetrix microarrays. Plasma samples were used for cytokine and chemokine profiling by Luminex multiplex assays.

**RESULTS:** We characterized the kinetics of different subsets of peripheral blood DCs, CD123+ (plasmacytoid DC), CD11c+ (myeloid DC) and BDCA3+ using a combination of multiple markers including a lineage cocktail and HLA-DR. Preliminary results show that at time of malaria diagnosis there is an increase of the percentage of CD40+, CD86+ and PD-L1 myeloid DCs, and a remarkable decrease of BDCA3+ DCs, while no differences were detected in the frequencies of plasmacytoid DCs. In addition a pro-inflammatory cytokine/chemokine profile in plasma was observed at the time of parasitemia detection. Transcriptional signatures will be correlated with plasma cytokines/chemokines and DC subset composition, and later to acquired cellular immune responses to elucidate how innate immune responses induce adaptive responses.

**CONCLUSIONS:** Comprehensive characterization of innate immune responses, particularly the DCs activation to Plasmodium infection in vivo will help understand the acquisition of immunity to malaria.

P460: From Malaria Control to Elimination in South Africa

**Professor Lucille Blumberg** and Dr Devanand Moonasar

**On behalf of the members of the South African Malaria Elimination Committee**

**1Deputy Director, National Institute for Communicable Diseases, National Health Laboratory Service Johannesburg, South Africa**

**2Director, Malaria, National Department of Health, Pretoria, South Africa**

South Africa is one of thirty-four Malaria-endemic Countries globally that are currently targeting elimination of the disease. In the southern African region this includes Namibia, Swaziland and Botswana. The goal for South Africa is to achieve zero local malaria cases by the year 2018. The objective of this Symposium is to share the successes of the Malaria Control Programme in South Africa, and to highlight the key lessons learnt and the priorities for elimination of the disease. The historical account of Malaria Control Interventions over 110 years details the significant roles played by South Africans in the fight against malaria locally, regionally and internationally. Presentations on vector control and the epidemiology of malaria highlight the period 1995 to 2012, with significant reduction in reported cases since 2001. The contributory factors include: the re-introduction of DDT for Malaria Vector Control a change to Artemisinin-containing Combination Treatment (ACT), and the adoption of Regional Malaria Control Strategies in South Africa, Swaziland and Mozambique through the Lubombo Spatial Development Initiative (LSDI). Effective case management, including both Diagnosis and Treatment, is key to reducing malaria-related morbidity and mortality in South Africa and quality assurance for diagnostic tests is pivotal to good case management and for accurately measuring programme indicators and interventions. Evidence-based Drug Policy has guided chemotherapy and has been critical in reduction of malaria morbidity and mortality in South Africa. As the continuum moves towards Malaria Elimination, a number of different strategies need to be adopted: strengthening of health promotion in partnership with communities to prevent and encourage early treatment-seeking behaviour; active case surveillance; consideration of the use of gametocytoidal drugs; increased vector surveillance and focusing Vector Control on ‘hot spots’; monitoring for mosquito insecticidal resistance and parasite drug resistance; and intensifying cross-border initiatives.

P461: Maintenance of antibody and cytokine responses against *Plasmodium falciparum* in immigrants after extended periods of interrupted exposure to malaria

**Gemina Moncunill, 1 Alfredo Mayor, 1,2 Alfons Jimenez, 1 Augusto Nhabomba, 2 Núria Casas-Vila, 1 Laura Puyol, 1 Joseph J. Campo, 1 Diana Barrios 1 María Nélia Manaca, 1 Ruth Aguilar, 1 María Jesús Pinazo 1 Mercé Almirall, 1 Cristina Soler, 1 José Muñoz, 1 Azucena Bardají, 1 Evelina Angov, 2 Sheetli Dutta, 1 Chetan E. Chitnis 1, 6 Pedro Alonso 1,5 Joaquim Gascón, 6 Carlota Dobaño 1,2**

1Barcelona Centre for International Health Research (CRESIB, Hospital Clinic-Universitat de Barcelona), Barcelona, Catalonia, Spain; 2Centro de Investigación en Salud de Maniça, Maputo, Mozambique; 3Hospital Arnau de Vilanova, Lleida, Catalonia, Spain; 4Hospital Santa Catarina de Salt, Girona, Catalonia, Spain; 5Walter Reed Army Institute of Research (WRAIR), Silver Spring, MD 20910, USA; 6International Centre for Genetic Engineering and Biotechnology (ICGEB), New Delhi, India.

**BACKGROUND:** Malaria immunity is commonly believed to wane in the absence of *Plasmodium falciparum* exposure. However, this assumption is based on limited epidemiological data and short-lived antibody responses in some longitudinal studies in endemic areas. In this study we analysed immune responses in immigrants to evaluate loss or maintenance of immunity.

**METHODS:** Plasma antibody and cytokine/chemokine responses against *Plasmodium falciparum* were quantified in sub-Saharan African adults residing in Spain for up to 38 years (immigrants), with or without clinical malaria. Immune profiles were compared to those of naïve adults (travelers) with a first clinical malaria episode and those of life-long malaria exposed adults from Mozambique (semi-immune adults), with or without clinical malaria. IgG levels against the erythrocytic antigens AMA-1 and MSP-1, 3D7 and FVO strains), EBA-175 and DBL-a were determined by Luminex. IgG levels to antigens on the surface of infected erythrocytes (IEs) and cytokines/chemokines were measured by flow cytometry.

**RESULTS:** Immigrants without malaria had lower IgG levels than healthy semi-immune adults regardless of the antigen tested (P≤0.026), but no correlation was found between IgG levels and time since migration. Upon re-infection, immigrants with malaria had higher levels of IgG against all antigens than immigrants without malaria. However, the magnitude of the response compared to semi-immune adults with malaria depended on the antigen tested. Thus, immigrants had higher IgG levels against AMA-1 and MSP-1 (P≤0.015), similar levels against EBA-175 and DBL-a, and lower levels against IEs (P≤0.016). Immigrants had higher levels of IL-
P462: Modulation of immune responses to a heterologous malaria parasite protein on Lactococcus lactis cell walls and related histopathological changes in mice

Sivagowry A. V. Moorthy¹, Ranjan Ramasamy²
¹Research Division, National Science Foundation, Colombo 07, Sri Lanka, ²Institute of Medicine, University Brunei Darussalam, Jalan Tungku Link, Gadong, BE1410, Brunei Darussalam

INTRODUCTION: Lactic acid bacteria are considered as safe vectors for oral and other mucosal vaccinations. Antibodies against a merozoite surface antigen (MSA2) of the human malaria parasite Plasmodium falciparum (PfMSA2) are associated with protection against malaria in field studies. One possibility is to investigate vaccine potential of mucosally immunised PfMSA2. We report on the oronasal immunisation of mice with the PfMSA2, a putative vaccine candidate and related histopathological changes.

METHODOLOGY: A malaria parasite protein (PfMSA2) was covalently attached to cell walls of live Lactococcus lactis (MSA2cP) and non-covalently attached to L. lactis cell wall ghosts (MSA2cA), and then used for immunisation of different mouse strains (ICR, Balb/c, C57 Black and C3H/HeJ). Effect of co-administering the inducer L. lactis strain together with the recombinant vector and histopathological changes in gut associated lymphoid tissue were also investigated.

RESULTS: Serum IgG antibodies to MSA2 were elicited by both immunogens in strain-dependent manner. The IgG isotype antibodies of these mice reflected the influence of Th1 and Th2 cells. Serum and faecal IgA antibodies against MSA2 antibodies were also detected. Antigen specific IFN-g producing T cells were detectable in spleens of all inbred mouse strains immunised with PfMSA2cA and in C57 mice immunised with PfMSA2cP. The co-inoculation of inducer L. lactis with the MSA2cP expressing bacteria, significantly improved the antibody response to MSA2. Enlargement of mesenteric lymph nodes, increased lymphatic infiltration of the lamina propria as well as germinal centre formation in the spleen were noted in mice fed with L. lactis.

CONCLUSIONS: Results suggest that mucosal immunisation of Pf MSA2 is able to generate protective levels of systemic antibodies and cellular immunity in mice dependent on strain and anchoring method and co-administration of an inducer strain elicited better immune responses. These findings are relevant for developing better vaccine delivery systems for human use.

P463: Mapping Malaria in South Africa: Informing Control and Targeting Elimination

Natasha Morris
South African Medical Research Council, nmorris@mrc.ac.za

BACKGROUND: Surveillance together with monitoring and evaluation of malaria intensity and distribution are critical for informed malaria control and programme management. Adding a spatial element to surveillance information enhances the value of data in indicating spatial locations of hotspots and probable foci of transmission. In South Africa, malaria burden was stratified demographically in order to describe the social strata amongst which the disease predominates.

RESULTS: The improved high resolution mapping of malaria cases allow data to be assessed at various levels, ranging from national distribution of malaria down to identifying hotspots and targeting interventions at a locality level. The benefits of targeting interventions like indoor residual spraying at potential hotspots in low transmission settings include increased efficacy and improved cost-effectiveness.

P464: Mapping Malaria and Targeting Elimination in KwaZulu Natal

Morris N¹, Maharaj R², Raman J², Raswiswi E², Seocharan P²
¹South African Medical Research Council; ²KwaZulu Natal Malaria Control Programme

BACKGROUND: Integrated malaria information systems are critical to the success of a malaria elimination agenda. The addition of a spatial element to decision support further enhances the usefulness of data in indicating spatial locations of hotspots and probable foci of transmission. One of three malarious provinces in South Africa, KwaZulu Natal has experienced marked declines in case burden over the past decade and stands to be the first to formally embark upon an elimination campaign. Spatially enabled data from the provincial malaria information systems were used to identify focal points of possible malaria transmission at which intervention might be targeted to successfully reduce local transmission towards the end goal of elimination.

METHODS: Local cases were mapped at the locality and facility levels and recurring high-case count entities highlighted to infer potential foci of transmission in the absence of routine entomological surveillance. Localities with higher imported case counts reporting were classified by their probable source of infection and mapped, and spatial patterns of their distribution observed in order to gauge potential streams of imported malaria. Population movement within the province was mapped against the context of economic opportunity. Finally, malaria burden was stratified demographically in order to describe the social strata amongst which the disease predominates.

RESULTS: The three northern-most districts of KwaZulu Natal all recorded less than 0.5 cases per 1000 population over the last five years, all reported cases considered, and local case incidences of less than 0.1. Fifteen localities and twelve facilities routinely reporting malaria cases over the last five seasons were identified as locales of potential local transmission. Localities frequently reporting imported cases were mapped and their distribution in relation to border points of entry, urban centres and commercial farming areas considered. The socio demographic character of most affected communities was evaluated.

CONCLUSIONS: Spatially enabled malaria information systems proved invaluable in informing the elimination campaign in KwaZulu Natal by enabling the identification of hotspots and probable foci of transmission. Such data can be used to successfully target appropriate intervention and response. An understanding of the socio-demographic and economic profile of most affected communities is further critical to their mobilisation and support during the drive towards elimination.
P465: Evaluating the impact of enhanced regulation and increased access to diagnosis in the private sector in Zanzibar

Alexandra Morris, Abdullah Ali, Bruno Moonen, Mwinyi Msellemi, Abdulwahidy Al-mafazy, Abdunoor Mulokazi, Abigail Ward, Laura Kelley, Anne Wilson, Justin Cohen
1Clinton Health Access Initiative; 2Zanzibar Malaria Control Program; 3Ifohara Health Institute

BACKGROUND: The malaria burden in Zanzibar has reached an historic low, but the 2011 malaria indicator survey suggests only about one quarter of children receive blood tests for febrile illness, meaning that many who receive anti-malarials may not truly need them. Newly low-endemic regions like Zanzibar require strategies for ensuring that anti-malarial drugs are targeted to patients who have a confirmed malaria infection.

METHODS: This study employed a controlled pre-post evaluation to assess an intervention aiming to improve the rational use of anti-malarials in Zanzibar. The intervention consisted of a policy banning sales of anti-malarials from over-the-counter (OTC) shops in May 2012 as well as the introduction of subsidised malaria rapid diagnostic tests in private health facilities. Testing was already widely available in public health facilities. The study evaluated whether the intervention changed 1) the proportion of those with suspected malaria who sought care in OTC shops and pharmacies, 2) the proportion of patients who received parasitological confirmation and 3) the proportion of OTC shops that continued to stock and sell anti-malarials. Following a baseline evaluation in April 2012, midline and endline evaluations were conducted at six and twelve months, respectively, to determine the short- and long-term effects of the intervention. Each evaluation consisted of 1,000 household surveys, 119 mystery client surveys, and 66 provider surveys.

RESULTS: After one year, the intervention had a significant effect on the removal of anti-malarials from OTC shops: the proportion of OTCs stocking anti-malarials fell from 85% at baseline to 30% at endline in the intervention region (Chi2 Test, p<0.001). However, during this same time period, the fraction of suspected malaria cases seeking treatment in OTC shops and pharmacies remained unchanged. Results from the final evaluation, conducted in April 2013, include qualitative investigation of patient perceptions of treatment seeking, malaria diagnosis, as well as the new regulations.

CONCLUSIONS: Results from this project suggest that government regulation of the private anti-malarial market is a viable path to encourage proper case management in an elimination setting.

P466: Increases in prescribing and stocking of artemisinin-based combination therapies (ACTs) in Madagascar following educational outreach

Abigail Ward, Alexandra Morris, Pierre-Loup Lesage, Aimée Miller, Felix Lam, Justin M. Cohen
Clinton Health Access Initiative, Boston, Massachusetts, USA

BACKGROUND: Although artemisinin-based combination therapies (ACTs) are the recommended first-line treatment for malaria in Madagascar, the 2011 malaria indicator survey indicated that fewer than 40% of anti-malarial drugs received by children under five for fever were ACTs. Approximately one third of suspected malaria is treated in the private sector where ACTs are often unaffordable or inaccessible.

METHODS: To encourage prescribing, stocking, and purchasing of ACTs in Madagascar’s private sector, trained educators shared scientifically accurate knowledge about ACT effectiveness with doctors and shopkeepers. Baseline cross-sectional surveys on factors related to prescribing, anti-malarial stocking, and consumer preferences were conducted in 234 outlets and 163 health facilities in five regions of Madagascar in July 2011, several months after implementation of the Affordable Medicines Facility for malaria (AMFm) subsidy in Madagascar. Doctors and outlets intervention regions were then visited by educators with ACT outreach materials over a six month period. Midline follow-up surveys in all regions were conducted after the educational visits and endline surveys were conducted eight months later to evaluate the sustainability of any observed changes. Changes in prescription, stocking, and consumer selection of ACTs were compared statistically between intervention and control outlets and health facilities.

RESULTS: Outlets that did not stock ACTs at baseline were 113% (Chi2=17.6, p<0.01) more likely to do so at midline if visited by educators, while doctors who had never prescribed ACTs were 107% (Chi2=5.9, p<0.02) more likely to prescribe ACTs after educational visits. At endline, 93.8% of shops that began to stock ACTs and 92.3% of doctors who began prescribing them continued to do so. The overall anti-malarial market share of ACTs in both control and intervention groups increased 122% between baseline and midline (27.7-60.4% intervention; 37-69.8% control, n=406) and remained constant between midline and endline (58.2% intervention; 69.7% control, n=391).

CONCLUSIONS: The substantial increase in ACT market share observed during this study suggest the strong influence of price reductions occurring through the AMFm. However, the low-cost intervention tested here suggests that educational activities can improve availability of ACTs in the private sector.

P467: Epidemiology of sub-patent Plasmodium falciparum infection: implications for detection of hotspots with imperfect diagnostics

1National Institute for Medical Research (NIMR), Mwanza Medical Research Centre, Mwanza, Tanzania; 2Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, UK; 3The Global Health Group, University of California, San Francisco, CA, USA; 4Department of Pediatrics, University of California, San Francisco, USA; 5Kilimanjaro Clinical Research Institute and Kilimanjaro Christian Medical College, Kilimanjaro Moshi, Tanzania; 6Department of Medical Microbiology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands

INTRODUCTION: At the local level, malaria transmission clusters in hotspots, which may be a group of households that experience higher than average exposure to infectious mosquitoes. Active case detection, often relying on rapid diagnostic tests for mass screen and treat campaigns, has been proposed as a method to detect and treat individuals in hotspots. Data from a cross sectional survey conducted in north-western Tanzania were used to examine the spatial distribution of Plasmodium falciparum and the relationship between household exposure and parasite density.

METHODS: Dried blood spots were collected from consenting individuals from four villages during a survey conducted in 2010. These were analyzed by PCR for the presence of P. falciparum, with the parasite density of positive samples being estimated by quantitative PCR. Household exposure was estimated using the distance-weighted PCR prevalence of infection. Parasite density simulations were used to estimate the proportion of infections that would be treated using a screen and treat approach with RDTs compared to targeted mass drug administration and MDA.

RESULTS: PCR analysis revealed that of the 3,057 blood samples analysed, 1,078 were positive. Mean distance-weighted PCR prevalence per household was 34.5%. Parasite density was negatively associated with transmission intensity with the odds of an infection being sub-patent increasing with household exposure (OR 1.09 per 1% increase in exposure). Parasite density was also related to age, being highest in children 5-10 years old and lowest in those >40 years. Simulations of different MDA strategies showed that treating all individuals in households where RDT prevalence was above 20% increased the number
of infections that would have been treated from 43% to 55%. However, even with this strategy, 45% of infections remained untreated.

**CONCLUSION:** The negative relationship between household exposure and parasite density suggests that DNA-based detection of parasites is needed to provide adequate sensitivity in hotspots. Targeting MDA only to households with RDT positive individuals may allow a larger fraction of infections to be treated. These results suggest that community wide MDA, instead of screen and treat strategies, may be needed to treat the asymptomatic, sub-patent parasite reservoir successfully and reduce transmission in similar settings.

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**P468: Community perspectives and practices related to outdoor malaria transmission in rural Tanzania**

Irene Moshi¹, Edith Madumla¹ and Fredros Okumu¹
Ijokara Health Institute

**BACKGROUND:** Indoor malaria prevention methods such as Long-Lasting Insecticide Treated Nets have significantly reduced the disease burden in Africa, yet transmission persists in many communities, partly driven by mosquitoes that bite people outdoors. It is essential to consider perspectives of local communities towards this outdoor transmission, so as to inform the development of new tools for malaria control.

**METHODOLOGY:** We assessed views and behaviors of rural and peri-urban communities in southern Tanzania, regarding outdoor mosquito bites and malaria prevention. A cross-sectional survey was conducted in two rural and two peri-urban villages in southern Tanzania, using semi-structured interviews and structured observations. A total of 40 households were studied. The interviews assessed whether malaria vectors also bite outdoors and transmission can also occur outdoors, while the observations were used to identify common outdoor activities that expose people to mosquito bites and current means of protection while the observations were used to identify common outdoor activities that expose people to mosquito bites and current means of protection against the outdoor bites. A prototype outdoor mosquito control device was then used to assess community responses towards such potential outdoor interventions in future malaria control.

**RESULTS:** More than 90% of the respondents knew about malaria and had regularly experienced outdoor mosquito bites and complained of malaria persistence, but most of them still believed that transmission occurs mostly indoors such that some use repellents or long cloths to prevent themselves from biting. Common outdoor activities included shopping and socializing (30% of people observed), storytelling (21%), cooking (18%), eating (16%), fetching water (15%), all of which took place between 6pm and 11pm, and also starting 5am and 6:30 am, matching times when outdoor biting mosquitoes are also known to be most active. The respondents were willing to use and contribute towards financing of the outdoor devices for malaria control.

**CONCLUSION:** The results show that people appreciate outdoor biting and the likelihood of outdoor transmission but use of intervention other than bed nets indoors, was rare. Providing well developed outdoor mosquito control devices that are acceptable will contribute substantially in reducing malaria transmission.

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**P469: In vivo /in vitro efficacy of artemether-lumefantrine and artesunate-amodiaquine in children with uncomplicated falciparum malaria in Bobo-Dioulasso, Burkina Faso**

Moussa Lingani¹, Léa Nadège Bonkian³, Isidore Yerbanga¹, Louis Arnaud Coluzzi and Jean Bosco Ouédraogo³

**BACKGROUND:** In a randomized, open label study carried out between 2008 and 2010, assessing both the in vivo and in vitro efficacy of artemether-lumefantrine (AL) and artesunate-amodiaquine (ASAQ) in children aged 6 months to 15 years with uncomplicated malaria. Venous blood sample was collected before treatment and at day of recurrent parasitaemia for in vitro test. Patients were followed-up for 42 days and the isotopic microtest technique was used to perform the in vitro assays. In vivo efficacy was assessed according to WHO standard protocol.

**RESULTS:** 440 children were enrolled (220 in each group). Both ACTs were highly efficacious though this was significantly higher for ASAQ (98.1%) than for AL (91.1%), p<0.0016. The 50% inhibitory concentration was higher at time of recurrence. Before treatment mean was 18.06 nM for monodesethylamodiaquine and 26.77 nM for lumefantrine and at time of recurrence it was 23.01 nM for monodesethylamodiaquine and 51.59 nM for lumefantrine. No increase was observed for dihydroartemisinin.

**CONCLUSION:** Recurrent infection had a lower sensitivity to artesinin partner drugs, indicating a selection of these parasites. Dihydroartemisinin is still very effective in Burkina Faso.

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**P471: Entomologic parameters assessment of transmission of human malaria after indoor residual spraying with bendiocarb at Diébougou Burkina Faso**

Sylla Moussa
Unit for Research on Malaria and Neglected Tropical Diseases Centre MURAZ Research Institute, Burkina Faso.

**BACKGROUND:** To combat the vectors of malaria and other dangerous insects for health, materials impregnated with long-lasting insecticides and indoor residual spraying are the most important means. After pyrethoids resistance, vectors control would be investigated in others alternatives insecticides as carbamate, pyrimiphos methyl and organophosphate insecticides. The goal of our study was to follow the impact of indoor residual spraying in reducing malaria transmission in the health district of Diébougou, to evaluate its effect on the composition of vectors, their density, the behavior of bites, and entomological inoculation rate. Also, to monitor the level of resistance during treatment.

**METHODS:** From June to November 2011, wild populations of females of An.gambiae, and females Kisumu strain from laboratory colonies was tested with Bendiocarb in wall treatment. Our study involved two areas in South West of Burkina Faso (Diebougou as intervention area, and Dano, control area). We also collected the mosquitoes by CDC and spray catches to estimate respectively the species composition, the behavior of bites, and entomological inoculation rate.

**RESULTS:** A total of 3600 mosquitoes tested, results showed that, mortality ranged from 80 to 100 % in the different villages of intervention. The Circumsporozoite protein rate was similar for the both areas before spraying and after spraying, dropped to zero the following month in intervention area. The PCR confirmed also the presence of An.gambiae Coluzzi and An. gambiae Giles. The entomological inoculation rate decreased in the village of intervention as village of control. The level of distribution of kdr gene was very increasing in both molecular forms.

**CONCLUSION:** After six months, study showed that bendiocarb could be recommended as good alternative insecticide for IRS in Diébougou, not to mention that a few alternative solutions can be found in organophosphates more durable than carbamate effect.
P472: Evaluation of the effectiveness of a training programme for school-teachers in performing and interpreting malaria rapid diagnostic tests safely and accurately in Zomba, Malawi.

Austin Mtali1, Stefan Witek-McManus2, John H. Sandle3, Don Mathanga4, Reuben Mwenda5, Charles Mazinga6, Tiye Se Chimuna7, Natalie Roschnik8, Katherine E Halliday9 & Simon J. Brooker10

1Save the Children International, Malawi; 2Faculty of Infectious and Tropical Diseases, London School of Hygiene & Tropical Medicine, UK; 3National Malaria Control Program, Ministry of Health, Malawi; 4Malaria Alert Centre, College of Medicine, Malawi; 5Health Technical Support Services-Diagnostics, Ministry of Health, Malawi; 6Ministry of Education, Science and Technology, Malawi; 7Save the Children International, US; 8Kenya Medical Research Institute, Nairobi, Kenya

BACKGROUND: With increasing levels of attendance, schools present a credible and pragmatic opportunity to improve the access to school children to timely diagnosis and treatment of malaria, increasingly recognised as a major health problem for this age group. The expanded use of malaria rapid diagnostic tests (RDTs) in communities by health surveillance sectorists and through the private sector has led to an interest in whether teachers can provide diagnosis for school children. We investigated the ability of school teachers to constitute safe, accurate and acceptable providers of malaria diagnosis and treatment using RDTs and artemisinin-based combination therapies (ACTs) – AL following training.

METHODS: A pilot, comprehensive skill focused, training was conducted in Zomba District, Malawi. Teachers were trained on the use of first aid kits including instruction on the principles and use of malaria RDTs by facilitators from the Ministry of Health. The four-day training followed a structured workshop, with manuals and job aids adapted from a range of existing materials pre-tested with community medicine distributors and health surveillance assistants. Teachers practiced use of RDTs on each other and role plays were used to train on treatment decisions, and referral based on RDT results.

RESULTS: We present results on the effectiveness of the training in relation to an increased knowledge and skill set through pre and post evaluation questionnaires, script concordance tests and checklist evaluations. Additionally we report on the acceptability from the community of teachers performing such a role in schools and the confidence of the teachers to provide this service in schools, assessed through focus group discussions.

CONCLUSIONS: To our knowledge, this is the first study training teachers to use RDTs. The results provide important evidence of the feasibility using teachers to diagnose malaria using RDTs in terms of safety, accuracy and confidence and make appropriate treatment decisions based on the results.

P473: Comparison of malaria morbidity before and after indoor residual spraying in Kanungu district, Uganda

Vincent Mubangizi1, John Bosco Ddamulira2

1Mbarara University of Science and Technology; 2College of Health Sciences Makerere University

BACKGROUND: Malaria remains one of the most serious global health problems and a leading cause of morbidity and mortality. Malaria causes more illness and death than any other single disease in Uganda. In Kanungu district, malaria accounted for 48% of burden of disease in 2005. The large scale use of (Indoor Residual Spraying) IRS is being introduced in Uganda and its effect on Malaria morbidity is not yet established. The objective of this study was to assess trends in malaria morbidity following the IRS exercise in Kanungu to generate information as a basis for future interventions.

A STATEMENT OF THE RESEARCH PROBLEM: The Kanungu district carried out IRS using ICON™ in March 2007. The effect of IRS on malaria morbidity in Kanungu was not known. Due to lack of supportive data, some people de-campaign the IRS exercise as a waste of resources and unnecessary exposure to chemicals, ICON™. It is important to ascertain the effect of IRS so that resources for malaria prevention are allocated with evidence.

METHODS: A before and after IRS with a control study was done in Kanungu. It utilized qualitative and quantitative methods of data collection. The study populations were medical records of residents of Kanungu who sought health care at government and private not for profit health facilities in 2006 and 2007, health unit in-charges, District malaria focal person and the HMIS staff at the district health office.

RESULTS: The results from this study indicated there was reduction in malaria case visits in both absolute numbers and percentages after IRS compared to the period before in Kanungu. There was a reduction in the proportion of blood slides read as positive for malaria in the six months immediately after IRS.

CONCLUSION: Malaria morbidity showed a decline following indoor spraying with ICON™

P474: Costs of antenatal care seeking including intermittent preventive treatment of malaria during pregnancy in two Tanzanian districts: a quantitative analysis

Godfrey M. Mubyazi
National Institute for Medical Research (NIMR) Headquarters, Dar es Salaam, Tanzania.

BACKGROUND: There is still inadequate empirical evidence documented on the influence of actual or perceived costs of antenatal care (ANC) seeking including intermittent preventive treatment of malaria during pregnancy (IPTp) in Tanzania. This paper describes how the actual and perceived costs of ANC influence pregnant women to seek and access ANC including malaria IPTp in Mkuranga and Mufindi districts in Tanzania. The study populations were medical records of residents of Kanungu who sought health care at government and private not for profit health facilities in 2006 and 2007, health unit in-charges, District malaria focal person and the HMIS staff at the district health office.

METHODS: A structured questionnaire was used to interview 417 and 406 women exiting ANC clinics in Mkuranga and Mufindi, respectively. Simple tabulation and logistic regression analyses were executed using STATA 8 software package to establish the evidence needed.

RESULTS: Generally, 66.2% and 89.3% of the interviewees in Mkuranga and Mufindi, respectively had contacted government ANC clinics before this study. But, less than 20% and 15% in those districts respectively had contacted private clinics. Having paid user-fees on the day of interview was reported by 36.7% and 7.0% of the respondents in these districts, respectively. Experience with unofficial payments to the clinic staff was reported by <2% of the interviewees in both districts. Long travel distance was identified as the main disappointing factor for seeking ANC in both districts, followed by prevalence of user-fees. Low quality of care at faith-based and other non-government clinics implementing user-fees lowly influenced the respondents to decide contacting the government clinics than the same factor did to influence the clients to visit private clinics. Respondents from wealthier families and those with decision-making power at family level on use of family income were less likely to have faced user-fee payment hardship than those without such power. Lack of money for user-fee or for transport payment made 12.6% and 12.4% of the respondents in Mkuranga and Mufindi districts respectively to start looking at the ANC clinics during the seventh month of their pregnancies, hence making them to delay receiving IPTp doses.

CONCLUSION: This study confirms that costs of user-fees, travel, waiting time at facilities, and perceived or actual low quality of care and low women’s decision-making power on family incomes hinder some pregnant women to seek ANC and use IPTp in Tanzania, hence a call for interventions.
P475: Measurements of insecticide susceptibility depend on the choice of standard bioassays and larval rearing conditions

Henry Frempong Owusu1, David Malone1, Nakul Chitnis2, Hilary Ranson1 and Pius Müller1
1Swiss Tropical and Public Health Institute, Basel, Switzerland; 2University of Basel, Basel, Switzerland; 4Innovative Vector Control Consortium, Liverpool, UK; 4Liverpool School of Tropical Medicine, Liverpool, UK

Susceptibility bioassays form a key step in insecticide resistance monitoring and the development of new insecticidal formulations and delivery modes against malaria vectors. The most frequently used assays are the WHO cylinder assay and the CDC bottle assay. These two bioassays employ different means of delivering the insecticide product: the WHO assay uses a filter paper containing the insecticide and the CDC assay uses an insecticide-coated glass bottle. The assays also measure resistance differently. In the WHO bioassay, insecticide susceptibility is assessed by the proportion of mosquitoes killed twenty-four hours after a one hour exposure to a diagnostic insecticide concentration. In contrast, the endpoint of the CDC bottle assay is median time to knock-down. There is much debate as to which bioassay would be more suitable to measure insecticide susceptibility. Often overlooked in this discussion are the effects of environmental factors that may also affect the comparability of resistance endpoints. Here, we set out to compare the two assays side by side and to evaluate the impact of larval rearing conditions on insecticide susceptibility under controlled laboratory conditions and through the analysis of published data. We measured the relationship between time to knock-down and 24 hours mortality in a series of experiments testing for a range of insecticides in several mosquito colonies showing different insecticide susceptibility profiles. The effects of breeding conditions and body weight on susceptibility were also investigated as possible contributors of heterogeneity in bioassay results. We will discuss the comparability of the two insecticide susceptibility assays and the impact of environmental factors on the end points and will give recommendations for improving the quality of such bioassay data.

P476: Selective sweeps and genetic lineages of Plasmodium falciparum multi-drug resistance (Pfdmr1) gene in Kenya

Peninah Muiruri1, Dennis Juma1, Luiser Ingasia1, Angela Omondi1, Nancy Jalaga1, Ndenga Mutwiri1, Lorna Lemosop1, Bidil Naglah1, Redemptah Yeda1, Agnes Cheruiyot1, Charles Okudo1, Hosea Akala1, Ben Andangalu1, Joseph Ng’ang’a1, Edwin Kamau1

1Global Emerging Infections Surveillance (GEIS) Program, United States Army Medical Research Unit-Kenya (USAMRU-K), Kenya Medical Research Institute (KEMRI) - Walter Reed Project, Kisumu and Nairobi, Kenya
2Department of Biochemistry, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya

BACKGROUND: Kenya adopted Artemisinin Combined Therapy (ACT) as first-line treatment for malaria in 2006. However, there are concerns that resistance against ACTs might emerge in the region as has been reported in Thailand-Cambodia border. Single-Nucleotide Polymorphisms (SNPs) in critical alleles of Pfdmr1 gene and increase in copy numbers has been associated with resistance to Artemisinin and its partner drugs. Studying drug-resistance-associated SNPs and their genetic lineages is critical in understanding of the evolution of antimalarial drug resistance. This study investigates evidence for a selective sweep around Pfdmr1 gene as a way of explaining the spread of adaptive polymorphisms and/or multiple copies of this gene in the Plasmodium falciparum parasite population in Kenya.

METHODS: Analysis of samples collected from diverse regions of Kenya with varying transmission intensities is in progress. DNA was extracted using Qiagen DNA extraction kit. Codons 86, 184, 1034 and 1042 in Pfdmr1 gene were genotyped using allelic discrimination assays and/or by sequencing. Copy number analysis was performed using real-time PCR. Characterization of 13 microsatellite loci flanking (±99 kb) Pfdmr1 in single-clone P. falciparum infections is underway.

RESULTS: From the 714 samples genotyped, 69 (9.6%), 173 (24.2%), 22 (3.1%) and 16 (2.2%) were mutants at codons Pfdmr1 86, 184, 1034 and 1042 respectively. In addition, 86 (12%), 88 (12.3%), 59 (8.3%) and 175 (24.3%) were mixed genotypes at codons specified respectively. The prevalence of 86 wild type allele was 78.4% and 184 mutant allele was 24.2%. Of the samples analyzed only 4 contained multiple copies of the gene. Genetic analysis for selective sweeps at loci flanking Pfdmr1 is ongoing.

CONCLUSION: Pfdmr1 86N and 184F alleles are associated with decreased sensitivity to ACTs. Data obtained here is worrisome because prevalence of these two alleles combined exceeded 24%. The multiple copies observed infer emerging resistance to Mefloquine a partner drug in ACT. We expect that genetic analysis of variation at microsatellite loci flanking Pfdmr1 and selective sweep at 86N and 184F alleles will reveal the pattern of selective sweeps. This will help track the origin and spread of ACTs resistant parasites and allow better containment and control of malaria.

P477: Study on malaria morbidity: Case of Kinshasa and Mbandaka cities

Papy N Mandoko1, Thierry L Bobanga2, Jean Pierre K Mukendi1, Solange E Umesumbu1, Jacques N Tshibamba1, Dieudonné N Mumba1, Célestin N Nsibu1

1Institut National de Recherche Biomédicales, 2Institut National de Recherche Biomédicales, 3University of Kinshasa, 4University Joseph Kasa-vubu, 5National Programme for Malaria Control

INTRODUCTION: Plasmodium falciparum malaria is a major public health problem and contributes to the morbidity due to malaria. This study aims to estimate malaria morbidity by comparing the cities of Kinshasa and Mbandaka based on their epidemiological facies which are tropical and equatorial respectively. We consulted the medical records of patients seen during year 2008.

RESULTS: Morbidity in Mbandaka is concentrated mainly in children less than 5 years (36.7% vs. 22.2%) and is more common in patients over 14 years in Kinshasa (56% vs 46.2%). More than 30% of patients in Mbandaka received quinine for uncomplicated malaria, whereas 2.25% of patients in Mbandaka against 0.9% in Kinshasa received quinine for severe malaria. At least 23% of patients in Mbandaka against 8.5% in Kinshasa have received ACT and SP for uncomplicated malaria. Any patient in Mbandaka against 0.9% in Kinshasa received chloroquine. Patients more than 14 years have received mostly the quinine for uncomplicated malaria.

CONCLUSION: The strategy of malaria control should be based on education, information and communication of healthcare personnel and strengthen the research to reduce malaria morbidity in the Democratic Republic of Congo.

P478: Failure of malaria control in Nchelenge, how much is due to insecticide resistance?

Mbanga Muleba1, Laura Norris2, Hunter Chilusu1, Mike Chaponda1, Timothy Shields2, Oliver Wood4, Richard Hunt1, Modest Mulenga1 Douglas Norris2, William Moss2, Maureen Coartze2

1Tropical Diseases Research Centre, 2Johns Hopkins Bloomberg School of Public Health and Molecular Microbiology, 4National Institute for Communicable Diseases

BACKGROUND: Insecticides play a major role in the control of malaria vectors. While the introduction of indoor residual spraying (IRS) and distribution of long-lasting insecticide nets (LLINs) in many districts of Zambia have led to a remarkable reduction in malaria transmission the scenario is quite different in Nchelenge district. An entomological study was carried out to assess the efficacy of a profile of insecticides commonly used on LLINs and in IRS campaigns against malaria vector populations.
METHODS: Mosquitoes were collected from Study participant households by CDC light trap, Aspiration and spray catch. Blood slides were collected and an RDT performed in the field on consenting individuals in these households. The ownership and usage of LLINs was noted in the field. Morphological and later PCR identification were performed on processed specimens. The standard WHO susceptibility tests were conducted on wild mosquitoes in the field and on F1s. Further assessments on the mechanisms of resistance were performed on Anopheles gambiae s.s. Malaria sporozoite rates in the vector were detected by the ELISA technique. No metabolic determination of resistance mechanisms was done.

RESULTS: A total of 5759 anopheline mosquitoes were collected with (76%) An. funestus s.s. and (23%) An. gambiae s.s. while (1%) other anopheles. The knock-down resistance ( kdr) gene responsible for DDT and pyrethroid cross-resistance in An. gambiae s.s. was 100%. The malaria sporozoite rates were 7.7% (An. funestus s.s.) and 8.7% (An. gambiae s.s.). The RDT positive rate over 45% with the age group 5-16 years most hit (66%). Net usage was found to be 89% and 50% in adults and children respectively. The indoor residual spray coverage was 25%.

CONCLUSIONS: Anopheles funestus s.s. is resistant to pyrethroids and carbamates but susceptible to DDT and organophosphates. Anopheles gambiae is resistant to deltamethrin, bendiocarb and DDT. The LLINs at best are just providing a barrier against malaria vectors for those using them. Limited options are available for vector control in Nchelenge and novel control strategies are needed. Close monitoring of efficacy of current vector control methods is required.

P479: Risk factors for malaria transmission in Engela District, of the Ohangwena region of Northern Namibia

J. Auala1, H. Sturrock2, I. Kleinschmidt3, I. Du Preez4, R. Bock5, R. Gosling5, S. Katokie1 and D.R. Mumbengewi1

1Multidisciplinary Research Centre, Science, Technology & Innovation Division, University of Namibia, Windhoek, Namibia; 2Malaria Elimination Initiative, Global Health Group, University of California, San Francisco, USA; 3Faculty of Epidemiology and Population Health, Dept. of Infectious Disease Epidemiology London School of Hygiene and Tropical Medicine, London, United Kingdom. 4Faculty of Science, Dept. of Biological Sciences, University of Namibia, Windhoek, Namibia; 5National Malaria control program, Ministry of Health and Social Services, Windhoek, Namibia.

BACKGROUND: Malaria transmission in Namibia has declined dramatically from 477,786 in 2000 to 1546 cases in the 2012/13 malaria season. Namibia has adopted a policy of malaria elimination by 2020 (zero local transmission). This presents new challenges as interventions that were successful in bringing down malaria cases may no longer be appropriate at low transmission settings. New tools and interventions are required to move to no local transmission of malaria. This study was conducted to determine the risk factors for malaria transmission in a low transmission setting by following up and interviewing, all malaria cases in Engela district in the Ohangwena region of Northern Namibia.

METHODS: All RDT confirmed malaria cases reported from the 17 clinics in the Engela district were recruited for this study. Four surrounding households were also selected and recruited into the study and this constituted a neighbourhood. All fever individuals testing malaria positive by RDT were visited at their homes; and interviewed about malaria risk factors such as use of mosquito nets, indoor residual spraying of sleeping structures, presence of mosquito breeding sites and travel history.

RESULTS AND CONCLUSION: Twenty two of malaria were reported including 4 death cases. One hundred and ninety four households were visited from 46 neighborhoods. Out of the 22 cases reported, 12 individuals had a history of travelling to Angola close to the time in which they were diagnosed with malaria. Forty six percent of households reported having a breeding site close to the sleeping structures. Thirty three percent of the houses did not have mosquito nets and almost 76% percent of all sleeping structures had a space between the roof and the wall where mosquitoes can pass freely at night. Only 16.5% of structures were not sprayed, 78.4% were not sprayed.

CONCLUSION: Importation of malaria is a major risk factor for malaria transmission in Engela district as persons travelling are potential reservoirs of the disease in their communities. The low usage of mosquito nets and low IRS coverage, poses a real challenge to stopping malaria transmission. Health check-ups following travelling to malaria-endemic areas should be made a priority.

P480: A variant within the CD14 promoter (-159C/T) is associated with increased susceptibility to pediatric malarial anemia in a holoendemic Plasmodium falciparum transmission area

Elly O. Munde1, Evans Raballah2, Winnie A. Okeya1, Caroline A. Owade1, Samuel B. Anyona1, Wilson Okumu1, John M. Vulule1, John M. Ong’ech2, Douglas J. Perkins4,1, Collins Ouma2,1

1Maseno University, Maseno, Kenya; 2Masinde Muliro University of Science and Technology, Kakamega, Kenya; 3Kenya Medical Research Institute, Centre for Global Health Research, Kisumu, Kenya; 4Centre for Global Health, Department of Internal Medicine, School of Medicine, University of New Mexico, Albuquerque, NM, USA.

Plasmodium falciparum malaria is among the leading causes of morbidity and mortality among sub-Saharan African children. In P. falciparum holoendemic transmission areas of western Kenya, malarial anemia [MA; hemoglobin (Hb) <8.0 g/dl, any density parasitemia] is common in pediatric populations. Cluster of differentiation 14 (CD14) plays a key role in inflammatory pathways. Previous studies have shown that the -159TT genotype in the promoter region of CD14 gene is associated with susceptibility to inflammatory diseases. However, the precise role of CD14 (-159C/T) polymorphism, particularly in modulating susceptibility to MA in children residing in holoendemic P. falciparum transmission regions is largely undefined. We hypothesised that the CD14 (-159C/T) promoter polymorphisms could be associated with MA. The current study therefore investigated the association between CD14 (-159C/T); rs2569190) and MA in parasitemic children (n=441, aged 3-36 months) presenting with malaria at a rural hospital in western Kenya. In all study participants, we determined both haematological and parasitological profiles. TaqMan 5’ allelic discrimination assay was used to determine the genotypic profiles of CD14 (-159C/T). The genotypic distributions in MA were 29.4%TT, 38.7%CT and 31.9%CC while in non-MA, it was 23.7%TT, 34.7%CT and 41.6%CC. Multivariate logistic regression analysis, controlling for co-variates revealed that relative to homozygosity at position -159 (-159CC), carriage of the TT genotype was associated with increased risk of developing MA (OR, 1.758; 95% CI, 1.007-3.069; P=0.047). Thus, CD14 -159C/T appears to be a genetic marker for increased susceptibility to MA in pediatric population resident in P. falciparum holoendemic transmission region of western Kenya.

P481: Sterile Insect Technique: field feasibility study site selection, species abundance and monthly distribution of Anopheles mosquitoes in northern Kruger National Park, South Africa

Givemore Munhenga1,2, Basil D Brooke1,2, Bellinda L Spillings1,2, Leyya Essop2,3, Richard H Hunt1,2, Stephen Midzi2, Danny Govender2,3 and Lizette L Koekemoer1,2,3

1Vector Control Reference Laboratory, National Institute for Communicable Diseases, Centre for Tropical, Opportunistic and Hospital Infections of the National Health Laboratory Service, Private Bag X4, Sandringham, Johannesburg 2131, South Africa. 2Wits Research Institute for Malaria, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa. 3Shangoni Section, Kruger National Park, Private Bag X402, Skukuza, 1350, South Africa. 4Scientific Services, South African National Parks, Private Bag X402, Skukuza, 1350, South Africa. 5Department of Parasitological Sciences, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110, South Africa.

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BACKGROUND: A comprehensive knowledge of the ecology and behavior of a target pest or disease vectors is a prerequisite for the successful development of a control strategy using sterile insect technique (SIT). Before implementation of an SIT programme for mosquito control it is essential to compile comprehensive information on mosquito bionomics in the targeted area. The aims of this study were to assess the relative anopheline species abundance at five sites in northern Kruger National Park, assess the impact of weather conditions on An. arabiensis population there and to establish the most suitable collection methods to use for future SIT activities in the area.

METHODS: A survey of Anopheles species was made between July 2010 and December 2012. Mosquitoes were collected from five sites within the Nkxanatseni region, Kruger National Park. Methods included carbon dioxide-baited traps, human landing catches and larval collections. Collected specimens were identified to species level using morphological characteristics and polymerase chain reaction assays.

RESULTS: A total of 3,311 specimens belonging to eight taxa were collected. Species collected were: Anopheles arabiensis (n=1,352), An. quadriannulatus (n=870), An. coustani (n=395), An. merus (n=349), An. pretoriensis (n=35), An. maculipalpis (n=28), An. rivulorum (n=19), An. squamosus (n=3) and An. rufipes (n=2). Members of the An. gambiae species complex comprised 74.7% of the collection and were the most abundant and widely distributed, occurring across all collection sites. The highest number of mosquitoes was collected using CO2 bait nets (58.2%) followed by human landing catches (24.8%) and larval collections (17%). Mosquito sampling productivity was influenced by weather conditions especially wind speed. The overall population densities fluctuated according to seasons.

CONCLUSIONS: Anopheline species occur in northern regions of the Kruger National Park and their population densities fluctuate between seasons. Species abundance and relative proportions within the An. gambiae complex samples varied between collection methods. There is a perennial presence of an isolated population of An. arabiensis at the Mahalalapanga site which declines in density during the dry winter months, making this site suitable for assessing the feasibility of SIT as an option for malaria vector control.

P482: Insecticide resistance monitoring in malaria vectors in Kilifi county, along the coastal Kenya.

Daniel Munyvoki1,2, Joseph Mwangangi3,1, Elizabeth Kokwaro1, Charles Mbogo1,2
1Kenya Medical Research Institute, Center for Geographic Medicine Research Coast, 2Kenya University, Department of Zoological Sciences, 3Malaria Public Health Group, KEMRI-University of Oxford-Wellcome Trust Programme

BACKGROUND: Vector control is an effective way of reducing malaria transmission. Control programmes in sub-Saharan Africa continue to rely heavily on indoor residual spraying (IRS) or insecticide-treated nets (ITNs), both of which depend on vector susceptibility to the insecticides used. The main vector control method along the coastal strip of Kenya is the use of long-lasting insecticide nets (LLINs). Unfortunately, the high level of pyrethroid resistance in Anopheles gambiae sensu lato (s.l.) is a threat to the success of pyrethroid treated nets. The status of resistance was investigated in Anopheles gambiae sensu lato and An. funestus (Diptera: Culicidae) mosquitoes from Kilifi county.

METHODOLOGY: Anopheles larvae were collected from five sites in Kilifi County. The mosquitoes were reared to adulthood in the insectary and the use of long-lasting insecticide nets (LLINs) was tested. Similarly mosquitoes from Ng’ombeni and Kidutani showed 100% mortality 24 h post-exposure for DDT and deltamethrin. However, deltamethrin resistance was found in Anopheles gambiae s.l. from Burangi where the 24 hours post exposure mortality was <80% but 100% for DDT, bendiocarb and fenithrothion bioassays.

CONCLUSIONS: The current study of insecticide resistance in Anopheles provides baseline information essential for monitoring the development of insecticide resistance in Kenya. Resistant management strategies need to be considered in order to contain the spread of the resistant populations.

P483: The efficacy and residual properties of crude botanical extracts against malaria vectors along the Kenyan Coast

Simon Muruli1,2, Salim Rhamadhan1, Joseph Mwangangi1 and Charles Mbogo1,2
1Pwani University, Kilifi, Kenya, 2Kenya Medical Research Institute/Wellcome Trust Research Programme, Kilifi, Kenya

BACKGROUND: Plant-derived natural products have been widely used in most cultures and communities around the world for medicinal purposes including the management of malaria. Current efforts in mosquito management practices are geared towards finding highly effective, low cost and ecologically friendly control tools that would be readily accepted by local communities. The objective of the study was to evaluate the efficacy and residual properties of crude botanical extracts against malaria vectors in semi-field mosquito environments.

METHODS: Laboratory and simulated field experiments were conducted in Kilifi, Coastal Kenya to test the effectiveness of crude plant extracts derived from neem tree (Azadirachta indica) leaf powder, sawdust, neem oil and a vegetable oil derivative (Larvex®). The efficacy was evaluated against mosquito larvae at varying concentrations and compared with that of Bacillus thuringiensis var. israelensis, (Vectobac®) corn-granulaires). Effective larvicides were tested for their residual properties under field conditions according to WHOPE's guidelines.

RESULTS: Neem leaf powder, neem oil and the vegetable oil derivative were the most efficacious larvicides with mortality rate of 80-100% within 3-days although Vectobac® resulted in total larval mortality within 24hrs. Neem sawdust was slow acting and resulted in stunted larval growth. Neem leaf powder persisted in the larval habitats for a fortnight while oil products (neem oil and Larvex®) remained effective up to 8-days post-treatment.

DISCUSSION AND CONCLUSION: Neem tree crude extracts are effective against mosquito larvae with leaf powder showing high efficacy rates and residual properties while neem sawdust causes delayed larval development. Larvex® showed similar results as neem oil with high mortality rates but with lower residual effects than neem leaf powder but comparable to those of Vectobac®. Thus, plant derived crude extracts have a role in the management of malaria vectors in tropical Africa and should be evaluated further for possible integration in vector management strategies in Kenya.

P484: Antibody responses against Plasmodium falciparum merozoite antigens and risk of severe malaria: - A matched case-control study of Kenyan children

L.M. Murungi1,2, D.Llewellyn1, A.R. Williams1, E.Ogada1, K.Lundblom2, J.Rono1,3, A.Farnert1, J.A.G.Scott1, K.Marsh3, S.J.Draper2, F.H.A.Osier4
1KEMRI–Wellcome Trust, Kilifi,Kenya; 2The Jenner Institute,Oxford University,UK; 3Karolinska Institute,Sweden

In malaria endemic areas severe malaria (SM) is rarely observed in children over 5 yrs, indicating that children acquire immunity. While antibodies are central in mediating protection, the putative targets
remain unclear. Cohort studies have been widely used to help identify protective targets but these have focused on uncomplicated malaria, to which immunity is acquired later in early adulthood. Furthermore, few studies have investigated antibody-mediated mechanisms that confer immunity to SM. We aimed to identify merozoite targets and potential antibody-mediated mechanisms associated with protection from SM. In a matched case-control study, nested within a longitudinal birth cohort of children recruited and followed up every three months, serum samples were obtained from 59 children aged 1-28 months who subsequently developed SM. Each index case was matched to 3 controls who did not develop SM during follow-up (n=141). We measured total IgG responses to AMA1, MSP-2, MSP-3, MSP-143, PRR2 and schizont extract, in vitro growth inhibition activity (GIA) and antibody-dependent respiratory burst (ADR) in the serum sample preceding the disease episode for cases and corresponding controls. Only anti-AMA1 antibodies were associated with protection against SM (OR 0.38; 95%CI 0.16-0.90; p=0.03). GIA and ADR were not correlated in both cases and controls. Neither ADR nor GIA was individually associated with protection against SM. However, children with high levels of both GIA and ADR were at a reduced risk of developing SM (OR 0.07; 95%CI 0.006-0.82; p=0.03) compared to children with low levels of both. Our findings further support the evaluation of AMA1 as a potential vaccine candidate. Antibodies tested in both functional assays appeared to be protective only in combination suggesting that multiple effector mechanisms contribute to immunity against SM.

P485: Entomological Investigation for Malaria vector Control in semi arid Borno State Northeastern Nigeria.

Samdi LM 1,2,3, Oduola D 4, Ologede J 2, Amajoh N 1, Iyanya P U 3, Awolola TA 1
1 University of Jos Department of Zoology; 2 Abt - USAID/PMI AIRS Project Lafia Nigeria; 3 Nigerian Institute for Medical Research Lagos

BACKGROUND: Although the Federal Ministry of Health estimates that Northeastern Nigeria has the highest malaria prevalence in pregnancy of 64.5% not much is known about the Anopheles mosquitoes of this region.

OBJECTIVE: To provide baseline entomological data on species composition, host preference, Human Blood Index (HBI), infectivity, Entomological Inoculation Rates (EIR), Man Biting Rate (MBR) and insecticide susceptibility status for malaria vector control.

METHODS: Mosquitoes collections were made by pyrethrum spray collection and identified based on morphology and by PCR. Blood meal preference and sporozoite rate(s) were determined by ELISA. Man biting rate (ma) and Entomological Inoculation rate (EIR) were calculated. Susceptibility to 0.1%, Bendiocarb 0.05%, Deltamethrin and 5% Malathion were also determined.

RESULTS: A total of 1030 female Anopheles mosquitoes were identified, namely, 1026 (99.6%) of Anopheles gambiae complex PCR showed An. arabiensis Patton was (95%) and An. gambiae s.s. (5%). Anopheles mosquitoes morphologically identified were An. pharaonis, An. squamosus and An. rhodesiense. An. arabiensis was higher than Anopheles gambiae s.s. (P<0.05). The P.falciparum sporozoite rate (s) was 2.4% and all were An.arabiensis. Indoor collection was higher than the outdoor collection (P<0.01). Human blood index was 0.98. for indoor collections and 0.02 for outdoors (P>0.01). Man Biting Rate (ma) was 2.49 bites per night and EIR 5.6 infective bites/person/month. 24h post exposure mortality indicated 100% susceptibility to 0.1%, Bendiocarb 0.05% Deltamethrin and 5 % Malathion and a resistance of 72.5% to 4% DDT.

CONCLUSIONS: An. arabiensis is the most efficient malaria vector mosquito species and is largely endophilic and anthropophilic. Integrated Vector Management (IVM) is advocated including scaling up Indoor Residual Spraying (IRS) as a complementary strategy to the use of ITNs.

P486: Overview of insecticide resistance patterns in Zambia and the potential impact on malaria vector interventions

Mulenga Musapa1, Lucy Muziwa1, Chadwick Sikala2
1 Zambia Integrated Systems Strengthening Program, Lusaka, Zambia; 2 National Malaria Control Centre, Lusaka, Zambia

BACKGROUND: In Zambia, a major insecticide treated net (ITN) distribution campaign was initiated in 1999 and indoor residual spraying (IRS) with DDT or pyrethroids was reintroduced in 2000. Prior to the IRS program or scaling of ITNs, there was no evidence of resistance to insecticides in malaria vectors. However, entomology data collected in 2010 from a transect through Copperbelt Province, where DDT was being used for IRS showed high levels of pyrethroids and DDT resistance. This observation prompted the National Malaria Control Program to expand insecticide resistance surveillance. The objective of this review was to assess insecticide resistance patterns in Zambia and how this would influence vector control decisions.

METHOD: Indoor resting mosquitoes were captured monthly by aspiration from six sentinel sites and once during the rainy season from 104 non-sentinel sites covering four ecological zones based on rainfall patterns.

- The vector mosquitoes were tested for insecticide resistance following WHO tube assay. F0 that laid eggs and insecticide resistant F1 were preserved in RNA later for determination of resistance mechanisms.

RESULTS: DDT resistance was detected in An. gambiae (20-50% mortality) while An. funestus were fully susceptible. The resistance to pyrethroids was detected in both Anopheles gambiae s.s. and An. funestus s.s (15-70% mortality). Within the pyrethroid class, resistance frequency was markedly diverse. All vector species tested were fully susceptible to bendiocarb, malathion and pirimiphos methyl. Detection of kdr mutation found only kdr (L1014F) in populations of An. gambiae s.s. s-form. A number of detoxification genes were found significant but diversely elevated in An. gambiae including P450s known to confer metabolic resistance to pyrethroids and DDT.

CONCLUSION: The sympatric existence of vector species with different resistance profiles to DDT and pyrethroids excludes their use in Zambia. More expensive insecticides using pirimiphos methyl and/or bendiocarb may be a viable option to maximum efficacy and limit evolution of resistance against pyrethroids and DDT. Future return to use of pyrethroids would be economically attractive if the shift leads to reversal of vector susceptibility to pyrethroids. Resistance surveillance needs to be expanded since the complex nature of resistance distribution requires a more granular and timely approach.

P487: Natural Aromatic Acids as New inhibitors of plasmodium falciparum Enoyl-ACP Reductase

Suyan Awadelkarim1, Sumaira Hareem2, Asaad Khalid2, M. Galal 2, Sean T. Prigge1 and M. Iqbal Choudhary2
1 Medical and Aromatic Plants Research Institute, National Center for Research, Khartoum, Sudan, P. O Box 2404, Khartoum, Sudan; 2 Health Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi; Karachi 75270, Pakistan

BACKGROUND: Malaria accounts for nearly 250 million clinical cases resulting in about 1 million deaths every year mostly children below the age of 5 in sub-Saharan Africa. The spread of resistance to the existing drugs such as chloroquine and the development of an alarming signs of resistance to the current malarial front line drug artemisinin have
created an urgent demand for new antimalarial agents. *P. falciparum* enoyl-ACP reductase (PfENR) which catalyzes the rate-limiting step of the fatty acid pathway (FAS) has been identified and validated as a potential antimalarial drug target especially during the liver stage of the parasite life cycle. The goal of the current study was to screen compound library of natural products for PfENR inhibitory activities.

**METHODS:** A modified in vitro enzyme inhibition assay was used in this study. All experiments were conducted in 96-well microtiter plates using SpectraMax microplate spectrophotometers. Compounds were tested in 100 µL reaction by following the oxidation of NADH to NAD⁺ for 10 min at 340 nm. Average kinetic rate of change in absorbance was calculated by Softmax PRO software ( Molecular Devices, Sunnyvale, CA) for test and control. Accordingly, the percent by which each compound inhibited the enzyme activity (% inhibition) was measured. Compounds which inhibited the PfENR by 50% and more were selected for IC₅₀ determination. Kinetic studies were conducted on the most active compounds to determine their Ki values (inhibitor dissociation constant) and to identify their inhibition mechanism. Molecular docking simulations were performed using Surflex-Dock software with aim of investigating ligand-protein interactions.

**RESULTS:** Two aromatic acids, (AAD226) and rosamicin acid (S10) isolated from *Lichen cladonia* and *Melissa officinalis* (Lemon balm), have been identified as PfENR inhibitors with IC₅₀ values of 33.4 µM and 40.2 µM respectively. In vitro kinetic studies and molecular docking simulations highlighted both compounds as competitive inhibitors with regard to the enzyme cofactor, NADH and a noncompetitive PfENR inhibitor with regard to the substrate, crotonyl CoA.

**CONCLUSION:** Due to the few number of natural compounds identified as PfENR inhibitors, our identified PfENR inhibitors could have potential for further development as new antimalarial leads.

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**P488: The influence of sprayable surfaces on the effectiveness of indoor residual spraying using Lambda-cyhalothrin against malaria vectors in Zanzibar and Mainland Tanzania**

Joshua Mutagagwya¹,², Harish B Pratap¹, Fabrizio Molteni³, Francis Mugurula¹, Jeremiah Ngondi¹, Stephen Magesa¹, Mohammed Ramsan¹, Jessica M. Kafuko¹, Elias Nyanza³, Osia Mwaiapwe³, Charles D, Mwalim¹, Jasper N. Ijumba³

¹RTI International, Dar es Salaam, Tanzania; ²Department of Zoology and Wildlife conservation of the University of Dar es Salaam Tanzania; ³Swiss Tropical and Public Health Institute, Dar es Salaam, Tanzania; ¹USAID/PMI Dar es Salaam, Tanzania; ²School of Public Health, Catholic University of Health and Allied Sciences, Bugando, Mwanza Tanzania; ³National Malaria Control Program - Tanzania; ⁴Nelson Mandela African Institute of Science and Technology, Arusha, Tanzania

**BACKGROUND:** The type of sprayable surface impacts on the residual efficacy of insecticide used in indoor residual spraying (IRS). However, there is limited data on common types of wall surfaces sprayed in Zanzibar and Mainland Tanzania. The study investigated residual efficacy of lambda-cyhalothrin sprayed on common surfaces in Zanzibar and Mainland Tanzania. Surfaces included cement plastered, mud-daub, white-wash, wood, palm-thatch, iron-sheet and burnt-bricks, limestone and oil-painted. Using WHO standards operating procedures for IRS, the study surfaces were sprayed with lambda-cyhalothrin at the dose of 20–25mg/m². Residual efficacy of the insecticide was monitored through cone bioassay using laboratory reared mosquitoes Kismu strain (R – 70) of *An. gambiae* ss. Cone bioassay was done every fortnight throughout 152 days using a total of 20–25 mosquitoes per cone. The World Health Organization Pesticide Evaluation Scheme (WHOPES) threshold (70% mortality) was set as cut point for acceptable residual efficacy.

**RESULTS:** A total of 5,000 mosquitoes were subjected to test residual efficacy of lambda-cyhalothrin. There was a statistically significant variation in residual efficacy between the different types of wall surfaces (F(=0.24; p-value=0.001). Residual efficacy decreased with increasing pH of the substrate (R²=0.2; p-value=0.027). Based on WHOPES standards, shorter residual efficacy (56–77 days) was found in wall substrates made of cement, limestone, mud-daub, oil paint and white wash. Walls made of burnt bricks; iron sheet, palm thatch and wood retained the recommended residual efficacy beyond 152 days.

**CONCLUSION:** The study found a wide variation in residual efficacy of micro encapsulated formulation of lambda-cyhalothrin across the types of wall surfaces studied. In areas where malaria transmission is bimodal and wall surfaces of short residual efficacy (2 – 3 months) composed > 20% of sprayable structure, two rounds of IRS using lambda-cyhalothrin should be considered. Further studies are required to investigate the impact of sprayable surfaces on residual efficacy of other insecticides commonly used for IRS in Zanzibar and Mainland Tanzania.

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**P489: Plasmodium falciparum antigenic variation: relationships between widespread endothelial activation, parasite PfEMP1 expression and severe malaria**

Abdirahman I. Abdi¹,², Greg Fegan¹,², Michelle Muthui³, Esther Kiragu³, Jennifer N. Musyoki¹, Michael Opiyo¹, Kevin Marsh³,⁴, George Warimwe³,⁴, and Peter C. Bull¹,²

¹KEMRI-Wellcome Trust Research Programme, P.O. Box 230-80108, Kilifi, Kenya; ²The Jenner Institute, University of Oxford, ORCRB, Roosevelt Drive, Oxford, UK; ³Nuffield Department of Clinical Medicine, John Radcliffe Hospital, University of Oxford, Oxford, UK; ⁴WHO/N Curtis, WHO/N Curtis, WHO/N Curtis

**BACKGROUND:** *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1) is a family of variant antigens thought to play a central role in the pathogenesis of severe malaria. These molecules are inserted into the surface of parasite infected erythrocytes (IE) and mediate the IE sequestration in host tissues through adherence to the capillary endothelial cells. Opinion is divided over the precise role of sequestration in the widespread endothelial activation associated with severe malaria and in turn the role of this endothelial activation in the extensive brain sequestration associated with cerebral malaria. Here, we explored the role of PfEMP1 subgroup expression in the widespread endothelial activation associated with severe malaria.

**METHODS:** We measured plasma Angiopoietin-2 (Ang-2) levels by ELISA; a marker of widespread endothelial activation, from children with severe and non-severe malaria and used logistic regression analysis to relate Ang-2, group A-like PfEMP1 expression and a common IE adhesion phenotype “rosetting”, to two major manifestations of severe malaria, impaired consciousness and respiratory distress.

**RESULTS:** Plasma Ang-2 levels were associated with both impaired consciousness and respiratory distress whilst rosetting did not show an independent association with severe malaria when adjusted for Ang-2 levels, group A-like var expression maintained an independent association with impaired consciousness.

**CONCLUSIONS:** The results suggest that Parasites expressing group A-like PfEMP1 may cause impaired consciousness in the absence of widespread endothelial activation. We suggest that either parasites expressing group A-like PfEMP1 can adhere to non-activated brain endothelial cells, or cause localized endothelial activation that is not detectable using the plasma Ang-2 assay.
P490: Study of the physicochemical variables of the water of larvae shelters associated with the resistance of Anopheles Gambiae S.L. to deltamethrine in the North Cameroon

N Mvondo
University Of Yaoundé

The impact of anthropological activities on the ecology of the larvae of anophelines and the consequences on the development of their resistance in insecticides used in public health present a particular interest for the fight against malaria. To identify the physicochemical factors of larval shelters susceptible to influence the sensibility of Anopheles gambiae s.l. to deltamethrine in the North Cameroon, a study took place during period from October 09th till November 10th, 2012.

Young larvae (L1) of anophelines were collected in shelters and put in breeding. Larvae stemming from every shelter were distributed in two prizes: those of the first prize were raised in the spring water and those of the second prize in the water of their shelter of origin. The adults stemming from larvae were submitted to the tests of sensibility to deltaméthrine 0,05 %, according to the protocol of the WHO (1998). The mortality rates of mosquitoes were registered 24 hours after exposure, and then compared between both prizes of mosquitoes by the test of Student. Besides, physicochemical analyses of the water of every shelter and some spring water were realized according to the recommendations of Rodier and al (2009).

On a total of 22 tests of sensibility realized with 2200 specimens of An. gambiae s.l., the analysis of the results showed one significant increase of the resistance to deltaméthrine in 10/11 larvae’s samples raised in the water of shelters (23-83% of mortality), compared with those raised in the spring water (42-95% of mortality) (p = 0,05). The analysis of the physicochemical showed a simultaneous association of several variables in this increase of the resistance. It is among others about conductivity (spring water = 2,6µS.cm⁻¹; water of shelters = 311,4±125,83 µS.cm⁻¹); bromine (spring water = 0,02mg.L⁻¹; water of shelters = 0,50±0,63 mg.L⁻¹) and ammoniacal nitrogen (spring water = 0,02mg.L⁻¹; water of shelters = 5,014±3,85 mg.L⁻¹).

The results of this study could allow improving the fight against the vectors of malaria in the North Cameroon through awareness campaigns on a management directed well and coordinated of environment.

P491: Surfacing malaria hazard hotspots using local hydrology and topography to sustain detection of transmission residuals in highly urbanizing cities in Tanzania

Victoria Mwakalinga1,2, Benn Sartorius1, Alex J Limwagw1, John Paliga3, Prosper P Chako1, Alpha D Malishee2,3, Maureen Coetzee1, Gerry Killeen4, and Stefan Dongus1

1School of Public health, Faculty of Health Sciences, University of the Witwatersrand 1 Jan Smuts Ave, Johannesburg 2001, South Africa; 2Environmental Health and Ecological Sciences Thematic Group, Ifakara Health Institute, Coordination Office, Kika Avenue, Mikocheni, PO Box 78373, Dar es Salaam, United Republic of Tanzania; 3College of Information and Communication Technologies, University of Dar es Salaam, PO Box 35194, Dar es Salaam, Tanzania. 4Liverpool School of Tropical Medicine, Vector Biology Department, Pembroke Place, Liverpool L3 9QA, United Kingdom

BACKGROUND: To utilize potentials of urbanization in malaria control, identification of fine-scales malaria transmission hazard hotspots is highly needed. Identification of these hotspots can enable detection of malaria transmission residuals which have been hindering elimination strategies in many countries. This study provides the surface of high Anopheles densities and high positivity rates as indicators of malaria hazard hotspots using local hydrology and topography at a very fine spatial scale i.e. Ten Cell Unit (TCU), a cluster of at least 10 – 20 houses; that will influence spatially targeted interventions in highly urbanizing city and low transmission settings.

METHODS: Basic Terrain Analysis tool in SAGA GIS was used to model hill shading, sink routes, height above channel, channel network, convergence index, elevation, slope and aspect. The topographic wetness index (TWI) was developed using TOPMODEL method and the topographic position index (TPI) was developed using Terrain Analysis- Morphometry; both in SAGA GIS. Using exploratory regression in ArcGIS, important hydrological and topographical variables that explain presence of mosquitoes and high malaria positivity rates were identified by fitting Ordinary Least Square (OLS) Regression. Candidate variables were further fitted in a Geographically Regression Model (GWR) to capture non-stationarity effect and also to lead the selection of suitable interventions in areas where a certain variable affect presence of mosquitoes strongly than others.

RESULTS: Preliminary results from OLS regression show that TWI is a very strong predictor of areas with high mosquito densities and high malaria positivity rates. Comparing to other variables it comprised 83% of significant tests in the exploratory regression analysis. Moreover in a GWR it appears to have strong association with identified hotspots of both anophenes densities and malaria positivity rates.

CONCLUSION: The strong association observed between TWI and mosquitoes as well as positivity rates, can significantly contribute to malaria elimination; if the identified surface is spatially targeted and suitable type of interventions identified to meet the nature of the relationship.

P492: Perceptions of mothers and hospital staff of paediatric care in 13 government hospitals in northern Tanzania

Rose Mwango, Clare Chandler, Anja Poulsen · Fortunata Nasuwa, Hilda Mbakiliwa, Ib Christian Bygbjerg, Hugh Reyburn

BACKGROUND: User and provider perceptions of quality care are likely to affect both use and provision of services. However, little is known about how health workers and mothers perceive the delivery of care in hospital paediatric wards in Africa.

METHODS: Paediatric staff and mothers of paediatric patients were interviewed to explore their opinions and experience of the admission process and conditions on the ward.

RESULTS: Overcrowding, unsanitary conditions and lack of food were major concerns for mothers on the ward. Fears that hospital admission posed a significant risk of exposure to infection deterred mothers from seeking treatment earlier. While most staff were seen as being sympathetic and supportive to mothers, a minority were reported to be judgemental and authoritarian. Health workers identified lack of trained staff, overwork and low pay as major concerns.

CONCLUSIONS: Staff shortages, lack of effective training and equipment are established problems but our findings also highlight a need for wards to become more parent-friendly, particularly with regard to food, hygiene and space. Training programmes focused on professional conduct and an awareness of the problems that mothers face in seeking and receiving care may result in a more supportive and cooperative attitude between staff and mothers.

P493: Using community knowledge and experiences to predict densities and distribution of disease-transmitting mosquitoes in rural Tanzania

Stephen Mwangungulu

BACKGROUND: The current lack of reliable techniques that can be used for large scale programmatic monitoring of distribution and densities of disease transmitting mosquitoes is a major challenge to public health authorities, especially in low and middle income endemic countries. We
P494: Genetic diversity of Plasmodium falciparum malaria in Mpunge

Sydney Mwanza; Justin Chileshe; Ng’andwe Kalungwana and Eric. Njunju

INTRODUCTION: Malaria control remains a challenge in Zambia with drug resistance worsening problems of infection with multiple parasite strains. Multiple strain infections play important role in strain specific immunity development. Understanding Genetic diversity in Plasmodium falciparum is important in vaccine development

OBJECTIVES: To determine the genetic diversity of plasmodium falciparum using Merozoite Surface Protein 1 (MSP1), Merozoite Surface Protein 2 (MSP2) and Glutamate Rich Protein (GLURP) and assess the multiplicity of infection

METHODS: Blood spot samples were collected from microscopy positive malaria <5 patients from where DNA was extracted. Oligonucleotide primers designed from published sequences were, to amplify block 2 of MSP-1 and block 3 of MSP-2 and GLURP by nested PCR, each amplification with conserved or family-specific primer pair, being done separately. CR products were viewed on agarose gels and fragments obtained compared by size with reference to DNA ladder

RESULTS: From the 81 patient isolates were analysed, under the MSP1 locus, 62 (76.5%) were of the K1 and 50.6% MAD20 family where as under the MSP2, 307 gave 77.8% and the FC27 65.4%. GLURP loci was found in 79% of the isolates. The proportion of patient isolates with mixed infections or samples having at least two strains of parasites was found to be 98.7%

CONCLUSION: The Study highlighted a problem of high Plasmodium falciparum allelic variation as we deal with children under 5, the most vulnerable population, whose immunity is yet to develop and gave useful baseline data for consideration testing malaria vaccines in Zambia

P495: High levels of pyrethroid resistant KDR allele 11014s in An. funestus population in Gembe location, Suba district, Nyanza province, Kenya

Cassian Mwatele1, Dunstan Mukoko2, Charles Wondji3, Gabriel Dida1, George Sonye1, Sammy Njenga1, Charles Mwandawiro1, Hitoshi Kawada1

1Eastern and Southern Africa Centre of International Parasite Control, Kenya Medical Research Institute, Nairobi, Kenya; 2Division of Vector Borne and Neglected Tropical Diseases, Ministry of Public Health & Sanitation, Nairobi, Kenya; 3Vector Group, Liverpool School of Tropical Medicine, Liverpool, United Kingdom

BACKGROUND: Anopheles funestus is more efficient in contracting and transmitting malaria. Although it is difficult to rear, it is important to analyze field populations for pyrethroid insecticide resistance in Kenya. In Suba district Kenya, Malaria is endemic and prevalence is as high as 40 %. In this study, we carried out small-scale field collections and rearing of An. funestus in Gembe location. The aim was to determine the pyrethroid insecticide resistance of wild caught populations in Suba District.

METHODOLOGY: Indoor-resting mosquitoes were sampled in five villages using aspiration and spray catches techniques. In the laboratories, blood fed, Hg and Gravid mosquitoes were put in individual egg laying cups to oviposit; after which egg batches were hatched and reared in the insectary. The WHO susceptibility assays were unsuccessful due to difficulty in rearing the F1. However, DNA extraction, PCR to separate An. funestus was done

PRINCIPAL FINDINGS: Out of 910 indoor-resting mosquitoes, 445 were An. funestus. Sequencing for Kdr gene-T1A (L1014S) expressed high levels (60-90%) of mutations; indicating remarkably high pyrethroid insecticide resistance in An. funestus population in Gembe location. The GPS distribution pattern for Kdr gene frequencies observed, were suggestive of selection pressure to pyrethroid insecticides (Deltamethrin) widely used in LLINs and IRS.

Conclusion/significance: The high levels of pyrethroid resistance observed in Gembe location, MBita division are not only alarming, but a big challenge for resistance management strategies such as insecticide rotation in Gembe location, western Kenya. The selection pressure to pyrethroid insecticides complements the high malaria prevalence observed in this area.

P496: Long-lasting activity of odour-dispensing nylon strips on attraction of mosquitoes: potential role of microbes


The need for robustness underpins the deployment of odour-baited technologies for routine sampling, surveillance and control of malaria vectors. We explored the residual activity of a synthetic blend of attractive chemicals to host-seeking Anopheles gambiae Giles sensu stricto once in a week over 52 nights post-treatment. The chemicals were released from either nylon strips or low density polyethylene (LDPE) sachets. Additionally, the possibility of microbial action in modulating mosquito responses to attractive chemicals impregnated on nylon strips was also investigated. Behavioural assays were evaluated through randomized 4 x 4 Latin Square and dual-choice experimental designs. Untreated and attractant-containing nylon strips and LDPE sachets were re-used throughout the study. However, LDPE sachets were replaced upon depletion of individual attractant compounds. Attractant-treated nylon strips were consistently more attractive to An. gambiae mosquitoes than LDPE sachets over time. After one year of intermittent exposure, additional
volatile organic compounds and different bacterial populations were found on attractant-treated nylon strips. The responses of host-seeking An. gambiae, An. gambiae s.s., and Mansonia spp. to baited traps was not influenced by autoclaving attractant-treated strips prior to the start of experiments. More female An. funestus and other anopheline mosquitoes were attracted to autoclaved than to non-autoclaved attractant-treated nylon strips. By contrast, more female Culex mosquitoes were attracted to non-autoclaved compared to autoclaved nylon strips that had been impregnated with attractants. Female An. gambiae s.s. and An. funestus trapped were predominantly unfed.

We conclude that nylon strips attracted An. gambiae up to one year post-treatment due to the residual activity of attractant chemicals applied on them. The volatile compounds derived from microbes that colonized nylon strips over post-treatment period may have interacted with odorants previously applied on them, thereby influencing the suitability and attractiveness of treated nylon strips to host-seeking mosquitoes. Autoclaving contributes to differential attractiveness of treated nylon strips to malaria and other mosquito vectors.

**P497: A countrywide cross-sectional survey of the prevalence of asymptomatic Plasmodium falciparum infection in The Gambia**

**Julia Mwesigwa, Joseph Okebe, Muna Affara, Davis Nwakanma Davis, Jallow Haddiyatou, Prom Aurelia, Ngwa Alfred Amambua, Umberto D’Alessandro**

**Medical Research Council Unit, The Gambia**

**BACKGROUND:** Over the last few years, several malaria indicators have decreased substantially in The Gambia, to the extent that attainment of the pre-elimination status is targeted for 2015. Nevertheless, asymptomatic Plasmodium falciparum infections can be an important obstacle towards this goal as asymptomatic carriers can maintain transmission.

**OBJECTIVE:** Determine the prevalence of asymptomatic Plasmodium falciparum infections among communities in the Gambia and describe its heterogeneity.

**METHODOLOGY:** A cross-sectional survey was conducted in 24 villages in 4 regions towards the end (November) of the 2012 transmission season. About 350 individuals in each village were sampled; in villages with less than 300 inhabitants, the whole population was included. A blood sample for microscopy, later molecular analysis and haemoglobin was collected by finger prick. Additional information on bed net use and previous treatment was also collected. Species-specific PCR was performed to determine the prevalence of *P. falciparum* infection in each village.

**RESULTS:** We screened 6127 individuals; 23.2% of them were <5 years old and the mean age was 19.6 years (SD 19.7). Malaria prevalence was 4.48% (range 0.57% - 13.91%) with marked heterogeneity between villages. Most people (92.5%) owned a bed net. Mean haemoglobin was 12.03g/dl and anaemia (Hb< 8.0g/dl) prevalence was 2.2%. Children 10-14 years old had a significantly higher risk of infection.

**CONCLUSIONS:** Despite high coverage of bed nets and use of indoor residual spraying, the prevalence of malaria infection is still substantial in these study communities. Asymptomatic carriers represent an important reservoir and any intervention aiming at interrupting transmission should target this important but hidden group.

**P498: Travel history and malaria infection risk in a low-transmission setting in Ethiopia: a case control study**

**Honelgn Nahusenay**

**BACKGROUND:** Malaria remains the leading communicable disease in Ethiopia, with around one million clinical cases of malaria reported annually. The country currently has plans for elimination for specific geographic areas of the country. Human movement may lead to the maintenance of reservoirs of infection, complicating attempts to eliminate malaria.

**METHODS:** An unmatched case–control study was conducted with 560 adult patients at a Health Centre in central Ethiopia. Patients who received a malaria test were interviewed regarding their recent travel histories. Bivariate and multivariate analyses were conducted to determine if reported travel outside of the home village within the last month was related to malaria infection status.

**RESULTS:** After adjusting for several known confounding factors, travel away from the home village in the last 30 days was a statistically significant risk factor for infection with *Plasmodium falciparum* (AOR 1.76; p=0.03) but not for infection with *Plasmodium vivax* (AOR 1.17; p=0.62). Male sex was strongly associated with any malaria infection (AOR 2.00; p=0.001).

**CONCLUSIONS:** Given the importance of identifying reservoir infections, consideration of human movement patterns should factor into decisions regarding elimination and disease prevention, especially when targeted areas are limited to regions within a country.

**P499: Impaired phospholipid synthesis contributes to the reduced intra-erythrocytic growth of glycerol kinase knockout Plasmodium falciparum parasites.**

**Kubendran Naidoo and Theresa L. Coetzee**

**Wits Research Institute for Malaria (WRIM), Department of Molecular Medicine and Haematology, Faculty of Heath Sciences, School of Pathology, University of the Witwatersrand, National Health Laboratory Service, 7 York Road, Parktown, 2193, Johannesburg, South Africa.**

**BACKGROUND:** Malaria is a devastating disease and *Plasmodium falciparum* is the most lethal parasite infecting humans. Understanding the biology of the parasite is vital in identifying potential drug targets necessary to develop novel treatments to combat the disease. During every 48-hour intra-erythrocytic asexual replication cycle, a single parasite can produce up to 32 progeny. This extensive proliferation implies that parasites require substantial amounts of lipid precursors for membrane biogenesis. Enzymes involved in parasite metabolism and phospholipid synthesis have long been considered attractive drug targets. Glycerol kinase is a highly conserved enzyme that functions at the interface of lipid synthesis and carbohydrate metabolism. *P. falciparum* glycerol kinase catalyzes the ATP-dependent phosphorylation of glycerol to glycerol-3-phosphate, a major phospholipid precursor.

**METHODS:** The *P. falciparum* glycerol kinase gene (PlasmoDB ID: PF3D7_1351600) was disrupted using double crossover homologous DNA recombination to generate a 3D7ΔPgfK knockout parasite line. Southern hybridization and mRNA analysis were used to verify gene disruption. The growth of highly synchronized ring stage parasites was monitored over one 48-hour intra-erythrocytic development cycle using
thiazole orange, a DNA staining dye, coupled to flow cytometry analysis for improved sensitivity. Radiolabelling studies were used to assess incorporation of glycerol into late stage parasite phospholipids. **RESULTS:** Disruption of the P. falciparum glycerol kinase gene produced viable parasites, but their growth was significantly reduced to 56.5±8% when compared to wild type parasites and a 3D7ΔEBA control line, in which one of the invasion proteins, EBA-175, had been disrupted. *1-C*-glycerol incorporation into the major phospholipids of the parasite membrane, phosphatidylcholine and phosphatidylethanolamine, was 48.4±10.8% and 53.1±5.7% relative to an equivalent number of wild type parasites.

**P500: Properties of 3-mono/dibromoacetyl, 6-halogenated coumarin analogues against Anopheles arabiensis**

Venugopala K. Narayanaswamy, Kasumbwe K., Odhav B.
Department of Biotechnology and Food Technology, Durban University of Technology,

Coumarin synthesis; larvicidal; adulticidal; repellent activity. Mosquitoes are the major vectors for malaria, filariasis, dengue fever, yellow fever, Japanese encephalitis and other fevers. Synthetic organic insecticides that are currently used to control mosquitoes have produced a feedback of environmental ill effect, non-target organisms being affected and most mosquito species are becoming physiologically resistant to synthetic insecticides. Coumarin (2H-1-benzopyran-2-one) is a plant-derived natural product known for its pharmacological properties such as anti-inflammatory, anticoagulant, antibacterial, antifungal, antiviral, antancer, antihypertensive, antitubercular, anticonvulsant, antiadiopgenic, anthyperglycemic, antioxidiant, and neuroprotective properties. Warfarin is a synthetic coumarin analogue (known as Coumadin) that is used as an anticoagulant and is commercially available in the market with a trade name coumadin. In this context seven of 3-mono/dibromo acetyl, 6-halogenated coumarin analogues have been synthesized and the yield of the product was found to be 61 - 95%. The purity of the compounds was ascertained by HPLC (> 99%) and characterized by IR, NMR, LC-MS and single crystal X-ray method.

The title compounds (CMRN1-CMRN7) were tested against *Anopheles arabiensis* using the protocols outlined for repellency, larvicidal and insecticidal assays (WHO). Compounds CMRN1, CMRN2, CMRN4, CMRN5 and CMRN7 which are having bromo/fluro at 6th position and mono/dibromo acetyl group at 3rd position of coumarin at 1 mg/mL exhibited 100% larvicidal activity when compared to standard Temephos. The repellency activity and insecticidal activity of the title compounds were significant against *A. arabiensis* when compared to standards DEET and K-Othrine. The results show that CMRN1, CMRN2, CMRN4, CMRN5 and CMRN7 compounds kill larvae of *A. arabiensis* and thus may offer protection against malaria and repel the mosquitoes thus assisting in the control of the malaria vector.

**P501: Insecticide resistance in malaria vectors at a mining site in the Democratic Republic of the Congo.**

Luisa Nardini1,2*, Maureen Coetzee1,2, Richard H. Hunt1,2 and Lizette L. Koekemoer1,2
1 Vector Control Reference Laboratory, Centre for Opportunistic, Tropical and Hospital Infections, National Institute for Communicable Diseases, a Division of the National Health Laboratory Services, Johannesburg, South Africa.
2 Malaria Entomology Research Unit, School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa.

**BACKGROUND:** The impact of malaria on the staff, and commercial success, of large companies operating in Africa are significant and consequently, it is necessary for these companies to assist in vector control operations, not only within the activities of the company, but also for the surrounding communities. In order to protect the work-force and their families from malaria, it is necessary to assess the local vector population in terms of species identity and their resistance status so that suitable and effective interventions can be implemented. The main aims of this study are to assess, in detail the insecticide resistance status, as well as the mechanisms of resistance, of malaria vectors collected in a mining area in the Democratic Republic of the Congo.

**METHODS:** Mosquitoes were collected and identified, and the insecticide resistance status was determined using World Health Organization bioassays. Biochemical assays of major classes of resistance enzymes, and molecular analysis by qPCR were used to determine specific enzyme families and genes that might be responsible for the resistance phenotype. In addition, the presence of target-site mutations was assessed.

**RESULTS:** *Anopheles gambiae* and An. funestus found in the region were resistant to DDT, deltamethrin and bendiocarb. The presence of target site mutations was confirmed and preliminary data from the biochemical assays indicates significant differences in the activity of enzymes belonging to the main enzyme super-families; and in important genes associated with the insecticide resistant phenotype.

**CONCLUSION:** These data highlight the challenges facing malaria vector control programmes in large-scale mining communities. In addition, such information is essential for implementing suitable vector control programmes that are based on the resistance profiles of the local mosquito populations in order to reduce, as far as possible, the prevalence of malaria in these regions.

**P502: Trends in malaria morbidity among children under 5 years observed at 6 sentinel sites in Uganda**

Ruth Kigoro1, Bryan K. Kapella2, Asadu Sserwanga3, Kizito Fred4, Stella Kakeeto1, Wanzira Humphrey1, Denis Rubahika, Peter Okui, Simon Kasasa4, Michelle Chang3, Steve Yoon3, Yeka Adoke5, Arthur Mpimbaza1, Sarah Staedke2, Grant Dorsey4, Moses Kamy1

1Infectious Diseases Research Collaboration, 2Centre for Disease Control, 3London School of Tropical Hygiene, 4University of California San Francisco, 5Makerere University

**BACKGROUND:** Over the last decade, malaria control efforts have intensified in Uganda, including expansion of indoor residual spraying, distribution of insecticide treated bednets, and scale-up of artemisinin-based combination therapies as first-line therapy for uncomplicated malaria. Despite these efforts, malaria remains one of the most important health problem in Uganda, and the need for high quality surveillance data to monitor the malaria burden and impact of control measures is great.

**METHODS:** In 2006, we established sentinel site surveillance in outpatient departments of five government-run health centres (level IV), adding a sixth site in 2008. The sites were selected to represent the spectrum of malaria epidemiology in Uganda. Data collected on children under five from 2007 to 2012 were evaluated. Trends in malaria morbidity were estimated using the test positivity rate (TPR), defined as the proportion of children testing positive (by microscopy or rapid diagnostic test) divided by the total number tested. The Mann-Kendell’s trend test was used to assess monotonic malaria morbidity trends by site.

**RESULTS:** Over 198,000 children under 5 years presented with primary illnesses at the six sites; 154,370 were suspected to have malaria. The proportion of children suspected to have malaria who received a diagnostic test increased from approximately 50% in 2007 to over 90% by the end of 2011. The TPR varied over the years at the different sites, ranging from a low of 19% in Walukuba ( JinjaDistrict, a peri-urban, low-medium transmission setting) to a high of 76% in Aduku (ApacDistrict, a rural, very high transmission setting). Although trends in TPR decreased in four sites from 49% in 2007 to 26% in 2012, two showed increasing trends 29% in 2007 and 33% in 2012. Overall, data from all six sites revealed a statistically significant decreasing trend (Tau = -0.282, p = 0.0002) in TPR among children under five from 47% in 2007 to 35% in 2012.

**CONCLUSION:** The decreasing trends may be attributable to the on-going malaria control efforts. High quality surveillance data from additional sites are needed to generalise our findings to the rest of Uganda.
P503: Murine Plasmodium virulence is affected due to oxidative stress status of the host.

Shirley Herbas1, Magloire Natama2, Hiroshi Suzuki2
1National Research Center for Protozoan Diseases, Obihiro, Japan, 2Clinical Research Unit of Nanoro, Nanoro, Burkina Faso

BACKGROUND: It has been reported that non-lethal Plasmodium strains or genetically modified parasites were capable to prevent next lethal infections in mice. In alpha-tocopherol transfer protein knockout mice (α-ttPA) with C57BL/6 (B6) genetic background, it has been demonstrated that α-ttPA gene disruption confers resistance to malarial infections. This study aimed to investigate whether α-ttPA mice immunized with Plasmodium yoelii NL17 (PyNL17) are resistant to lethal infection caused by Plasmodium berghei NK65 (PbNK65) and the possible mechanisms such as parasite characteristic variation likely virulence or immune response of the host.

METHODS: α-ttPA mice were firstly infected with PyXL17 and then challenged with PbNK65 on day 15-post primary infection. In addition, B6 mice and α-ttPA mice were single infected with PyXL17 and with PbNK65 as controls during the first and the second infection time points respectively. Survival rate was monitored daily and parasitemia every 2 days. Parasites virulence was assessed using SCID mice. IgG levels in serum were determined by ELISA. mRNA expression of INF-γ, TNF-α and IL-10 in liver and spleen were assessed by RT-PCR. Liver histological analysis was also performed.

RESULTS: Immunization with PyXL17 in α-ttPA mice induced protective immunity against PbNK65. Inhibition of PbNK65 proliferation was associated to extremely low levels of parasitemia. Those parasites were not virulent even in SCID mice. IgG levels were higher in immunized mice than in controls. mRNA expression of INF-γ, TNF-α and IL-10 in liver and spleen revealed that cytokine response was delayed during the acute phase of the infection as compared to controls single infected with PbNK65. This cytokine response was delayed and up-regulated on day 15-post secondary infection with PbNK65. There was no infiltration of inflammatory cells in liver.

CONCLUSIONS: α-ttPA mice immunized with PyXL17 exhibited resistance to PbNK65. This resistance might due to alteration of parasite virulence caused by DNA damage in α-ttPA mice inflicted by oxidative stress and the enhancement of acquired immunity.

P504: Determination of DDT in contaminated indoor air and outdoor soil in a malaria area of South Africa – a new environmental forensics tool.

Yvette Naude1, Egmont R. Rohwer2
1University of Pretoria, Department of Chemistry, Lynwood Road, Hatfield, Pretoria, South Africa
2National Research Center for Protozoan Diseases, Obihiro, Japan

BACKGROUND: The organochlorine insecticide DDT (1,1,1-trichloro-2,2- bis(p-chlorophenyl)ethane) is used for malaria vector control in certain areas of South Africa. Traditional dwellings are sprayed on the inside with DDT. DDT is present in air, either adsorbed onto dust particles or as free gas phase molecules. Exposure may be via inhalation of gas and particulate phase DDT, or by ingestion. In rural villages contaminated dust presents an additional pathway for exposure to DDT. α,p'-DDT may show enantioselective oestrogenicity and biodegradability and therefore it is important to analyse enantiomers of α,p'-DDT and its chiral degradation product, α,p'-DDD, for both health and environmental- forensic considerations.

METHODS: A novel solvent free technique is described for the determination of DDT in indoor air where vapour phase and particulate phase samples were collected separately in a single step with a miniature denuder configuration of a multichannel open tubular silicone rubber (polydimethylsiloxane (PDMS)) trap combined with a micro quartz fibre filter. The multichannel PDMS trap section of the denuder concentrates vapour phase insecticide whereas particle associated insecticide is transferred downstream where it is collected on a micro-fibre filter. A cheap and simple soil sampling procedure is also presented: a length of PDMS tubing is fashioned into a loop and the loop placed in soil samples for sorptive extraction of compounds. Solvent extraction and sample clean-up are not required. Sampling materials are designed to fit a commercial thermal desorber for direct introduction of sorbed analytes into a gas chromatograph coupled to a mass spectrometer (GC-MS) or a comprehensive two dimensional gas chromatograph coupled to a time of flight mass spectrometer (GCxGC-TOFMS).

RESULTS: Airborne enantiomers of R(-)α,p'-DDT and S(+)-α,p'-DDT and of S(+)α,p'-DDD and R(-)α,p'-DDD were measured in indoor vapour and particle phases after indoor residual spraying (IRS) with DDT during 2007. Two very different enantiomeric profiles were revealed: indoor air vapour phase displayed a racemic composition, while indoor airborne particulate phase displayed a non-racemic composition. Ratios of airborne p,p'-DDT/p,p'-DDD and of α,p'-DDT/p,p'-DDD were unusual and do not match the ideal certified ingredient composition required of commercial DDT.

CONCLUSION: Results show that the DDT products used for IRS contained very little p,p'-DDT (only p,p'-DDT is effective against the malaria carrying mosquito), demonstrating the power of this new environmental forensics tool. Compromised insecticidal efficacy of technical DDT products is cause for concern.

P505: Epidemic Planning and Response (EPR) Tool for preventing and tracking Malaria in South Africa

Nawm J.B.1, Seocharan I.2, Groepe M3& Moonasar D4
1National Institutefor Communicable Disease of the National Health Laboratory Services, Private Bag x1, Sandringham, 213, South Africa (seconded to the National Department of Health, South Africa); 2Malaria Research Programme, Medical Research Council, Durban, South Africa; 3World Health Organisation, Pretoria, South Africa; 4National Department of Health, Pretoria, South Africa

BACKGROUND: South Africa is targeting malaria elimination by 2018. For this reason 2 critical indicators are important. Notification of malaria cases should be within 24 hours and when an outbreak occurs the response should be within 48 hours. This is important to prevent secondary malaria cases from occurring. An alert and action thresholds are calculated to detect and respond to outbreaks. Originally ten years of data was used for these calculations. Since the number of malaria cases has declined by 41 % in the last 10 years it was decided to use the last 5 years of data to calculate the thresholds which is updated yearly based on WHO standards (Mean plus 1.5 standard deviation and Upper 3rd Quartile). The aim of this paper is to describe the EPR tool for monitoring and responding to malaria outbreaks in South Africa.

MATERIALS AND METHODS: Weekly malaria cases data is collected by districts and entered into the Malaria Information System at provincial level and synchronised with the national Malaria Data Management System (MDMS). The MDMS will be the departure point for exploring the thresholds sensitivity through adjusting the number of years for the calculation. The EPR Tool will be described in more detail as part of the paper. South Africa is embarking on malaria elimination, the sensitivity of the thresholds will have to be adjusted and result in 1 malaria case considered an outbreak. For this tool to work, the current challenges of the notification of the malaria case within 24 hours, quality data entered into the MDMS, response within 48 hours from a threshold being exceeded and the sensitivity of the EPR thresholds at lower geographical levels must be addressed.

RESULTS: The Malaria Data Management System provides the opportunity for an automated process of notification of any exceedes of the epidemic thresholds. When a threshold is exceeded an e-mail/ text message is sent to the relevant person(s) to respond to the alert and/or action threshold. An important function of the tool would be to ensure that the response to the outbreak is clearly understandable and implementable at all level from facility level to provincial level.
**P506: Open Source and Android phones: Mapping with mSpray - the solution for fighting Malaria**

**Nawn JB, Seto E1, Ntimbane T2, Kruger P1, Eskenazi B2, Quiros-Alcala L2, Lipsitt LM3, Wu LD2, Bornman R4**

1. National Institute for Communicable Diseases, National Department of Health, Pretoria, South Africa; 2. Centre for Environmental Research and Children’s Health, School of Public Health, University of California, Berkeley, CA, USA; 3. Limpopo Provincial Government: Department of Health, Tzaneen, South Africa; 4. Department of Urology and Centre for Sustainable Malaria Control, University of Pretoria, South Africa

**BACKGROUND:** As part of the VHEMBE (Venda Examination of Mothers, Babies and their Environment) study, funded by National Institute of Environmental Health Sciences (NIEHS), we will be studying the exposure of 750 pregnant women and their children to chemicals used in Indoor Residual Spraying (IRS) and the potential health and developmental effects of these chemicals. The aim of the VHEMBE study is to recruit women half from sprayed and half from unsprayed villages. In addition, under a diversity supplement to NIEHS, the project team aimed to map the relation of spray locations with blood levels in the 750 pregnant women. For this reason the smartphone application, mSpray, was tested in a pilot study towards capturing GPS data and accurate attribute data related to IRS activities. Recognizing the mutual needs for the VHEMBE study and for the Malaria Control Program to better monitor spray activities, different methods to document the spraying of the area have been considered. The pros and cons of each method have been considered and an innovative method with the full cooperation and collaboration of the Limpopo Malaria Control Program is now tested by means of android based smartphones and the application called mSpray.

**METHODS:** Android-based smartphones was provided to 10 Spray Foremen in the Vhembe district in Limpopo. Each smart-phone was equipped with a purpose built application (app). The relevant data was captured with this app and a GPS point with a time and date stamp as part of the data collected. The phone stores the collected data in an integrated comma-delineated text file that can be further analysed in other software, which will allow for ease in use for various analyses, e.g., obtaining statistical summary data and importing directly into GIS software for easy visualization.

**RESULTS:** The results obtained were maps and attribute data of where DDT was used for spraying as well as pyrethroid spraying. Differentiation between structures and methods used for spray on maps provide valuable information for planning of malaria spray operations in the future and further exploration using GIS can add even more value. We would like to present the multitude of valuable lessons learnt for both the VHEMBE study and the Limpopo Malaria Control Programme.

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**P507: Predicting the start and end of the malaria transmission season in KwaZulu-Natal, South Africa**

**Nawn J.B.**

1. National Institute for Communicable Disease of the National Health Laboratory Services, Sandringham, 213, South Africa (seconded to the National Department of Health, South Africa)

**BACKGROUND:** The complexity of malaria as a disease is well documented and further highlighted by this study. This complicates the prediction of the onset, peak and end of the malaria transmission season and is further complicated by the difficulty in eliminating other factors involved in increased malaria transmission, e.g., drug resistance. Many of the factors affecting the vector and transmission of malaria are not constant; neither is they straight-forward cause-and-effect related factors. The techniques investigated in this study were chosen because of their practical and potential implementable value in southern Africa. Ongoing research is required to further explore other possible predictors.

**METHODS:** Various climate-malaria linked techniques were used to investigate the relationship between climate and the start and end of the malaria season. For KwaZulu-Natal this was done using the relationship between temperature, rainfall and malaria was tested in order to predict the oncoming seasons. The lead time before the start of the transmission season was examined in detail.

- The predictors that were investigated in this study are:
  - The effect of long term temperature on malaria case numbers
  - The effect of long term rainfall on malaria cases
  - The epidemic preparedness and response plan threshold values
  - The effect of El Nino and its association with droughts in South Africa and malaria cases
  - The previous season’s September temperature as a predictor
  - The use of early season malaria cases as a predictor for malaria epidemics
  - Degree days as a predictor

**RESULTS:** Results obtained re-emphasise the fact that malaria is a complex disease where no clear cause-and-effect scenario can be used for prediction. A multi-factorial approach is the only solution, Some of the factors can be ruled out are the previous season’s temperature as being predictive of the following season’s malaria cases as well as the accumulative effect of rainfall on malaria transmission. The most positive predictor for KwaZulu-Natal seems to be the use of degree days in identifying the start and end of the malaria transmission season.

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**P508: Out of Pocket Treatment for Malaria - a major Cost constrain in the Fight Against malaria in less developed countries**

**Nazah Gwat**

Malaria inflicts significant costs on households and on the economy of malaria endemic countries. There is evidence that the economic burden is higher among the poorest in a population, and that cost burdens differ significantly between wet and dry seasons. What is not clear is whether, and how, the economic burden of malaria differs by disease endemicity. The need to account for geographical and epidemiological differences in the estimation of the social and economic burden of is well recognized, but there is limited data, if any, to support this argument. Malaria is considered as one of the poverty related diseases and is the number one killer disease in sub-Saharan Africa. This is due to the fact that the environments in which they live in are enabling environments which enable the breeding grounds of malaria. The greater part of money spent on health care often comes from the private pockets of those who are directly or in indirectly suffering from the burden of the illness concern. When planning on healthcare expenditure, it is important for governments to strive as much as possible to put in place strategies that will cut down on the out of pocket payment of the cost of health care by the population. In a country where close to 45 % of the population leave on less than a dollar per day, and where the population has to battle with this ancient Scotch –malaria on daily bases, it becomes imperative for a cost effective intervention model to be put in place that will provide acceptable and affordable standard of care as far as malaria prevention and treatment is concern. All the indirect cost involves in the treatment of those suffering from malaria has to be well analyzed in order to effectively quantify the financial burden posed by this disease on the population. The aim of this study is to reduce or if possible eliminate out of pocket payments for malaria treatment and promoting a cost-effective malaria case management.
**P509: Incrimination of anopheline mosquitoes as malaria vectors in Guinea Savanna zone of central Nigeria**

1S. Ndams, I.H. Nock, and C.G. Vajime
2Department of Biological Sciences, Main Campus, Ahmadu Bello University, Zaria, Nigeria. 2Department of Biological Sciences, Benue State University, Makurdi, Nigeria

BACKGROUND: The incrimination of the major anopheline mosquitoes transmitting malaria in the guinea savannah of Nigeria has not been clearly stated for control purposes. Currently, control of malaria vectors, by indoor residual spraying pyrethroids and ITNs and LLNs distributions, is broad-based. The study aimed to incriminate anopheline transmitting malaria for selective control in the guinea savanna of Nigeria for.

METHODS: Female indoor-resting Anopheles were caught with mechanical aspirator and identified by microscopy. The PCR diagnostic assays was performed to delineated gambiae sI sibling species and further to S and M molecular form. No PCR was performed on An. funestus because sample size and cost. All mosquito samples collected were tested for Plasmodium circum-sporozoites by ELISA.

RESULTS: An. gambiae ss was more preponderant than An. arabiensis, An. rufipes respectively, while An. funestus was least abundant. The An. gambiae ss collected was significantly higher (P<0.05) in the southern Guinea Savanna than those collected in the northern Guinea. Conversely, An. arabiensis , An. funestus and An. rufipes collected were significantly higher (P<0.05) in the northern Guinea than in the southern Guinea Savanna. The S molecular form collected was significantly higher (P<0.05) than the M form. ELISA results revealed that 2.18% overall infection of mosquitoes tested for Plasmodium circum-sporozoite 2.08% infection due falciparum circum-sporozoites and 0.995% malariae circum-sporozoites. One (1) sample failed the test and was excluded from further analysis. The 2.66% falciparum infection were An. gambiae ss M forms, and 12.95% S forms, whereas 1.49% infection in An. arabiensis, 0.50% infection in An. arabiensis was P malariae circum-sporozoite. All the An. funestus ss and An. rufipes samples tested negative for the two circum-sporozoites assayed.

CONCLUSION: The study has demonstrated that An. gambiae ss S and M molecular forms and An. arabiensis were the major anopheline vectors transmitting P. falciparum and P malariae in the guinea savanna vegetation zones of Nigeria. The study lends credence for the use of LLNs, ITNs and ACT therapy for vector control in human habitations and malaria treatment on going in these two zones of Nigeria.

KEYWORDS: Anophelines gambiae, Anophelines arabiensis, molecular forms, Plasmodium Circumsporozoites

**P510: Surveillance of polymorphisms in Pfmrp1 gene in western Kenyan Plasmodium falciparum isolates and their implications on in-vitro drug susceptibility**

Mutwiri Ndegwa1,2, Angela Omondi1, Luiser Ingasia1, Dennis Juma1, Jelagat Cherutich1, Peninah Muiruri1, Lorna Jemosop1, Bidii Ngalah1, Redemptah Yeda1, Agnes Cheruiyot1, Charles Okudo1, Hoseah M. Akala1, David Onyango1, Fred Eyase1, Ben Andagalu1, Edwin Kamau1

1Global Emerging Infections Surveillance (GEIS) Program, United States Army Medical Research Unit-Kenya (USAMURE-K), Kenya Medical Research Institute (KEMRI) - Walter Reed Project, Kisumu and Nairobi, Kenya; 2Faculty of Science, Department of Zoology, Maseno University, Kenya.

BACKGROUND: Plasmodium falciparum malaria is a major public health burden in sub-Saharan Africa. The use of chemotherapy to fight malaria has been hampered by omnipresent polymorphisms in genes of the deadly parasite that are crucial for their biology. Multidrug-resistance proteins (MRP) are adenosine triphosphate (ATP) dependent efflux pumps whose mutations lead to drug resistance in many organisms due to lowered intracellular drug concentration. This study aimed at determining the association between Pfmrp1 mutations at codons 191, 437, 876 and 1390, and chemosensitivity on artesinin (ART), lumefantrine (LUM) and amodiaquine (AMQ) in western Kenya.

METHODS: In vitro drug sensitivity on ART, LUM and AMQ was assessed using SYBR green assay on 61 samples from P. falciparum positive subjects from Kisumu in western Kenya. Pfmrp1 gene was sequenced at codons S191A, H437Y, 876V and F1390L. Correlation was determined between the phenotype and genotype.

RESULTS: The IC50 median values for ART, LUM and AMQ were 1.772µM, 1.856µM and 14.74µM respectively (n=61 and CI=95%). Percentages of samples with SNPs at codons 191, 437, 876 and 1390 were found to be 3.2%, 3.2%, 35% and 16.3% respectively. There was slight association of the variation at codon 876 and in vitro ART and LUM susceptibilities (P=0.046).

CONCLUSIONS: The association of variation at codon 876 and increased IC50 values for ART and LUM confirms that it is an important marker in western Kenya. In the era of artemisinin combination therapy (ACT) emerging reduced efficacies it is prudent to carry out an in vivo study in Kenya incorporating the codon 876 and the components of ACT.

Juliet Nlubaza1, Chris Drakeley1, Simon Brooker2, Gayvilia Nkurunziza1, Helen Akurut1, Medi Kakande1, Pascal Magnusson3, Birgitte Vennewald1, Alison Elliott1,2

1MRC/UVRI Uganda Research Unit on AIDS, P.O. Box 49, Entebbe, Uganda; 2London School of Hygiene and Tropical Medicine, Keppel Street, London, WC1E 7HT, UK; 3Institute for International Health, Immunology and Microbiology, Faculty of Health and Medical Sciences, Copenhagen University, Denmark; 4Section for Parasitology and Aquatic Diseases, Faculty of Health and Medical Sciences, Copenhagen University, Denmark

BACKGROUND: Identifying populations with the highest malaria risk can be a valuable preliminary stage in directing targeted malaria control and elimination programmes. Improving malaria surveillance in regions where malaria burden is greatest is essential. We hypothesised that serological markers in pregnancy could be used to identify spatial variation in childhood malaria transmission in highly endemic regions. Methodology: In a randomised trial on anthelmintic use in pregnancy (ISRCTN32849447) 2,507 women were enrolled between April 2003 and November 2005, and 2,345 live births recorded. Participants’ addresses were geo-referenced using a handheld global position system (GPS). Childhood malaria episodes, defined as a history of fever or axillary temperature of ≥ 37.5°C and any parasitaemia, were recorded prospectively from birth to two years. Childhood asymptomatic P. falciparum parasitaemia, defined as a positive malaria slide in the absence of fever was determined at age one and two years. Maternal blood was collected at delivery and an enzyme immunoassay (EIA) used to detect total IgG antibody concentrations (µg/ml) to Apical Membrane Antigen-1 (AMA-1) and Merozoite Surface Protein-1 (MSP-1). SatScan™ software (version 9.0) was used for the detection of spatial clusters: a discrete Poisson based model to identify clusters of high childhood malaria risk, and the Normal model to identify clusters of high maternal serological titres. Maps were produced using ArcGIS software (ESRI® ArcMapTM 10.1(CA, USA)).

RESULTS: The incidence of childhood malaria was 47 per 100 child-years, and the mean prevalence of asymptomatic parasitaemia was 10.4%. The mean log(95% CI) maternal antibody levels for AMA-1 and MSP-1 were 6.26 (95% CI: 6.19-6.34) and 6.65 (95% CI: 6.58-6.71), respectively. Two consistent hotspots were identified. Hotspots of maternal antimalarial responses to AMA-1 and MSP-1 overlapped hotspots of childhood clinical and asymptomatic malaria.

CONCLUSION: Serological markers in pregnancy might be useful in identifying spatial variation in childhood malaria transmission at micro-geographic levels in highly endemic regions. Simple descriptive mapping using routine data collected at maternal and child health units could be used as a preliminary tool in the detection of malaria hotspots initiating the implementation of targeted control activities.
**P512: Bayesian Spatio-temporal Modelling of Malaria Incidence in KwaZulu-Natal.**

Noluthando Ndlou
Malaria Research Unit, South African Medical Research Council, Durban, KwaZulu-Natal, South Africa

**BACKGROUND:** Malaria is an ancient disease that has been affecting people since the beginning of recorded time. It poses serious economic, social and health burdens in tropical and subtropical countries where it is predominantly found. The level of malaria risk and transmission intensity exhibit significant spatial and temporal variability related to variations in climate, altitude, topography, and human settlement pattern. Spatial and temporal mapping of malaria disease can help in the detection of populations at risk. The Bayesian approach to spatio-temporal modelling has been identified to be the superior method in analysing malaria transmission and mortality. The aim of this study was to develop statistical models for identifying which climatic variables drive malaria transmission in KwaZulu-Natal and subsequently produce incidence maps based on the climatic variable that is significantly correlated with transmission in the area.

**METHODS:** Bayesian spatio-temporal modelling was employed in this study for cases from 2000 to 2011. The variable selection method was used for analysis and implementation was conducted in OpenBUGS 3.2.1. The models were fitted using MCMC techniques. The variables that were identified by the variable selection methods were used for prediction of incidence. The incidence maps were created using R version 2.15.2.

**RESULTS:** Incidence rates obtained from the prediction maps ranged from a low of 0.2 to 5 per 1000 inhabitants for the year 2010. Preliminary results revealed that the area with the highest incidence rates is in the Umkhanyakude district in Northern KwaZulu-Natal. The variable selection method chose temperature and land use as being the most significant factors in driving malaria transmission in the study area.

**CONCLUSIONS:** By understanding the complex dynamics of malaria transmission, early warning systems can be developed to ensure that communities at risk are provided with the adequate resources needed to protect themselves against the disease and control programs will thus be more effective and efficient. Efforts should be concentrated in the hotspots identified by statistical modelling with the highest incidence rates.

**P513: Mitochondrial phylogeny of the Anopheles moucheti group supports speciation between morphological forms**

Cyrille Ndo1,2,3, Pierre Kengne1,2, Frédéric Simard2, Didier Fontenille2, Christophe Antonio-Nkondjio1,4
1 Laboratoire de Recherche sur le Paludisme, Organisation de Coordination pour la lutte Contre les Étendues en Afrique Centrale, Yaoundé, Cameroon. 2 Institut de Recherche pour le Développement (IRD), UMR IRD224-CNRS5290-Université de Montpellier 1-Université de Montpellier 2 MIVEGEC (Maladies Infectieuses et Vecteurs : Écologie, Génétique, Évolution et Contrôle), Montpellier, France. 3 Faculty of Medicine and Pharmaceutical Sciences, University of Douala, Douala, Cameroon. 4 Faculty of Health Sciences, University of Bamenda, Bambili, Cameroon.

**INTRODUCTION:** Anopheles moucheti is an efficient malaria vector in forest regions of equatorial Africa, but few studies have been conducted on this species. With the recent finding suggesting the possible implication of A. moucheti in the circulation of Plasmodium parasites between human and great apes in Central Africa, it becomes crucial to better characterize this group of mosquitoes.

**METHODS:** We used two mitochondrial markers to assess the current and historical relationships among populations of two A. moucheti morphological forms collected in Cameroon and Uganda (A. m. moucheti), and in the Democratic republic of Congo (A. m. bervoetsi).

**RESULTS:** Graphical analysis of the genealogical relationships showed a clear separation between An. m. moucheti and A. m. bervoetsi haplotypes, which differed by large number of fixed mutations within their sequences suggesting that no genes flow occurs between the two forms. While A. m. moucheti samples from Cameroon and Uganda formed a distinct homogeneous cluster, with minor differences between sequences, at the level of intraspecific genetic differentiation.

**CONCLUSION:** The results were in straightforward with previous studies using various genetic markers and prompted for elevation of A. m. bervoetsi to a full specific rank within the A. moucheti group, namely A. bervoesti. Further investigations of A. bervoesti biology and behavior will allow to precise its implication in transmission of human malaria as well as its putative role in the circulation of Plasmodium parasites between human and great apes in the equatorial forest block of Central Africa.

**P514: Molecular evidences of additional species within the Anopheles nili group of malaria vector in the equatorial forest region of Cameroon, Central Africa**

Cyrille Ndo1,2,3, Frédéric Simard1, Pierre Kengne1,2, Parfait Awono-Ambene1, Isabelle Morlais1,2, Igor Sharakhov1, Didier Fontenille2, Christophe Antonio-Nkondjio1,4
1 Laboratoire de Recherche sur le Paludisme, Organisation de Coordination pour la lutte Contre les Étendues en Afrique Centrale, Yaoundé, Cameroon. 2 Unité de Recherche Mixte Maladies Infectieuses et Vecteurs: Écologie, Génétique, Évolution et Contrôle, Institut de Recherche pour le Développement, Montpellier, France. 3 Faculty of Medicine and Pharmaceutical Sciences, University of Douala, Douala, Cameroon. 4 Department of Entomology, Virginia Tech, Blacksburg, Virginia, United States of America. 5 Faculty of Health Sciences, University of Bamenda, Bambili, Cameroon.

**INTRODUCTION:** The Anopheles nili group includes important vectors of human malaria in equatorial forest and humid savannah regions of sub-Saharan Africa. However, despite its important epidemiological role, it remains largely understudied. With the recent finding suggesting the possible implication of forested mosquitoes in the circulation of Plasmodium parasites between human and great apes in Central Africa, it becomes crucial to better characterize this group of vectors.

**METHODS:** Mosquitoes were collected in different ecological settings across Cameroon and species were identified both morphologically and molecularly. A combination of nuclear (microsatellite and ribosomal DNA) and mitochondrial DNA markers were used to explore and compare the level of genetic polymorphism and divergence among populations and species of the group in the savannah and forested areas of Cameroon.

**RESULTS:** Graphical analysis of the genealogical relationships showed a clear separation between the four known species of the An. nili group and detected additional genetic clusters in deep equatorial forest environment. Nuclear markers detected four highly divergent genetic lineages in this environment, ever described within An. nili, which differed by 1.8 to 12.9% of their Internal Transcribed Spacer 2 (ITS2) sequences, implying approximate divergence time of 0.82 to 5.86 million years. However, mitochondrial data only detected three major subdivisions, suggesting different evolutionary histories of the markers.

**DISCUSSION/CONCLUSIONS:** The study enlightened additional cryptic genetic diversity within An. nili group, at the level of interspecific divergences in Anopheles and other insect groups. This reflects a complex demographic history for this important group of malaria vectors in the deep equatorial forest of South Cameroon and likely suggests coexistence of additional An. nili species in this environment. Further studies will shed light on the distribution and epidemiological role of these new putative species, as well as on the evolutionary history of An. nili group in the African rainforest.
P515: Efficacy and tolerance of artesunate-amodiaquine and artemether-lumefantrine for uncomplicated falciparum malaria

Mathieu Ndounga

BACKGROUND: Congo Brazzaville adopted a new antimalarial treatment policy in 2006, with artesunate–amodiaquine (ASAQ) and artemether–lumefantrine (AL) as the first- and second-line drugs, respectively. Only four clinical studies were conducted in the country before the change. All these studies were conducted in the south of the country, in or near Brazzaville, in Pool Department. We report here the results of the first randomized study on these two drugs conducted from November 2012 to February 2013, in Owando city located in the Congo Basin, in the north of the country.

METHODS: Children aged six months to 11 years with uncomplicated malaria were randomly assigned to co-formulated ASAQ (Coarsucam®) or AL (Coartem®) treatment groups. Patients were followed for 28 days according to the 2003 WHO standard protocol. Plasmodium falciparum recrudescence isolates were compared to pre-treatment isolates by polymerase chain reaction to distinguish between re-infection and recrudescence.

RESULTS: Of 123 patients, 62 were treated with ASAQ and 61 with AL. The PCR-uncorrected overall efficacy of ASAQ and AL on day 28 was 92.7% and 94.2%. After PCR correction, the efficacy of the two drugs was 100%. From day 1 to day 7, few patients treated with ASAQ reported fatigue, headache, vomiting, abdominal pain, and pruritus. Patients treated with AL reported only fatigue.

CONCLUSION: This first study in northern Congo showed an excellent efficacy of ASAQ and AL. High malaria transmission was associated with new infections in few patients.

P516: A Longitudinal Cohort Study of Malaria in Cameroonian Children Living in a malaria endemic area in South Western Cameroon.

Erica Neh, Clarisse Njua-Yafi1, Judith Anchang-Kimbi1, Tobias Apinjoh1, Regina Murgri1, Hanesh Chi1, Roland Tatah1, Emmanuel Nkock1 and Eric Achid1

1Malaria Research Laboratory, Faculty of Science, University of Buea, Buea, Cameroon; 2Department of Animal Biology and Physiology, University of Yaounde I, Yaounde, Cameroon

BACKGROUND: Understanding the relationships between transmission intensity, prevalence and incidence of malaria is crucial in developing and testing tools for malaria control. Longitudinal studies of malaria in well-defined cohorts offer a unique opportunity to achieve this objective. Methodology: A cohort of 357 children from Mutengene aged ten years and below recruited from randomly selected households were followed up bi-monthly for 12months. The incidence of malaria was determined by active case detection and sampling was done every trimester. An episode of malaria was defined as fever with an axillary temperature >37.5°C and the presence of malaria parasitaemia. Participants were also screened for intestinal helminthes and Hb levels were measured.

RESULTS: A total of 138 (97.9%) of the sick children had at least one malaria attack with a mean of 1.46 ± 0.73 (range-1-4) episodes/year. Children < 5 years had significantly (P=0.034) higher mean malaria episodes than those 5-10 years. The percentage of participants positive for malaria parasitaemia increased from 18% at enrolment (dry season) to 19.3% 6months later(rainy season) and decreased to 12% 12months later (dry season), P=0.05. Geometric mean malaria parasitaemia density/μl at enrolment was 4948 (range45-146636). Interestingly, 71.5% were anaemic (Hb=11g/dl) at enrolment and significantly (P<0.01) reduced to 60.1% 6months later and 43.5% 12months later. Mean Hb at enrolment was 10.5g/dl (range:9.3-14g/dl). The mean Hb level progressively increased significantly (P<0.01) during the 12 months trimestral surveys. 74.4% (116/156) of malaria parasitaemic cases were anaemic while 13.6% (n=12) were positive for helminthes. Furthermore, 58.8%(47/80) of helminth positive cases were anaemic.

DISCUSSION AND CONCLUSION: Majority of children 10 years in the study area have atleast 1 episode of malaria per year which is associated with anaemia. More than 50% of the children infected with malaria have helminthes co-infection.

P517: Major genome region underlying artemisinin resistance in malaria parasite isolates from Kisumu county, western Kenya.

Bidii Ngalah1, Liuser Ingasia1, Angela Omondi1, Dennis Juma1, Nancy Jegalat1, Ndegwa Mutwiri1, Lorna Jemosop1,2, Peninah Muiruri1,2, Redemptah Yeda1, Agnes Cheruiyot1, Charles Okudo1, Hoseah Akala1, Ben Andangaluu1, Ziporrah Ng’ang’a1, Edwin Kamau1,2

1Global Emerging Infections Surveillance (GEIS) Program, United States Army Medical Research Unit-Kenya (USAMRU-K), Kenya Medical Research Institute (KEMRI) - Walter Reed Project, Kisumu and Nairobi, Kenya; 2Institute of tropical medicine and infectious diseases, Jomo Kenyatta University of Agriculture and Technology, Kenya.

BACKGROUND: Emergence of clinical resistance to artemisinin and its derivatives is now well established in Southeast Asian (SEA). Studies from SEA have identified single nucleotide polymorphisms (SNPs) which are significantly associated with delayed parasite clearance rates. However, more studies are required to confirm and validate these markers elsewhere. This study aims to establish the molecular mechanism associated with artemisinin resistance in Kenya.

METHODS: DNA was extracted from 31 archived samples collected in Kisumu between 1995-1996. Seventeen of the 30 SNPs that were recently shown to be associated with artemisinin resistance in SEA were genotyped by Sanger sequencing. Plasmodium falciparum multi-drug resistance gene 1 (pmdr1) copy number variation was quantified using real time PCR. As we proceed, ~200 samples collected before artemisinin became widely available in Kenya will be analyzed to provide background information on the SNPs associated with artemisinin resistance. An ongoing efficacy trial in western Kenya will provide samples that will have accompanying parasite clearance rate data. It is estimated 120 samples will be analyzed from this study. Sequenom platform will be used to assess the SNP profile of all the samples with a subset of samples analyzed by Sanger sequencing. Microsatellite analysis of MSP1/MSP2 will be used to establish recrudescence verses new infections in samples obtained from the efficacy study.

PRELIMINARY RESULTS: Data showed that all the 31 samples contain wild type alleles in all the SNP positions analyzed. Two of the 31 samples analyzed (7%) contained >1 pmdr1 copy numbers. The IC₅₀ obtained for 2 samples analyzed was ~8.0 nM for artemisinin. Based on the in vitro data collected in more than 200 samples in Kisumu in the last 5 years, in vitro data from these archived samples was less than half of what the average of the last 4 year at 4.21 nM presents.

CONCLUSION: The preliminary data indicate samples collected before the introduction of artemisinin contain wildtype alleles in all SNP positions analyzed. They are sensitive to artemisinin compared to samples collected after the introduction of artemisinin treatment. The value of these molecular markers as indicators of artemisinin resistance is yet to be established.


Lindokuhle Ngomane1, Christiaan de Jager2

1University of Pretoria Centre for Sustainable Malaria Control, Faculty of Health Sciences, Pretoria, South Africa

BACKGROUND: Malaria remains an epidemic threat in Mpumalanga Province. In order to appropriately target interventions to achieve substantial reduction in the burden of malaria and ultimately eliminate
the disease, there is a need to track progress of malaria control efforts by assessing the time trends and evaluating the impact of current control interventions. This study aimed to assess the changes in the burden of malaria in Mpumalanga Province during the past eight malaria seasons (2001/02 to 2008/09) and whether indoor residual spraying (IRS) and climate variability had an effect on these changes.

METHODS: This is a descriptive retrospective study based on the analysis of secondary malaria surveillance data (cases and deaths) in Mpumalanga Province. Data were extracted from the Integrated Malaria Information System. Time series model (Autoregressive Integrated Moving Average) was used to assess the association between climate and malaria.

RESULTS: Within the study period, a total of 35,191 cases and 164 deaths due to malaria were notified in Mpumalanga Province. There was a significant decrease in the incidence of malaria from 385 in 2001/02 to 50 cases per 100,000 population in 2008/09 (P < 0.005). The incidence and case fatality (CFR) rates for the study period were 134 cases per 100,000 and 0.54%, respectively. Mortality due to malaria was lower in infants and children (CFR <0.5%) and higher in those >65 years, with the mean CFR of 2.1% as compared to the national target of 0.5%. A distinct seasonal transmission pattern was found to be significantly related to changes in rainfall patterns (P = 0.007). A notable decline in malaria case notification was observed following apparent scale-up of IRS coverage from 2006/07 to 2008/09 malaria seasons.

CONCLUSIONS: Mpumalanga Province has achieved the goal of reducing malaria morbidity and mortality by over 70%, partly as a result of scale-up of IRS intervention in combination with other control strategies. These results highlight the need to continue with IRS together with other control strategies until interruption in local malaria transmission is completely achieved. However, the goal to eliminate malaria as a public health problem requires efforts to be directed towards the control of imported malaria cases; development of strategies to interrupt local transmission; and maintaining quality surveillance and reporting system.

P519: Evolution of frequency of PFCRT and PFMDR1 alleles in Plasmodium falciparum in Gabon

Ndong Ngomo Jacques Mari; Mawili-Mboumba Denise; Maboko Francois; Kombila Maryvonne; Bouyou-Akotet Marielle
Department of Parasitology-Mycology: Faculty of Medicine, Université des Sciences de la Santé, Libreville, BP 4009 Gabon

BACKGROUND: In Gabon, the high frequency of Plasmodium falciparum strains resistant to chloroquine led to the adoption of artesminin-based combination therapies (ACTs) such as artesunate-amodiaquine for the treatment of uncomplicated malaria. The rapid spread of drug-resistant Plasmodium falciparum strains requires a careful monitoring of the parasite resistance level to the molecules associated to artesminisin.

The present work assessed the evolution of the frequency of molecular markers of P. falciparum to resistance 4-amino-quinolines PfCRT and PfMDR1 genes in isolates collected in 2005 and 2008 at the Regional Center Hospital of Oyem (CHRO) located in the North of Gabon.

METHODS: PfCRT codon 76 and PfMDR1 codon 86 were genotyped using the Polymerase Chain Reaction Restriction Fragment Length Polymorphism (PCR-RFLP) in 29 samples collected in 2005 and 78 in 2008 samples.

RESULTS: The mutant alleles PfCRT 76T and PfMDR1 86Y were detected in 75.8% and 79.3% of the isolates collected in 2005 and respectively 70.5% and 64.1% of those from 2008. The proportion of isolates carrying wild type alleles PfCRT76K and pfmdr186N increased from 17.2 to 29.5% and from 13.8 to 20.5% for pfmdr186N codon for PfCRT 76K codon. In 2005, the double wild type alleles 76K-86N was not found; while in 2008 it was carried by the 8.9% of the analyzed isolates. The proportion of parasites carrying the double mutation 76T-86Y tend to be higher in 2005: 58.6% vs 47.4% in 2008. According to the drug use PfCRT 76T mutant isolates were more frequently observed in patients with antimalarial drug. Indeed its frequency was of 94.5% in the group of patients with antimalarial drug (SM+) and 68.2% in those without antimalarial drug (SM-) (p<0.01).

However, the proportions of parasites the carrying pfmdr186N mutations were comparable in both groups. In the group of patients without antimalarial selfmedication, isolates with wild-type alleles were two (pfmdr1 N86) to five (pfcr T76) fold more frequent (p<0.01).

CONCLUSION: The present study shows that three years after the withdrawal of chloroquine as treatment for uncomplicated malaria in Gabon, a gradual increase of the frequency of wild type alleles of Pfmdr186N and Pfcrt T6K was observed. A selection of isolates carrying mutant alleles was observed in the group of patients with antimalarial drug.

P520: The influence of sprayable surfaces on the effectiveness of indoor residual spraying using Lambda-cyhalothrin against malaria vectors in Zanzibar and Mainland Tanzania

Joshua Mutagaphwa1, Harish B Pratap2, Fabrizio Molteni3, Francis Mugurula4, Jeremiah Ngondi5, Stephen Magesa6, Mohammed Ramsan7, Jessica M. Kafuko8, Elias Nyanza9, Oisia Mwalipape10, Charles D, Mwalimu11, Jasper N Ijumba12

1RTH International, Dar es Salaam, Tanzania; 2Department of Zoology and Wildlife conservation of the University of Dar es Salaam, Tanzania; 3Swiss Tropical and Public Health Institute, Dar es salaam, Tanzania; 4USAD/PMI Dar es salaam, Tanzania; 5School of Public Health, Catholic University of Health and Allied Sciences, Bugando, Mwanza Tanzania; 6National Malaria Control Program - Tanzania; 7Nelson Mandela African Institute of Science and Technology, Arusha, Tanzania

BACKGROUND: The type of sprayable surface impacts on the residual efficacy of insecticide used in indoor residual spraying (IRS). However, there is limited data on common types of wall surfaces sprayed in Zanzibar and Mainland Tanzania. The study investigated residual efficacy of lambda-cyhalothrin sprayed on common surfaces in Zanzibar and Mainland Tanzania where IRS is one of the malaria vector control interventions.

METHODS: An experimental hut was constructed with materials simulating common sprayable surfaces in Zanzibar and Mainland Tanzania. Surfaces included cement plastered, mud-daub, white-wash, wood, palm-thatch, iron-sheet and burnt-bricks, limestone and oil-painted. Using WHO standards operating procedures for IRS, the study surfaces were sprayed with lambda-cyhalothrin at the dose of 20–25mg/m². Residual efficacy of the insecticide was monitored through cone bioassay using laboratory reared mosquitoes Kismu strain (R = 70) of An. gambiae ss. Cone bioassay was done every fortnight throughout 152 days using a total of 20–25 mosquitoes per cone. The World Health Organization Pesticide Evaluation Scheme (WHOPES) threshold (70% mortality) was used as cut point for acceptable residual efficacy.

RESULTS: A total of 5,000 mosquitoes were subjected to contact bioassay to test residual efficacy of lambda-cyhalothrin. There was a statistically significant variation in residual efficacy between the different types of wall surfaces (R²=0.24; p-value=0.001). Residual efficacy decreased with increasing pH of the substrate (R²=0.2; p-value=0.027). Based on WHOPES standards, shorter residual efficacy (56–77 days) was found in wall substrates made of cement, limestone, mud-daub, oil paint and white wash. Walls made of burnt bricks; iron sheet, palm thatch and wood retained the recommended residual efficacy beyond 152 days.

CONCLUSION: The study found a wide variation in residual efficacy of micro encapsulated formulation of lambda-cyhalothrin across the types of wall surfaces studied. In areas where malaria transmission is bimodal and wall surfaces of short residual efficacy (2 – 3months) compose > 20% of sprayable surface, two rounds of IRS using lambda-cyhalothrin should be considered. Further studies are required to investigate the impact of sprayable surfaces on residual efficacy of other insecticides commonly used for IRS in Zanzibar and Mainland Tanzania.
**P521: Enhanced malaria surveillance in Zanzibar: initial results of nine months of malaria case notification and response in pre-elimination settings**

Issa A. Garimo1, Michael McKay2, Abdul-wahid Al-mafazy1, Abdullah S. Ali3, Mohammed Ali3, Wahida Hassan1, Mahdi M. Ramsan1, Uche Ekenna4, Richard Reithinger5, Jessica M. Kafuko6, Jeremiah M. Ngondi6


**BACKGROUND:** Active case detection and treatment has the potential to reduce parasite reservoir and reduce transmission of malaria. Zanzibar Malaria Control Programme (ZMCP) introduced malaria case notification (MCN), an active malaria surveillance system, in August 2012. Through the MCN surveillance system details of individual malaria cases are collected and transmitted in real time through the use of mobile phones and android tablets to a central database. We report initial results of malaria cases notified over a nine month period: September 2012 to May 2013.

**METHODS:** Malaria case data were reported via Unstructured Supplementary Service Data (USSD) mobile phones from health facilities to a Central database. For each case, a massage was sent from Health Facility to a Central server and the server generated an SMS alert

**PRELIMINARY RESULTS:** Over the nine-month period, a total of 1,439 index malaria cases were notified, of whom 1,013 (70.4%) were followed up at the household level. Loss to follow-up was due to incorrect address information and delays in follow-up following increased notification of cases during peak transmission. A total of 4,627 household members were tested with mRDT, of whom 349 (7.5%; range by district 2.6% to 13.8%) were positive for malaria.

**CONCLUSION:** Zanzibar MCN surveillance system demonstrates that active case detection and treatment of malaria cases is feasible. Routine targeted testing and treatment of at risk populations alongside other preventative interventions is likely to reduce malaria transmission and malaria morbidity, and enhance malaria elimination efforts in Zanzibar.

**RESULTS:** This study revealed that, macerated extracts and decocted powder from *Cupressus macrocarpa*, *Cupressus arizonica* and *Cupressus evergreen* contained saponins, phenols, alkaloids, terpenes, tannins and flavonoids. Larvicidal bioassays showed a 100% mortality of the *Anopheles gambiae* larvae at a concentration of 0.250 g / ml for macerated extracts and 80% mortality at a concentration of 0.250 g / ml for the extracts by decoction. The LC 50 and LC 80 measured for all extracts indicated the same order of reactivity. However, macerated extracts were more effective than extracts by decoction.

**CONCLUSION:** These results imply that these extracts possess larvicidal properties and could be exploited in the fight against malaria through vector control.

**P522: Larvicidal effects of extracts from three plants of the cupressaceae family on *Anopheles gambiae* larvae, the main malaria vector in Cameroon**


1Molecular parasitology and disease vector research laboratory, Biotechnology center, University of Yaounde I. 2Laboratory of Phytochemistry and Medicinal plant study, University of Yaounde I.

**BACKGROUND:** Malaria is a major public health problem in Cameroon. Despite all the efforts by the National Malaria Control Program to reduce this burden, it remains the leading cause of infant mortality. This failure is due to both the development and the spread of drug-resistant parasites and insecticide resistant vectors. Thus, new control tools are needed. This study therefore aimed to assess the lethal effect of extracts of three species of *Cupressus* on larvae of the *Anopheles gambiae* vector of the *Plasmodium* parasite in order to better contribute to the fight against malaria through vector control.

**METHODOLOGY:** To achieve this, three species of *Cupressus* (*Cupressus macrocarpa*, *Cupressus arizonica* and *Cupressus evergreen*) were harvested and aqueous extracts prepared by maceration and decoction. Phytochemical tests were carried out to determine the composition of secondary metabolites of the various extracts. The larvae of *Anopheles gambiae* were collected in Yaounde by the dipping technique using a scoop. Larvae were used to assess the mortality at various concentrations of the extracts using the World Health protocol for assessing larval mortality on natural plant products.

**RESULTS:** This study revealed that, macerated extracts and decocted powder from *Cupressus macrocarpa*, *Cupressus arizonica* and *Cupressus evergreen* contained saponins, phenols, alkaloids, terpenes, tannins and flavonoids. Larvicidal bioassays showed a 100% mortality of the *Anopheles gambiae* larvae at a concentration of 0.250 g / ml for macerated extracts and 80% mortality at a concentration of 0.250 g / ml for the extracts by decoction. The LC 50 and LC 80 measured for all extracts indicated the same order of reactivity. However, macerated extracts were more effective than extracts by decoction.

**CONCLUSION:** These results imply that these extracts possess larvicidal properties and could be exploited in the fight against malaria through vector control.

**P523: Behaviour modifying effects during and after exposure of pyrethroid-susceptible and resistant *Anopheles gambiae* to transfluthrin and metofluthrin coils: potential for malaria transmission control.**

Raphael N’Guessan1,2, Achille Oumbouke1, Augustin Founngnikin1, Mark Rowland1, Martin Akogbeto1, Sarah J Moore1

1Department of Disease Control, London School of Hygiene & Tropical Medicine, Kepel Street, London, UK; 2Centre de Recherche Entomologique de Cotonou, Cotonou, Benin

**BACKGROUND:** Tackling malaria vectors indoor with Insecticide Treated Nets (ITNs) and Indoor residual Spraying (IRS) is leading to behavior change of vectors and build up of residual outdoor malaria. Spatial repellents (SR) applied outdoor could be the solution but most of them are pyrethroid-based products. The objective of this study is to determine any difference in spatial repellence between susceptible and pyrethroid-resistant strains of *A. gambiae* to show SRs potential to control malaria transmission in diverse epidemiological settings including areas with pyrethroid resistance.

**METHODS:** We used Semi-Field Tunnel (SFT) in Benin to assess the repellence range (up to 65m) and toxicity that pyrethroid-based coils (transfluthrin and metofluthrin) used by man would confer against pyrethroid-susceptible and resistant *A. gambiae* bearing the knock down resistance (*kdr*) gene. The ability of mosquitoes to take subsequent blood meal after surviving the SFT space containing vapors of the SRs was evaluated in laboratory.

**RESULTS:** Metofluthrin and transfluthrin induced repellence rate of pyrethroid-susceptible *An. gambiae* similar to that of the resistant strain and the trends at all distance range were not distinguishable. Pyrethroid-resistant individuals recovered from SR exposure and bloodfed on the animal sooner than their susceptible counterpart. Forty hours after exposure, between 40-60% of susceptible *A. gambiae* were unable to bloodfeed compared to nearly 100% feeding success with resistant *A. gambiae*. Transfluthrin but not so for metofluthrin, delivered sublethal deposit, killing no greater than 25% of both strains.

**CONCLUSION:** The data supports the hypothesis that Metofluthrin and transfluthrin coils have potential for malaria transmission control and suggest they would do so by creating a vector-free space, even in areas with pyrethroid resistance. The fact that detoxification of SR molecules was faster in *An. gambiae* carrying the *kdr* mechanism suggests possible association/interaction between the target site of pyrethroids and specific target sites within the mosquito antennae where SRs bind to trigger repellence. There is a need to confirm this trend under more realistic condition using experimental huts and explore this hypothesis further by electrophysiology.
P524: Community perceptions of malaria and malaria treatment seeking behaviours in Cameroon

Joel N. Ambellaa, Abanda N Njei1, Ignatius N. Cheng3, Sarah N Ndive1, Rachel Nguela2, Joelle Pamen-Ngakoa, Ojong Barnabasa, Leile Albertine, Theresia N. Metchi1, Lindsay J Mangham2, Clare Chandler2, Virginia Wiseman2, Wilfred F Mbach1a.

1 The Laboratory for Public Health Research Biotechnology, The Biotechnology Centre, University Of Yaounde I; 2 London School of Hygiene and Tropical Medicine

BACKGROUND: One of the most important factors in the success of any malaria management programme is to understand what malaria is within the household. This study investigates the local communities’ understanding of malaria transmission, recognition of signs and symptoms, perceptions of cause, treatment-seeking patterns, preventive measures and practices in Cameroon.

OBJECTIVES: The aim of this study was to assess treatment-seeking behaviours for reported malaria among all age groups in order to reorient the country’s National Malaria Control Programme on appropriate intervention strategies to ensure appropriate treatment for malaria and provide good quality care to patients.

METHODS: Focus Group Discussions were held with demographic groups of community members who attend health centers most frequently (primary caregivers, adult women and adult men). In all, 14 focus group discussions were held with 150 participants.

RESULTS: The main findings of this study were that there was a high score of participants perceiving fevers as malaria. There was a high level of automedication and the use of ‘heath shock therapy’ for the treatment of malaria. Most of the participants recognized malaria as an acceptable disease compared to another disease like HIV. Participants preferred to consult at mission health facilities compared to cheaper public health facilities.

CONCLUSION: This study has shown the importance of home base management of malaria and the importance of seeking malaria treatment from non public health facilities such as mission health facilities and Patent Medicine Dealers as first resort to malaria. This necessitates the importance of strengthening community based interventions and the private sector in the fight against malaria. Community members should be informed and educated about the importance of early diagnosis and appropriate treatment of fevers within 24 hours of symptom onset.

P525: Immune responses to Plasmodium falciparum in relation to age, exposure and clinical outcomes in a prospective cohort of Mozambican children

Nhabomba A1, Guinovart C2,3, Manaca N1, Jimenez A1, Mayor AG1,2, Quintó I1, Aguilar R2, Barbosa A1, Bassat Q1,2, Aponte J1,2, Chitnis C1,2, Alonso PL1,3, Doballo C1,2

1Barcelona Centre for International Health Research (CRESIB-Hospital Clinic, Universitat de Barcelona), Catalonia, Spain; 2Manhiça Health Research Centre (CISM), Mozambique; 3Queensland Institute of Medical Research (QIMR), Brisbane, Australia; *International Centre for Genetic Engineering and Biotechnology (ICGEB), New Delhi, India

BACKGROUND: In malaria endemic areas of sub-Saharan Africa the negative outcomes of Plasmodium falciparum (Pf) infection concentrate primarily in infants and pregnant women. Understanding mechanisms of naturally-acquired immunity and identifying immune correlates of protection will contribute to the rational design and deployment of novel malaria vaccines.

METHODS: We used prophylactic treatment in three groups of infants between 2.5 to 5 months, 5 to 10.5 months and 2.5 to 10.5 months of age to control for the age of first blood stage infection with P. falciparum, defining early exposure, late exposure and continuous exposure. Children were followed up to 24 months of age for malaria morbidity and immunological responses. We used ELISA to measure total IgG, IgG subclasses and IgM responses to MSP-1, AMA-1, EBA-175 parasite antigens, and flow cytometry to measure IgG against variant surface antigens. We assessed the factors affecting antibody responses in relation to chemoprophylaxis and past, present and future malaria episodes.

RESULTS: The magnitude and breadth of antibody responses in children were significantly affected by exposure to Pf, age, season and neighbourhood of residence, and in general were not associated with reduced clinical malaria incidence, except for anti-EBA-175 IgG (IRR 0.67, p=0.018). Overall, antibodies were markers of Pf exposure rather than protection and only IgG to EBA-175 correlated with reduced risk of malaria in children.

CONCLUSIONS: The age of first parasite exposure did not influence the magnitude and breadth of IgG responses, but previous exposure was critical for antibody acquisition. IgG responses to EBA-175 were the strongest correlate of protection against clinical malaria.

P526: Monitoring of first line antimalarials drugs in 5 sentinel sites in Mozambique

Abel Nhama

The World Health Organization (WHO) currently recommends the use of artemisinin-based combinations (ACTs) for the treatment of uncomplicated P. falciparum malaria. With the first evidence of emergence of artemisinin resistance in South East Asia, it has now become critical to conduct robust surveillance of in vivo efficacy of ACTs in malaria-endemic areas where they have been deployed as first line treatment. We conducted two consecutive clinical surveillance studies of the efficacy and safety of two ACTs (artemether-lumefantrine, AL and amodiaquine-artesunate AQ-AS, first and second line policy respectively) in five sentinel health posts across Mozambique. Directly observed administration of the study drugs to children aged 6 months–5 years was conducted, followed by 3 days consecutive, and weekly visits for 28 days, following the WHO 2009 protocol. Of 2587-screened children, 439 (17%) fulfilled inclusion criteria, signed an IC and were recruited for the AL arm of the study in the 5 sites. Crude (PCR uncorrected) efficacy was 89.1% (330/370), increasing to 93.8% when adjusting by PCR for new infections. 16% of the patients (69/439) were lost during follow-up. For the AQ-AS arm, a total of 2154 patients were screened in the three sites with highest malaria incidence, and 261 (12.1%) recruited to the study. PCR-uncorrected and corrected efficacy for AQ-AS was very high (225/226, 99.6%). One death not deemed related to the study drug occurred in the AQ-AS group, in an 1 year old child who was treated at home (according to verbal autopsies) with traditional medicine after hospital discharge. Serious adverse events were rare (only 7), and in general both drugs were very well tolerated.

CONCLUSIONS: Although the efficacy of AL seemed slightly lower than that of AQ-AS, both drugs remain highly efficacious and safe and can be routinely used for the treatment of uncomplicated malaria in Mozambican children.

P527: Partial reproductive isolation between the M and S molecular forms from the westernmost of their range

El Hadji Amadou Niang1, Lassana Konaté3, Mawlouth Diallo1, Ousmane Faye5 & Ibrahim Diaw4

1Unité d’Entomologie Médicale, Institut Pasteur de Dakar; 2Laboratoire d’Écologie Vectorielle et Parasitaire, Université Cheikh Anta Diop de Dakar

BACKGROUND: Throughout west and central Africa, Anopheles gambiae M and S molecular forms are characterised by largely overlapping geographical/temporal distributions and high levels of reproductive isolation. However, at the westernmost extreme of their range, a secondary contact zone between these forms has been recently
revealed based on the finding of putative M/S hybrid frequencies higher than in the rest of their range (i.e. 3-7% in The Gambia and >20% in Guinea Bissau), mainly in coastal areas. But, the cross-sectional nature of these studies raises the question of transitional nature, mainly the stability of frequencies over time but also the extent of this phenomenon in inland.

METHODS: Indoor-resting females were sampled from July to December 2010, along two transects in the inland extreme eastern zone of Senegal that differ in their socio-demographic status such as the presence or absence of Rivers' flood plain, agricultural practices and insecticide pressure. Specimens were identified to species and molecular forms by PCR-RFLP and Kdr mutation molecular detection was performed.

RESULTS: A total of 2356 An. gambiae s.l. were sampled from 20 sites surveyed in the two transects. In each site, M and S molecular forms co-existed sympatrically and substantial proportions of M/S hybrid forms were seen (3.8 and 3.9 respectively in Transect 1 and Transect 2). At spatial level, a large spectrum of estimated inbreeding coefficient values for this putative speciation from 0.63 to 1 in transect 1 and 0.55 to 1 for transect 2 was recorded, whereas temporally, these frequencies ranged from 0.71 to 0.89 and 0.27 to 0.92. In both transects, M/S hybrids forms were observed only from July to September. Thus, the proportions of M/S hybrids were less than expected under Hardy-Weinberg equilibrium with complete panmixia. The mutation L1014F was observed in the two molecular forms and for both transects. But, the kdr alleles frequencies were not significantly higher in transect 1 as expected. Significant differences were, however, noted within the S form between the two transects (inbreeding coefficients respectively 0.08 and 0.27).

CONCLUSION: These results show a partial reproductive isolation and indicate that speciation process is underway in comparison to coastal areas where interbreeding and minimal genetic differentiation was observed.

P528: Efficacy of the combinations Artemether-Lumefantrine and Artesunate/Sulfadoxine-Pyrmethamine and quiescence of *P. falciparum* isolates tolerant to artemisinin in Mali.

Karamoko NiABE1, Antoine DARA1, Antoine BERRY2, Abdoulaye DIIMDE3, Ogbara K. DOUMBO1

1Malaria Research and Training Center- University of Sciences, Techniques and technologies of Bamako, Mali; 2Service Universitaire de Parasitologie-Mycologie CHU Toulouse, France

BACKGROUND: It has been showed that *P. falciparum* entered a state of quiescence in response to stress caused by exposure to artemisinin (ART) and its derivatives. Recently, some laboratory strains resistant to artemisinin have been selected. The quiescence character was demonstrated to be implicated in the mechanism of resistance to ART. However, we have not been able to demonstrate the direct relationship between concentrations of 50 and 100nM of ART and the occurrence of a given parasite stage. The PCR-corrected *in vivo* efficacy rates of both combinations AL (98.75%) and AS/SP (100%) were comparable.

CONCLUSION: Considering the results obtained, further studies are necessary to understand the tolerance of parasite isolates to ART by the phenomenon of quiescence in Mali.

P529: Submicroscopic gametocytes and reservoir of Malaria in relation to age after new malaria control strategies implementation in different areas of Gabon


Department of Parasitology-Mycology, Faculty of Medicine, Université des Sciences de la Santé, BP 4009 Libreville, Gabon.

BACKGROUND: Following the introduction of malaria new strategies control in Gabon in 2005, the scaling of malaria control to achieve national coverage requires a better understanding of the population sub groups that constitute barriers to interrupt transmission, being presumably gametocytes reservoir. The aim of the present study was to evaluate the proportion of submicroscopic trophozoites and gametocytes carriers in febrile patients living in different areas of Gabon, Central Africa.

METHODS: Blood samples from febrile patients living at Libreville (CHL), at Port-Gentil (CHR), at Oyem (CHRO) and at Melen (HREM), were collected between June 2011 and February 2012. Asexual and sexual forms of *P.falciparum* were identified using microscopy and Nucleic Acid Based Amplification (NASBA) methods. NASBA 18S mRNA and Pfs25 mRNA amplification was performed to determine trophozoites and gametocytes carriage by the Nuclisens EasyQ analyser (bioMérieux, Lyon, France) after nucleic acid extraction with the guanidium isothiocyanate silica procedure.

RESULTS: Microscopically, 173 (55.8%) out of 310 samples had *P. falciparum* asexual forms whereas three (0.96%) harbored gametocytes. Among the 137 samples with negative blood smears, 24.1% had submicroscopic trophozoites. Within the 307 samples without gametocyte on blood smears, *P25 mRNA* was amplified in 32%. The proportion of asexual and sexual parasite detected by either microscopy or NASBA was the lowest at Port-Gentil while it was the highest at the three others sites. Patients younger than 5years old were less infected. The lowest proportion of patients aged 0-4 years harboring trophozoites and gametocytes was found at Port-Gentil (25% and 8.3% respectively) (p<0.01).

CONCLUSION: These data indicate a high frequency of submicroscopic *P.falciparum* trophozoites and gametocytes carriage. Children older than 5 years are more frequently concerned. Immunological assays will complete the analysis to evaluate the level of exposition and the naturally acquired immunity level in the different age groups.

P530: Evaluation of Malaria Ag Pf (HRP2/PLDH) And Its Role on Monitoring Persistent Antigenicity.

Denise Niama-Meya1, Pascal Magnussen2, Richard Ndyomugenyi3, Anthony Mbonye1, Kristian S. Hansen4, Charles Karamagi1

1Makerere University College of Health Sciences, Kampala, Uganda; 2Centre for Medical Parasitology, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark; 3Vector Control Division, Ministry of Health, Uganda 4School of Public Health, Makerere University and Commissioner Health Services, Ministry of Health, Uganda; 5London School of Hygiene & Tropical Medicine, United Kingdom

BACKGROUND: The introduction of rapid diagnostic tests (RDTs) has provided a means for improving the diagnosis of malaria. RDTs based on *Plasmodium falciparum* histidine-rich protein 2 (PfHRP2) have been rolled out in Uganda and other regions where the predominant parasite is nodded based on the finding of putative M/S hybrid frequencies higher than in the rest of their range (i.e. 3-7% in The Gambia and >20% in Guinea Bissau), mainly in coastal areas. But, the cross-sectional nature of these studies raises the question of transitional nature, mainly the stability of frequencies over time but also the extent of this phenomenon in inland.
**P531: Efficacy and safety of three Artemisinin-based combination therapies (ACTs) for uncomplicated malaria in Cameroon**

N.T. Meto1,2, G.P. Fon1, P. Gandji1, M. Ekoko1, S.R. Moyou1,4, X-N. Zhou1

1National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention; Key Laboratory of Parasite and Vector Biology, MOH; WHO Collaborating Center for Malaria, Schistosomiasis and Filariasis, Shanghai 200025, China; 2Faculty of Science University of Bamenda, West Region Cameroon; 3University of Twente (ITC) Hengeloestraat 99 7514 AE Enschede ACE Division (Alumni) Netherlands; 4Medical and Health Services Department Cameroon Development Corporation (CDC) Bato-Limbe, S.W. Region, Cameroon; 5Institute of Medical Research and Medicinal Plants-IMPM, Yaounde, Cameroon; 6Faculty of Medicines and Biomedical Sciences, PO Box 812 University of Yaounde I, Cameroon

**BACKGROUND:** Artemisinin-based Combination Therapies (ACTs) are reported to be effective against multidrug-resistant Plasmodium falciparum malaria. As their consumption increases, the efficacy may have another profile in the management of malaria suspected cases. Besides, it is the problem of auto-medication usually under dosed by most malaria patients whose consequences are recrudescence of resistance parasite. This study is aimed at monitoring ACTs efficacy in order to assist the NMCP in its search for alternative treatment options, when these ACTs will no longer be effective and safe in malaria management.

**METHODS:** We evaluated the efficacy and safety of dihydroartemisinin-piperazine (DHPA), artemunate-mefloquine (ASMQ) and artemether-lumefantrine (AL), in the treatment of malaria in one of the high malaria transmission areas of S.W. Region of Cameroon and particularly within the health facilities of Cameroon Development Cooperation (CDC). Outpatients having amongst other criteria, a pre-treatment parasite density of ≥2000 μL⁻¹ of blood were enrolled for the study following the WHO protocol. Informed Consent was obtained and ACTs given on days 0, 1 and 2, with 42 days follow-up for each patient.

**RESULTS:** Out of 1555 patients screened within 3 months on the field, 216 met the enrolment criteria but 207 completed the trial. The success rates, PCR uncorrected for AL on D28 were 97.10%, 97.14% and 98.57% for AL, ASMQ and DHAP respectively. On D42, the success rates were 95.65% for AL, 95.58% for ASMQ and 97.14% for the DHAP group. Parasite clearance time was shorter in the DHAP group 28.7±9.64 hours as compare to AL, 34.3±18.40 hours (CI 95% 3.25-8.08;P=0.0001) and ASMQ 39.7±24.14 hours(CI 95% 7.34-14.66;P=0.0001). There were no major adverse reactions in the AL and DHAP group except of mild but higher frequency of, loss of appetite, nasal congestion and pruritus while vomiting, abdominal pain were reported couple with mild hallucination and dizziness in the ASMQ group.

**CONCLUSION:** These findings provide evidence that DHAP is the most efficacious, safe and well tolerated ACT as compare to AL and ASMQ. It is therefore recommended as a better alternative treatment of malaria in Cameroon and in Africa in general.

**P532: Anopheleline fauna and its implication in the transmission of malaria in Pitoa health district, Northern Cameroon**

Molecular Parasitology and Disease Vector Research Laboratory, the Biotechnology Centre, University of Yaounde I.

**BACKGROUND:** The northern part of Cameroon, because of its humid climatic conditions coupled to its marshy areas used for rice cultivation and a large number of stagnant pools resulting from the river Benoue, has been noted to be a zone heavily infested with mosquitoes. This is directly reflected in the high prevalence and an increasing incidence of clinical malaria in this area. In this study we aimed at examining the role of the Anopheles fauna in the transmission of malaria in Pitoa Health District in order to improve upon anti vector control measures.

**RESULTS:** A total of 2250 Anopheles mosquitoes were collected, with Anopheles gambiae s.l representing the principal Anopheles vector (77.2%). Anopheles rufipes (7.8%), Anopheles funestus (6.4%), and Anopheles paludis (5.1 %) were minor vector species. Averagely, individuals received 3.5 ib/p/n. However, the entomological inoculation rate was as high as 8.5 ib/p/n in some villages. Anopheles gambiae showed a parity index of 82.5%.

**CONCLUSION:** Anopheles gambiae remains the principal Anopheles vector species in Pitoa. This results show that existing anti vector control measures have to be reinforced and new methods implemented in order to scale down transmission in this zone.

**P533: Antibodies to crude Plasmodium falciparum Blood Stage Antigens in a Cohort of Children Living in Mutengene**

Clarisse Nju Ya-Ni, Judith Anchang-Kimbi, Tobias Apinjoh, Regina Mugri, Hanesh Chi, Roland Tatabi, Emmanuel Nkock and Eric Achidi

Malaria Research Laboratory, Faculty of Science, University of Buea, Buea, Cameroon

Background: Decades of research have shown that naturally acquired antibodies are important for protection against blood stage malaria parasites. Previous studies demonstrated an association between cytotoxic antibodies with protection against Plasmodium falciparum malaria. In other studies, IgG2 antibodies to some P.falciparum antigens have been associated with protection, indicating that the role of IgG subclasses in malaria protection still needs to be elucidated.

Methodology: The study was carried out in Mutengene, a semi-urban community located in Fako division of South West Cameroon. A cohort of 357 children aged ten years and below recruited from randomly selected households were followed for 12months. The incidence of malaria was determined by bi-monthly morbidity surveys and blood sampling every trimester for malaria parasitaemia examination and antibody ELISAs.

Results: Participants positive for malaria parasites increased from...
P534: Resistance to insecticides and bionomics of urban populations of Anopheles gambiae in the cities of Douala and Yaoundé Cameroon.

Christophe ANTONIO-NKONDJO1,2, Billy TENE FOSSOG1,2, Cyrille NGO1,2, Parfait AWONO-AMBENE2, Charles S. WONDJI1, Carlo COSTANTINI1 and Hilary RANSOM2

1Laboratoire de Recherche sur le Paludisme, Organisation de Coordonnation pour la lutte Contre les Endémies en Afrique Centrale (OCEAC), P.O. Box 288, Yaoundé, Cameroon; 2Faculty of Sciences, University of Yaoundé I, P.O. Box 337, Yaoundé, Cameroon; 3Vector group Liverpool School of Tropical Medicine Pembroke Place, Liverpool UK L3 5QA; 4Institut de Recherche pour le Développement (IRD), UR 016, 911, avenue Agropolis, P.O. Box 64501, 34394 Montpellier cedex 5, France.

BACKGROUND: Urban malaria is becoming a serious challenge for malaria control. We report about a series of studies conducted in the cities of Douala and Yaoundé to assess malaria vectors bionomics and insecticide resistance.

METHODS: Anopheline breeding sites distribution was assessed by monthly investigations in the cities of Douala and Yaoundé. Physicochemical parameters of breeding sites were analyzed and compared across classes (urban vs peri-urban localization; polluted vs unpolluted vs cultivated sites). Malaria transmission dynamic was assessed using human landing catches and light traps. The level of susceptibility of mosquito to various insecticides and xenobiotics was determined using bioassays on larvae and adults. Mosquitoes resistant to insecticides were screened to detect the presence of the kdr alleles and carbamates. No significant differences (potassium, ammonia, total hardness and conductivity). Adult mosquito collections in the city of Douala detected a seasonal pattern for malaria transmission with an annual Entomological Infection Rate of 31 infected bites/human/year. Mosquitoes originating in urban agriculture areas generally appeared highly tolerant to almost all insecticide compounds. A close association was demonstrated between selection by xenobiotics and increase tolerance to insecticides. Resistance to DDT and pyrethroids was associated to a high prevalence of kdr alleles and overexpression of several detoxification enzymes such as CYP6M2, CYP6P3, GSTD1-6 known to confer resistance to pyrethroids. No specimen was recorded with the ace-1R mutation conferring resistance to carbamates.

CONCLUSION: Our data confirm the current adaptation of An. gambiae to urban areas and the huge diversity of resistance mechanisms. The present study calls for more attention on the evolution of malaria and vectors in urban areas.

P536: Perceptions, Uptake and Impact of Indoor Residual Spraying in Urban Setting in North-western Tanzania

Soori Nnko

BACKGROUND: Malaria is one of the greatest health challenges facing the developing world. In Tanzania, malaria remains a major cause of hospital admissions and deaths especially in children < 5 years. In order to complement other malaria control interventions in the country, the Tanzania Ministry of Health and Welfare recently introduced indoor residual spraying (IRS). IRS is the application of long-lasting chemical insecticides on the walls and roofs of all houses and domestic animal shelters in a given area in order to kill the adult mosquitoes that land and rest on these surfaces.

OBJECTIVE: To determine community members perceptions, attitude and uptake of indoor residual spraying intervention, and evaluate the impact that IRS has on malaria outcomes.

METHODS: To explore perceptions and attitudes we conducted Focus Group Discussions and In-depth interviews with community members. To validate the diagnosis of malaria we re-examined blood samples that had been collected and tested by laboratory technicians from the selected health facilities in the study area. Furthermore we reviewed health facility based records to establish the changing patterns of malaria prevalence for a period of two years prior and following introduction of IRS intervention.

FINDINGS: One month after the spraying, we managed to collect only few (4) Anopheline mosquitoes. The number of mosquitoes increased as time elapse after the spray of insecticide. Hospital based record indicated the sharp decline of malaria cases following the introduction of IRS intervention. People were enthusiastic about the impact of IRS and because of the impact that IRS has on mosquitoes, some community members have stopped using other preventive measures against malaria. Although the majority of community members appreciated the intervention and willingly allowed their houses to be sprayed, some community members refused their houses to be sprayed for fear that IRS could be harmful. It was rumoured that IRS can cause infertility, TB and other respiratory tract infections, whereas other suspected that IRS was not efficacious to kill mosquitoes. Skeptical persons who accepted their houses to be sprayed only did so for fear that refusal could be punishable by the state machinery.

P537: Mass drug administration in malaria hotspots following increased seasonal transmission of malaria in 2013: Zanzibar experience

Abdullah S. Ali1, Ritha Willilo2, Bakar Khathi1, Hajj H. Amieri1, Joseph Shija1, Mwinyi Mslemu1, Ally Khambi1, Abdul-wahid Al-mafazy1, Issa A. Garimo2, Mahdi M. Ramzan3, Jessica M. Kafuko4, Jeremiah M. Ngondu4

1Zanzibar Malaria Control Program, Ministry of Health, Zanzibar, Tanzania; 2RTI International, Dar es Salaam, Tanzania; 3United States Agency for International Development/President’s Malaria Initiative, Dar es Salaam, Tanzania.

BACKGROUND: Mass drug administration (MDA) with anti-malarial drugs is effective in clearing malaria parasitaemia and thus interrupts malaria transmission. We report Zanzibar Malaria Control Programme (ZMCP) experience in implementing MDA with artesasmine-based combination therapy (ACT) to mitigate increased seasonal transmission of malaria in selected hotspots.
METHODS: MDA was done in four Sheinia selected on the basis of high malaria incidence detected through malaria case notification. Communities were sensitized through public address system and local community leaders. Teams of health care workers distributed MDA using a house to house approach. All household members, except pregnant women and children aged under 2 months, were provided with the treatment. The first dose was dispensed as directly observed treatment (DOT) for household members present. ACT doses for absent members were provided for absentee in a labelled package. Long lasting insecticidal nets (LLIN) were distributed in households to cover all sleeping spaces. Following MDA, information reminding communities about second and third dose was provided through public address systems and community leaders. Two weeks after MDA campaign, a survey was undertaken to investigate completion of ACT doses.

PRELIMINARY RESULTS: A total of 9,655 people in 2,001 households received treatment with ACT and 5,125 LLIN distributed to ensure 100% net access. During post MDA surveys, A total of 1,944 people were interviewed of whom: 93.7% reported having completed MDA doses; 3.4% did not complete dosage; and 5.4% did not take treatment. Reasons for failure to complete treatment were: fear of side effects; hunger or fasting; forgot to take treatment; and busy with daily chores. Survey participants not taking treatment reported reasons, including: fear of side effects, pregnancy, and insufficient information about MDA. Majority (94.2%) of survey participants reported that they would take part in MDA in the future.

CONCLUSION: This activity demonstrates that MDA for malaria was highly accepted by communities at high risk of malaria in Zanzibar with high participation and completion rates. Together with preventative measures, MDA has the potential to interrupt transmission of malaria and accelerate ZMCP efforts towards malaria elimination. Use of a single dose of anti-malarial drugs could enhance completion of treatment. (withdraw)

P538: Malaria chemotherapy increases the possibility of elimination of the disease in low transmission areas.

Paul Noah H1,2, Midzi Nicholas1, Chidzwoondo Farisai1, and Mduulza Takafira1.
1Biochemistry Department, University of Zimbabwe, P O Box MP 167 Mount Pleasant, Harare; 2Research Council of Zimbabwe, Delken Complex. Mount Pleasant. P O Box CY 294 Causeway, Harare; 1Food and Biomedical Technology Institute, Scientific and Industrial Research and Development Centre, P O Box 6640, Hatcliffe, Harare, npaulnaul@gmail.com/ npaul@sirdc.ac.zw

INTRODUCTION: Plasmodium falciparum malaria and schistosomiasis are found to be co-existent in the developing countries and also in Zimbabwe. These diseases have been associated with malnutrition and poverty. It has been hypothesized that schistosomiasis co-infection with Plasmodium falciparum malaria may modulate the immune response to schistosomes leading to increased susceptibility to clinical malaria severity of other cases. We investigated the immunological profiles and the effects of treatment during co-infection in an exposed community.

MATERIALS AND METHODS: The study was conducted in the Mashonaland East Province of Zimbabwe at schools where previous prevalences showed that S. haematobium is endemic and there is moderate P. falciparum transmission. A community based study and sampling was conducted cross sectionally at baseline and longitudinally involving examination and treatment of the study population at baseline, 6 weeks, 6 months, and 12 months follow up surveys. Blood samples were obtained at every sampling time point and the serum was separated and used to determine immunological profiles against diverse anti-parasite (S. haematobium and P. falciparum) antigen antibody through the ELISA method.

RESULTS AND DISCUSSION: There was no malaria infection as observed using thick blood smears on 200 fields examination and confirmed by the rapid test kit Paracheck Pf™ probably due to low parasite density. The population exhibited auto-antibodies which could not be attributed to an active malaria infection but to schistosomiasis. There was no relationship between the IgM directed against MSP1-19 and MSP2 (χ²=0.293 df=1, p=0.588) and χ²= 0.336, df=1, p=0.562, respectively. The results showed that children as young as 6 months had been exposed to malaria parasites, and also that children as young as 1 year were already developing immune responses directed against malaria vaccine candidates, confirming observations that children gradually develop acquired resistance to malaria parasites.

CONCLUSION: The study has shown the anti-malaria drug currently in use Coartem-Ether™ is highly effective due to the gametocidal effects; thereby continued usage may reduce malaria incidences in regions of low transmission. The results show the possibility of elimination of malaria due to the break in the transmission cycle at the human stage.

P539: Widespread immunity against targets of transmission blocking immunity in a malaria moderate transmission region in Zimbabwe.

Noah H Paul1, Vinay Kumar1 Dibyaduti Datta3, James Chipeta1 Nibhay Kumar3 and Takafira Mduulza1.
1University of Zimbabwe, Biochemistry Department, Mount Pleasant, Harare; 2Scientific and Industrial Research and Development Centre, Hatcliffe, Harare; 3Tulane University, Department of Tropical Medicine and Public Health. New Orleans; 4University of Zambia, Lusaka, Zambia

BACKGROUND. Malaria remains a leading killer disease among Africa. The main focus has been on control and eradication of malaria. While artemisinin combination therapy and insecticide treated mosquito nets are some of the ways to prevent malaria, development of vaccines remains a goal still to be achieved. Pre-clinical studies on transmission blocking vaccines (TVB) have shown marked efficacy, 96-100%. A TVB works by blocking the sexual development of parasites in the mosquito and as a result no oocysts are produced. There are reports on the presence of natural immunity against some of the antigenic targets of TVB and a successful TVB is expected to play critical role in the malaria elimination goal. We therefore aimed to screen for transmission blocking immunity from a population that is exposed to P. falciparum malaria in Zimbabwe.

MATERIALS AND METHODS: The study was carried out in school-going children (7-16 years age, N=150) in Makoni District, Zimbabwe, Prevalence of malaria diagnosed by slide and rapid diagnostic kit (Paracheck™) through diagnosis was 5.2 % and 30.3 % had been treated recently using Coartem™. Additionally, the prevalence of schistosomiasis was S. haematobium 59.3%; S. mansoni 38.1%. Schistosomiasis was screened using urine filtration and the Kato-Katz method. Serum and whole blood samples were used for ELISA and membrane feeding assays for malaria transmission blocking immunity evaluation.

RESULTS AND DISCUSSION: Analysis of serum samples by ELISA revealed that almost the entire population had previous exposure to P. falciparum malaria as evidenced by the presence of against the crude parasite lysates 95.3 % at baseline and 83.9% (104) 6 weeks post schistosomiasis treatment. Most significantly 99.3 % responded both to the recombinant Pf 48/45 protein and the Pf 47 proteins. A few randomly selected sera samples from this population were also tested in mosquito membrane feeding assays (MFA). The MFA demonstrated measurable transmission blocking activity- 4 out of 20 sera revealed 56 to 84% transmission reducing activity.

CONCLUSION: The results show that there TVB targets of natural transmission blocking immunity in individuals from low-moderate transmission area of malaria. A TVB vaccine induced immunity further boosted by natural immunity may play significant role in the elimination of malaria transmission.

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**P540: Antibody immune responses to NTS-DBL1α domain of Plasmodium falciparum erythrocyte membrane protein 1 (PFEMP1) in paired maternal cord blood and babies from Fako Division, Cameroon.**

*Anong D. N.*, *Ndamukong E. A.*, *Ayonghe J.A.*, *Apinjo T.*, *Achidi E.*, *Rowe J*A*

1 *Department of Microbiology & Parasitology, University of Buea; 2*Department of Biochemistry & Molecular Biology, University of Buea; *3*Centre for Immunology, Infection and Evolution, Institute of Immunology and Infection Research, School of Biological Sciences, University of Edinburgh, Edinburgh, UK

**BACKGROUND:** Antibodies to the NTS-DBL1α domain of PFEMP1 which is strain specific is responsible for acquired immunity to malaria by disrupting rosetting, a phenomenon whereby PFEMP1 on the surface of infected erythrocytes binds to human receptors on uninfected erythrocytes, forming clumps that block blood flow in vessels resulting in severe malaria. The present study was to find out the extent to which NTS-DBL1α from different parasite strains elicits antibodies in pregnant women in Fako, if these antibodies can be transferred transplacentally to the foetus and if they remain in babies up to 1 year of age.

**METHODS:** Blood was collected from Sixty eight (68) pregnant women enrolled in the study after informed consent and antibody production against NTS-DBL1α proteins from 3 *Plasmodium falciparum* strains (TM180, PAR+ and MUZ12) measured from plasma by ELISA. The level of parasitaemia, gravidity, maternal age, gestation period and fever history during pregnancy were determined and related to total IgG levels in blood from mothers, maternal cord and their respective babies up to 1 year old.

**RESULTS:** Mean IgG response to TM180 was shown to be significantly higher than IgG responses to the proteins from the other two strains, PAR+ (P<0.05) and MUZ12 (P<0.0001). DBL1α IgG antibody responses to the three recombinant proteins were significantly higher in pregnant mothers than in their maternal cord and babies (P<0.0001). There was also a significant correlation between IgG levels in blood of mothers and that in their corresponding cords for all the recombinant antigens with PAR+ having the highest significance (P<0.0001, r=0.548).

**CONCLUSION:** We conclude that: there is acquired immunity to NTS-DBL1α domain in pregnant women NTS-DBL1α IgG antibodies can be transferred transplacentally to the foetus; These antibodies are present in babies until the age of one year, and strain diversity influences NTS-DBL1α IgG antibody levels. This molecule has potentials as a vaccine target against malaria.

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**P541: Operational assessment of physical integrity of Insecticide-Treated Nets (holes index) by the use of new and specific Anopheles salivary biomarker evaluating the human-vector contact.**

*Herbert Nkouko*1,2, *Georgia Damien*1,2, *Papa Drame*1,2, *Evelyne Chaffa*3, *Emmanuel Elanga*1,2, *Olayide Bousari*1,2, *Martin Akogbéto*1,2 & *Franck Remoué*1,2

1 *Centre de Recherche Entomologique de Cotonou (CREC), Ministère de la Santé, Cotonou, Bénin; 2*Institut de recherche pour le développement (IRD), Maladies Infectieuses et Vecteurs, Écologie, Génétique, Évolution et Contrôle (MIVEGEC), UM3 IRD 224 Cotonou, Bénin; *3*Programme National de Lutte contre le Paludisme (PNLP), Ministère de la Santé, Cotonou, Bénin; *Faculty of Sciences and Techniques (FAST) Laboratoire d’Etude et de Recherche en Statistique Appliquée et Modélisation (LERSAM), de l’Université d’Abomey Calavi (UAC), Benin

**BACKGROUND:** The widespread implementation of insecticide-treated nets (ITN) is a major intervention method to significantly reduce morbidity and mortality of malaria. Although the increase in ITN coverage has well documented, weak information is reported on physical condition and integrity of ITNs used by exposed populations. This study was included in a multidisciplinary project funded by Benin NMCP-World Bank and aimed to assess the human IgG specific to *Anopheles g*SG6-P1 salivary antigen as pertinent biomarker for evaluating the real physical integrity (holes index) of ITNs in the field.

**METHODS:** The data used for this analysis came from the 2011 of the household survey undertaken as part of the monitoring and evaluation of the NMCP campaign of mass ITN distribution (2007). Two health districts in Benin were selected in north and in south. The study population concerned 280 children (<5 years) using ITN, and randomly selected from 30 villages. IgG responses of children specific to salivary P1-gSG6 peptide were compared with ITNs physical integrity evaluated by holes index (HI), calculated according to WHO recommendations.

**RESULTS:** A strong positive correlation (r=+0.342 Spearman; p<0.0001) was observed between specific IgG level and Holes Index. The results showed three categories of ITN hole index according to anti-P1-gSG6 IgG level: i) when HI=0, it could represent the “ideal” situation where ITNs are in “good physical integrity” and human specific IgG level is the lowest, suggesting that children are very low exposed to Anopheles bites ii) HI[1,100] nets are in “moderate/intermediate physical integrity” and iii) HI>100 where ITNs are in “bad physical integrity”, corresponding to higher IgG level, suggesting that children are high exposed to *Anopheles* bites.

**CONCLUSIONS:** This preliminary study showed, the first time, a strong correlation between human exposure to *Anopheles* bites and ITNs physical integrity (Holes Index). These results suggest that human IgG to gSG6-P1 salivary peptide, previously demonstrated as a pertinent biomarker for measuring the real human-vector contact, could be a reliable alternative tool for accurately assessing the ITNs physical integrity. This approach can be a credible indicator for the pertinent monitoring and surveillance of malaria vector control.

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**P542: The Current Status Efficacy of Artesunate/ Sulphadoxine Pyrimethamine Tablets for the Treatment of Uncomplicated Plasmodium falciparum Malaria in Sud Anong Medani Locality, Gezira State, Sudan. Maha Mirghani Abdalla Maatoug1, Mirghani Abdelrahman Yousif*, Bakri Yousif Mohmmed Nour2,3

1,2*Faculty of Pharmacy, University of Gezira, Wad Medani, Sudan; 3*Blue Nile National Institute for Communicable Diseases, University of Gezira, Wad Medani, Sudan; *Faculty of Medical Laboratory Sciences, University of Gezira, Wad Medani, Sudan

**INTRODUCTION:** In Sudan the National Malaria Control Program adopted the use of artesunate + sulphadoxine-pyrimethamine (ASP) as the first line of treatment for uncomplicated malaria since 2004. This study was done in Medani town, Gezira State, Central Sudan to evaluate the current efficacy of ASP among infected patients within the national monitoring of antimalarial drugs.

**METHODS:** From October to December 2011, 81 Patients with uncomplicated *P. falciparum* malaria who met the study inclusion criteria were enrolled, treated with ASP and monitored for 28 days. The follow-up consisted of a fixed schedule of check-up visits and corresponding clinical and laboratory examinations. On the basis of the results of these assessments, the patients were classified as having therapeutic failure (early or late) or an adequate response to ASP according to the WHO(2005) susceptibility to antimalarial drugs protocol.

Blood samples from each patient were taken on Whatman filter paper (3M) on days 0, 7, 14, 21 and 28 and also the day when the parasite and symptoms reappeared for Polymerase chain reaction (PCR) to distinguish between a true recrudescence due to treatment failure and reinfection.

**RESULTS:** At the end of the follow up period (28 days) and before the PCR correction, 76/80(93.9%) of the 81 patients enrolled in the study were classified as had adequate clinical and parasitological response (ACPR), two (2.5%) as had late clinical failure (LCF) and late parasitological failure (LPF), two (2.5%) as lost to follow-up and only one patient (1.2%) as had early treatment failure.

The two patients whom were considered to have had late clinical failure (LCF) and late parasitological failure (LPF) when subjected to PCR correction. The result revealed that the late clinical failure and...
lack parasitological failure were due to reinfection rather than due to recrudescence. Therefore the parasitological and clinical efficacy of ASP was found to be 98.7% (77/79).

**CONCLUSION:** The finding of this study concluded that the first line treatment (ASP) for uncomplicated P. falciparum is still effective and monitoring its efficacy is recommended annually in all Sudan states.

**P543: Epidemic malaria outbreaks in Kasai occidental in DR Congo: Situation in three health districts.**

Célestin N Nsibu 1,2, Gauthier K Mesia 1, Dieudonné Mumba 1, Thierry L Bobanga 1, Célestin de Paul Maniangs 1, Clarisse K Mbo 1, Gaston T Lutete 1, Samuel M Mampunza 1.

1 Department of Pediatrics, University of Kinshasa, Kinshasa, DRC; 2 Pharmacovigilance Center, Pharmacology Unit, University of Kinshasa, DRC; 3 Tropical Medicine Department, Service of Parasitology and Entomology, University of Kinshasa, DRC; 4 Anthropology Department, University of Kinshasa, DRC; 5 National Malaria Control Program, Ministry of Medicine, DRC

**BACKGROUND:** Epidemic malaria has been defined as an acute exacerbation of disease out of proportion to the normal to which the community is subject. This suggests a decentralized approach based on data collection from individual health facilities, analysis and interpretation before any aggregation. Historical morbidity patterns are used as the monitoring basis of anomalies within prospective data. Epidemic situations were reported from January to May 2013 in four districts health in Kasai Occidental in the Democratic Republic of Congo.

**METHODS:** An investigation for malaria was carried out in three of the four districts targeting selected general hospitals, health centers and some households. A blood sample was obtained by finger prick or venipuncture from 90 people and anticoagulated with heparin for malaria testing by RDTs and microscopic examination (Giems-stained thin and thick smears), pan-species and specific-species polymerase chain reaction (PCR) (salmonella, amari virus, chikungunya, filovirus, measles) and Elisa (dengue, trypanosomiasis). Records of visited households (prevalence of fever the last two weeks and death of less than five years old) were collected on questionnaire.

**RESULTS:** From January to May 2013, a two-fold increase of malaria morbidity was found in these three districts when compared to the same period of 2012 (29983 versus 14668 cases, P<0.05) with an incidence of 5.87%. An increased incidence was observed in children <5 than in others (children and adults). From 90 blood samples collected in health facilities, Plasmodium falciparum (PF) was found in 93% of them when considered RDTs or 85% in microscopic test. Among 147 samples collected in the households, 35% were found positive to PF by microscopic examination. PCR was positive for PF in 81 patients (90%). Elisa was positive for dengue (1case): In the 100 households visited, the prevalence of fever in the last two weeks was 23% in general and 39% in under five. An average of 5.18% of specific malaria lethality was observed in health facilities last two weeks was 23% in general and 39% in under five. An average of 5.18% of specific malaria lethality was observed in health facilities (118/2304 cases of severe malaria) in children<5 in the epidemic period. Among the 100 households visited, 42 deaths of less than five years have been declared the last five months.

**CONCLUSION:** Malaria epidemic outbreaks were confirmed in these health districts in Kasai-Occidental.

**P544: Mosquitocidal and antifecundity effects of coumarin and betulinic acid isolated from Cassia siamea (Fabaceae) stem bark chloroform extract on female Anopheles stephensi (Diptera: Culicidae).**

G.F. Nsone Ndou 1, 2, A. Habluetzel 1, J.T. Banouzi 1, J.M. Ouamba 1, L. Luca Toni 1, A.A. Abena 1.

1 Département de Biologie et Physiologie Animale, Faculté des Sciences et Techniques, Université Marien NGOUABI, B.P. 69, Brazzaville, Congo; 2 Unité de Chimie du Végétal et de la Vie, Faculté des Sciences et Techniques, Université Marien NGOUABI, Brazzaville, B.P. 69, Congo; 3 Unité de Biochimie et Pharmacologie, Faculté des Sciences de la Santé, Université Marien NGOUABI, B.P. 69, Brazzaville, Congo

**BACKGROUND:** Mosquito acts as vector for various pathogens that cause malaria, filariosis, schistosomiasis, Japanese encephalitis, and yellow fever. Mosquito control is the key measure to prevent spreading these diseases are still a major problem in the developing countries. The strategy against malaria in the world includes the destruction by chemical, physical or biological agent of larvae, adult mosquitoes and reducing the number of human mosquito’s contacts. The aim of this study is to contributed to the development of new tools to fight against malaria.

**METHODS:** The bark of Cassia siamea were subjected to phytochemical investigation, which led to the isolation of coumarin and betulinic acid. We conducted a chronic administration in the form of food and natural product lead generation programs.

**RESULPS:** The bark of Cassia siamea were subjected to phytochemical investigation, which led to the isolation of coumarin and betulinic acid at the concentrations of 2000, 800 and 1600 ppm respectively, once every two days, for 21 days corresponding to the sporogonic cycle. 800 ppm of coumarin and betulinic acid. We conducted a chronic administration in the form of food and natural product lead generation programs.

**CONCLUSIONS:** Coumarin and betulinic acid showed an interesting antifecundity and mosquitocidal activities on the female anopheles and may be potential candidates to the development of new insecticides.

**P545: AfroMalariaDb: building a database for anti-malarial agents derived from African medicinal plants for virtual screening.**


1 Chemical and Bioactivity Information Centre, Department of Chemistry, Faculty of Science, University of Buea, Cameroon; 2 Department of Pharmaceutical Sciences, Martin-Luther University of Halle-Wittenberg, Wolfgang-Langenbeck Str. 4, 06120, Halle (Saale), Germany; 3 Department of Chemistry, Faculty of Science, University of Buea, Cameroon; 4 Department of Chemistry, Faculty of Science, University of Douala, Douala, Cameroon

**BACKGROUND:** Computer-aided drug design (CADD) often involves virtual screening (VS) of large compound datasets and the availability of such is vital for drug discovery protocols. We assess the medicinal value and “drug-likeness” of a relatively small but structurally diverse dataset (containing ~700 compounds, from which >1,000 compounds could be derived synthetically) from African medicinal plants, which have been tested against malaria or anti-malarial drug targets.

**METHODS:** The geographical regions of collection of the medicinal plants cover the entire continent of Africa, based on data from literature sources and information from traditional healers. For each isolated compound, the optimized 3D structure has been used to calculate physicochemical properties which determine oral bioavailability on the basis of Lipinski’s “Rule of Five”.

**RESULTS:** A comparative analysis has been carried out with the “drug-like”, “lead-like”, and “fragment-like” subsets, as well as with the Dictionary of Natural Products. A diversity analysis has been carried out in comparison with the ChemBridge diverse database. Descriptors related to absorption, distribution, metabolism excretion and toxicity (ADMET) have been used to predict the pharmacokinetic profile of the compounds within the dataset.

**CONCLUSIONS:** Our results prove that drug discovery, beginning with natural products from the African flora, could be highly promising. The 3D structures are available and could be highly useful for virtual screening and natural product lead generation programs.
P546: Integrating primaquine into national policy in Swaziland

Simon Kunene1, Joseph Novotny2, 1, Mark Newman3, 1, Sarah Darteh1, Mbongiseni Mathobela2, Zulilise Zulu2, Nyasatu Ntshalintshali2, 1

1 National Malaria Control Programme, Swaziland Ministry of Health, Mbabane, Swaziland; 2 Global Health Group at the University of California, San Francisco, California, United States of America; 3 Clinton Health Access Initiative, Boston, Massachusetts, United States of America

BACKGROUND: In October 2012, the World Health Organisation (WHO) updated their policy to recommend a single low dose of primaquine (0.25mg/kg on day 0), to be administered in conjunction with artesinin-based combination therapy (ACT), to kill mature P. falciparum gametocytes in settings targeting malaria elimination. Potential side-effects to primaquine include severe haemolysis, especially in patients with the inherited enzyme deficiency of glucose-6-phosphate dehydrogenase (G6PD). Due to safety concerns, limited evidence on the extent of impact, and expected challenges in procuring and distributing low-dose primaquine, elimination countries have been slow to implement this new policy.

DESCRIPTION: Consultations were held with multiple malaria stakeholders in Swaziland including representatives from WHO, medical practitioners, and various members of the National Malaria Control Programme to obtain opinions on the use of primaquine as radical cure for malaria.

LESSONS LEARNED: Stakeholders consulted agreed that primaquine is the only commercially available drug that can eliminate stage V gametocytes, which can contribute to onward transmission. In spite of the evidence suggesting that the risk of severe haemolysis from primaquine is low, stakeholders are reluctant to implement the new policy due to lack of knowledge of the prevalence of G6PD in Swaziland. Healthcare providers are also concerned about the side effects associated with primaquine among anaemic and HIV positive patients in Swaziland. Limited guidance on the dosing regimen for primaquine, especially among children, and the lack of commercially available low-dose tablets were identified as barriers to rolling-out primaquine in the near future.

RECOMMENDATIONS: To effectively integrate primaquine into national guidelines, there is a need to investigate the prevalence of G6PD in Swaziland. If primaquine is rolled-out at health facilities, the country would need to develop procedures to monitoring patient safety following administration of primaquine. Further clarifications on dosing by weight bands should be sought from WHO and other technical experts. Countries should liaise with manufacturers to ensure the future availability of lower strength doses conducive to treating all weight bands under the new policy.

P547: Moving from Clinical to Confirmed Diagnosis for Malaria to Achieve Elimination: The Roll Out of Rapid Diagnostic Tests

Simon Kunene1, Joseph Novotny2, 1, Nyasatu Ntshalintshali2, 1, Sarah Darteh1

1 National Malaria Control Programme, Swaziland Ministry of Health, Mbabane, Swaziland; 2 Clinton Health Access Initiative, Mbabane, Swaziland; 2 Global Health Group at the University of California, San Francisco, California; United States of America

BACKGROUND: Prior to 2010, the majority of health facilities in Swaziland clinically diagnosed malaria based on signs and symptoms of the disease rather than through parasitological diagnostic tests, like microscopy or rapid diagnostic tests (RDTs). To achieve malaria elimination, all suspected malaria cases must be parasitologically confirmed using one of these methods to ensure the reduction of parasite reservoirs that contribute to onward transmission.

DESCRIPTION: To facilitate confirmation of all suspected malaria cases, RDTs were distributed to all public and some private health facilities in February and March 2010. The RDT rollout was accompanied by national health care worker training workshops and the implementation of a national quality assurance programme, which was created to ensure that malaria diagnostic services met a predetermined technical and management standard. Prior to the RDT rollout, suspected and confirmed malaria cases were both reported to the Health Management Information System (HMIS). In August 2010, an Immediate Disease Notification System was launched to allow for the rapid reporting of confirmed malaria cases.

LESSONS LEARNED: Between 2008-2009 and 2011-2012, malaria cases reported to the HMIS decreased 91%, from 7507 to 643. Over the same period, the proportion of reported malaria cases confirmed by RDT and/or microscopy increased from 1% to 57%. Results indicate that prior to 2010, malaria was over diagnosed and over reported in the absence of diagnostic tools such as RDTs. Improved diagnosis and reporting of malaria improved the understanding of transmission patterns, which has improved the targeting of malaria prevention interventions.

RECOMMENDATIONS: Countries aiming to achieve elimination must expand access to parasitological diagnostic tests to all health facilities. In the absence of diagnostic tests, low-endemic countries risk misdiagnosing patients and delaying appropriate treatment. To achieve elimination certification, Swaziland must continue to increase the rate of confirmation of malaria in the country’s health facilities.

P548: Reactive case detection for malaria elimination in Swaziland: Factors associated with the detection of secondary Plasmodium falciparum infections

Nyasatu Ntshalintshali*, 1, 2, Michelle S. Hsiang*, 1, 2, Simon Kunene1, Zulilise Zulu1, Patrick Msibi1, Joseph M. Novotny2, 1, Alanna Schwartz2, Hugh Sturrock3, Iveth J. Gonzalez2, David Bell3, Grant Dorsey4, Roly Gosling1, Bryan Greenhouse1

1 Global Health Group, California, United States of America; 2 Departments of Pediatrics, University of California, San Francisco, United States of America; 3 Clinton Health Access Initiative, Mbabane, Swaziland; 4 Swaziland National Malaria Control Programme, Swaziland Ministry of Health, Mbabane, Swaziland; 5 Foundation for Innovative New Diagnostics (FIND), Geneva, Switzerland; 6 Department of Medicine, University of California, San Francisco, United States of America

BACKGROUND: Reactive case detection (RACD), the screening of household members and neighbors of passively detected malaria cases for infection, is recommended for malaria elimination but there is little evidence to guide practice.

METHODS: A prospective surveillance study was conducted in Swaziland to identify factors associated with an index case leading to the detection of secondary cases through screening. Rapid diagnostic tests (RDT) were compared to a molecular method, Loop-mediated isothermal amplification (LAMP), for detection of secondary cases. RDT and/or microscopy confirmed index cases reported from 245 health facilities were targeted for follow-up. If there was potential for local acquisition (area receptive to malaria transmission), family and neighbors residing within a 1 km radius were targeted for RACD. Dried blood spots (DBS), GPS coordinates, and information on demographics, travel, vector control, housing and coverage of RACD were collected. Bivariate analyses of potential relationships between risk factors and secondary case detection by LAMP were performed using t-test or logistic regression.

RESULTS: From August 2012 to April 2013, 165 index cases were identified; resulting in 1481 household members and neighbors who were screened. Secondary cases were more likely to occur when the index case was LAMP positive at follow-up (OR 6.9, 95% CI 1.3-36.8, median follow-up at 5 days, 95% CI 1-29), timely RACD (within 4.9 days, 95% CI 2.7-8.8, vs. 11.6, 95% CI 8.3-16.1, p=0.009), and more subjects screened (mean 28 people, 95% CI 12.6-43, vs. 10, 95% CI 7-15, p=0.008). Among individuals screened, LAMP positivity was associated with travel outside Swaziland (OR 12, 95% CI 5.1-28.2) and closer distance to the index case (mean 39.8m, 95% CI 13.6-116.4, vs. 151.0m, 95% CI 134.6-169.4, p=0.001). To date, LAMP has detected 5 fold more infections than RDT (2.5%, 27/1093, LAMP positivity vs. 0.5%, 6/1093, RDT true positivity using LAMP as gold standard).

CONCLUSIONS: Post-treatment LAMP positivity in index cases is likely due to gametocytes and points to a potential role for additional gametocidal agents to prevent onward transmission. The effectiveness of RACD to detect secondary infections can be improved using LAMP and by optimizing response time, screening radius size, and target population.

6th MIM Conference 2013
**P549: Source of Infection and Other Factors Associated with Malaria in Swaziland: Results of the First Three Years of the Active Surveillance Programme**

**Joseph Novotny**, Zulislile Zulu, Bongani Dlamini, Nomcebo Mkhonta, Sabelo Dlamini, Sicelo Kunene, Nysatsu Ntsalintshali, Steven Mthethwa, Sibonakaliso Vilakati, Simon Kunene

**BACKGROUND:** Malaria elimination requires a robust active surveillance system to facilitate case investigation, where a standardized questionnaire is administered to a person diagnosed with malaria to identify the source of infection and risk factors associated with transmission, which inform the development of a prompt response to halt potential outbreaks.

Swaziland’s National Malaria Control Programme (NMCP) launched an active surveillance programme in the malaria-at-risk region in October 2009 and expanded the programme nationally in November 2010.

**METHODS:** Confirmed malaria cases are reported by health facilities through a toll-free hotline to a central database. Following data entry of the case’s residential location and contact details, a text message is sent to the mobile phones of the NMCP surveillance team alerting them of the case. An NMCP surveillance agent then contacts the case and attempts to conduct an investigation within 7 days of the case being reported.

Demographic details, GPS coordinates of each case’s household, recent travel history, and information on the utilization of personal protection measures are collected during the investigation. The source of infection is determined by collecting a detailed travel history of the patient over the previous two weeks both within and outside of Swaziland.

**RESULTS:** A total of 671 out of 1076 confirmed malaria cases were investigated between October 2009 and June 2012, 56% within 7 days of diagnosis. On average, cases were investigated 8.3 days after presentation. Over 51% of cases (344) were classified as imported, with 97% of imported cases reporting travel to Mozambique. Nearly 40% of all local cases occurred in just 10 localities, or foci, during this period. Local cases reported low coverage of IRS (31%) and bed net ownership (19%), which may have contributed to infection.

**CONCLUSIONS:** Case investigation has improved understanding of malaria transmission in Swaziland. Regional collaboration with Mozambique is required to reduce the imported case burden in Swaziland. Targeted vector control in focal transmission areas may greatly reduce local transmission. Swaziland must achieve a higher rate of investigation among confirmed cases to achieve elimination.

**P550: Use of passive and active surveillance to assess the role of housing as a potential risk factor for local transmission of Plasmodium falciparum infection in Swaziland**

**Nysatsu Ntsalintshali**, Zulislile Zulu, Joe Novotny, Simon Kunene, Patrick Msiibi, Iveth Gonzalez, David Bell, Grant Dorsey, Justin Cohen, Roly Gosling, Bryan Greenhouse, Hugh Sturrock, Michelle S. Hsiang

**BACKGROUND:** Poor quality housing may contribute to malaria infection. Understanding the role of housing as a potential risk factor for local transmission in Swaziland may highlight new areas for intervention as the country aims for malaria elimination by 2015.

**METHODS:** As part of prospective study evaluating Swaziland’s active case detection program (investigation of index cases and screening of nearby residents), we performed a case-control analysis to evaluate the relationship between housing structures and symptomatic or asymptomatic infections acquired locally. Symptomatic infection among index cases was diagnosed by P. falciparum-specific rapid diagnostic tests and asymptomatic infection by Loop-mediated isothermal amplification (LAMP). Source of infection was determined based receptivity of person’s residence and travel history within the past 4 weeks. Controls consisted of LAMP-negative subjects screened in reactive case detection. Data was collected through in-person interviews and observation. Chi-squared, t-test, logistic regression analyses were performed. The study period was from August 2012 to May 2013.

**RESULTS:** A total of 350 malaria cases were reported in passive surveillance. Of 1829 household members and neighbors of index cases screened in active case detection, LAMP was completed for 1493 subjects with 22 infections identified (1.4%). We found a higher risk of symptomatic or asymptomatic local infection among subjects living in a house with windows (100% vs. 83.6%, p=0.008) and higher quality external walls (OR 4.4 for cement block or brick vs. mud, cane, grass, or shrub, 95% CI 3.7 to 5.1), internal walls (OR 4.8 for cement block or brick or plaster vs. mud, cane, grass, or shrub, 95% CI 4.1 to 6.7), and roof (OR 3.2 for metal sheets or tile vs. grass or palm, 95% CI 0.4 to 24.7). There were no associations with age, sex, occupation, presence of window screen, use of an insecticide treated bed net, or sleeping under a sprayed structure in the past year.

**CONCLUSIONS:** Poor quality housing was not associated with infection. It is possible that in this low transmission setting, housing is not associated with infection. However, the study is nascent and has not yet included all wet season data; sample sizes for infected subjects were small.

**P551: Entomological surveillance following a long-lasting insecticidal net universal coverage campaign in mid-western Uganda**

**Anthony Nuwai**, Tarekegn A Abeku, Michelle Helinski, Natacha Protopopoff

1 Malaria Consortium, Kampala, Uganda; 2 Malaria Consortium, London, UK; 3 Department of Disease Control, London School of Hygiene & Tropical Medicine, Keppel Street, London WC1E 7HT, UK

**BACKGROUND:** A universal coverage campaign (UCC) was implemented in the mid-western region of Uganda to distribute long-lasting insecticidal nets (LLINs) in 2009-2010. Entomological surveys were carried out to monitor vector density, behavior, and malaria transmission at baseline before the campaign and over a period of four years following the campaign.

**METHODS:** The study took place in four sentinel sites located in four districts. All but one of the sites received LLINs as part of the UCC. Six sentinel houses were selected in each site. *Anopheles* mosquitoes were collected using CDC light traps quarterly in three houses and human landing catch twice a year in the three remaining houses. *Plasmodium falciparum* sporozoite enzyme-linked immunosorbent assay tests were performed to determine infection rates.

**RESULTS:** The UCC resulted in an increase in net coverage from 19 to 92% and net use from 9 to 66% when measured 7-11 months post-distribution. The dominant malaria vector in all sites was *Anopheles gambiae s.l.* (92%) followed by *A. funestus*. In all sites that received nets, a decrease in the entomological inoculation rate (EIR) was observed after LLIN distribution compared to baseline, primarily the result of decreased vector densities. The reduction was most pronounced in the year following the UCC. In the site with the highest transmission, the EIR dropped from 5.84 infectious bites per person per night at baseline to 0.35 and 0.97, two and 14 months after the UCC, respectively. In subsequent years, an increase in the EIR was observed, although values did not reach baseline figures (EIR of 2.46, 26 months following UCC). There was no indication of any change in feeding behavior as a result of the UCC compared to baseline, and both *A. gambiae* s.l. and *A. funestus* continued to seek hosts primarily after midnight (on average 77% of the bites indoors occurred after midnight).

**CONCLUSION:** The entomological surveys indicate that there was a reduction in transmission intensity coinciding with a large scale increase...
in coverage and use of LLINs and other antimalarial interventions in midwestern Uganda. There was no indication of a change in vector feeding habits following the UCC.

**P552: Assessing the prevalence of asymptomatic malaria in eligible blood donors: A major but neglected transmission route in the North West Region of Cameroon**

Ndibmum Carl Nwana

Malaria is a major public health problem and cause of much suffering and premature death in the poorer areas of tropical Africa, Asia and Latin America. In many endemic areas it is becoming increasingly difficult to control because of the resistance of the parasite to anti-malarial drugs and the failure of effective vector control measures. Malaria is not routinely screened by blood donating centers in Cameroon. A study was designed to determine the prevalence of asymptomatic malaria amongst eligible blood donors and to provide useful data to hospital care givers required for reformulating policies on blood transfusion practices in the North West Region of Cameroon. A total of 200 eligible donors were screened for malaria parasite by the thick and thin blood film using the Giemsa staining technique, from 10 randomly selected hospitals and 20 eligible donors from each of these hospitals. Questionnaires were used to collect data from the donors on use of mosquito treated bed nets, repellants or chemoprophylaxis or if the donor had been sick of malaria a month prior to donation. From the randomly selected hospitals, only 30% (that is 3 out the 10 hospitals) did screening for malaria in the eligible blood donors. The results showed that the prevalence of asymptomatic infected donors was 17% (34/200) all between the ages of 18 to 50 years old. In addition to blood transfusion being unsafe in most developing countries for various reasons, malaria further complicates it, since plasmodium can survive well in stored blood and even in frozen blood. Therefore in the wake of fighting and eradicating malaria, screening parasitaemia in eligible blood donors in malaria perennial regions is prordial. While it may be difficult but not impossible to screen all donors in this milieu for malaria parasite, it is strongly recommended that recipients are tested for malaria parasitaemia immediately after they are transfused.

**P553: Multiple insecticide resistance mechanisms in Anopheles gambiae s.l. populations from Cameroon, Central Africa**

Philippe Nwane, Josiane Etang, Mouhamadou Chouaibou, Jean Claude Toto, Alphonseine Koffi, Rémy Milmpfoudi and Frédéric Simard

**BACKGROUND:** Increasing incidence of DDT and pyrethroid resistance in *Anopheles* mosquitoes is seen as a limiting factor for malaria vector control. The current study aimed at an in-depth characterization of *An. gambiae* s.l. resistance to insecticides in Cameroon, in order to guide vector control interventions.

**METHODS:** *Anopheles gambiae* s.l. mosquitoes were collected as larvae and pupae from six localities spread throughout the four main biogeographical domains of Cameroon and reared to adults in insectaries. Standard WHO insecticide susceptibility tests were carried out with 4% DDT, 0.75% permethrin and 0.05% deltamethrin. Mortality rates and knockdown times (kt50 and kt95) were determined and the effect of pre-exposure to the synergists DEF, DEM and PBO was assessed. Tested mosquitoes were identified to species and molecular forms (M or S) using PCR-RFLP. The hot ligation method was used to depict kdr mutations and knockdown times (kdt50 and kdt95) were determined and the effect of exposure to synergists partially restored insecticide knockdown effect and increased mortality rates, suggesting a role of detoxifying enzymes in increasing mosquito survival upon challenge by pyrethroids and, to a lower extent DDT. The distribution of kdr alleles suggested a major role of kdr-based resistance in the S form of *An. gambiae*. In biochemical tests, all but one mosquito population overexpressed P450 activity, whereas baseline GST activity was low and similar in all field mosquito populations and in the control.

**CONCLUSION:** In Cameroon, multiple resistance mechanisms segregate in the S form of *An. gambiae* resulting in heterogeneous resistance profiles, whereas in the M form and *An. arabiensis* insecticide tolerance seems to be essentially mediated by enzyme-based detoxification. Synergists partially restored susceptibility to pyrethroid insecticides, and might help mitigate the impact of vector resistance in the field. However, additional vector control tools are needed to further impact on malaria transmission in such settings.

**P554: Pilot Survey of malaria parasites and molecular markers in Port Harcourt, Nigeria**

Chijioke Nwauche1; Hefinwa Chiijoke-Nwauche2; Colin Sutherland2; Mary Oguile1; Omotayo Ebon1; Kaladada Korubo1; Lucy Yaguo-Iden1

1Centre for Malaria Research and Phytomedicine, University of Port Harcourt, Port Harcourt, Nigeria; 2London School of Hygiene and Tropical Medicine, London, United Kingdom

**BACKGROUND:** The burden of malaria on the public health sector in Nigeria is enormous and has been complicated by the challenge of antimalarial drug resistance. Resistance to antimalarial drugs results from genetic plasticity of the parasite therefore the investigation of molecular markers is a major tool for assessing drug resistance. This study was designed to address the paucity of data regarding the mapping of resistance markers, and identification of prevalent Plasmodium species in our environment.

**METHODS:** Finger prick samples were taken from adult blood donors presenting at the University of Port Harcourt Teaching Hospital and Braithwaite Memorial Hospital. Blood group, thick and thin film microscopy for malaria parasite and filter paper blood spots for molecular genotyping were carried out on all samples.

**RESULTS:** Pilot study of blood spots collected from 207 blood donors showed that only 8.2% (17) were positive by PCR. Species identification showed: 14 *P. falciparum*, 2 *P. ovale* and 1 *P. malariae*; two samples had mixed infection as *P. falciparum* / *P. malariae* and *P. falciparum* / *P. ovale*. Amplification of the falciparum samples showed mutations on the *pfmdr1* and the *pfhps* genes. Polymorphisms on the *pfmdr1* gene revealed some changes from the wild type to the mutant on codons N86Y and Y184F, but 1246 remained the wild type. The *pfhps* gene showed a variety of mutations presenting as different haplotypes with the double mutant A437G and A581G; in addition to the rare S436F, and the I431V mutation that has been only identified in Nigerian samples. There was no K540E.

**CONCLUSION:** There is need to monitor markers like mdr86N mutation which has been implicated in Artemether-Lumefantrine slow clearance. The absence of K540E suggests the continued use of SP for IPTp in Nigeria. It is important to evaluate other species of plasmodium in the environment.

**P555: Public health implications of health-seeking behaviour of pregnant women on malaria parasitemia in South-Eastern Nigeria.**

Eugene C. Nwosu1, Florence O. Nduka2, Paschal E. Etusim2, And Kalu M. Kalu2

1Federal College of Agriculture Ishiagho Ebonyi State, Nigeria; 2Department of Animal and Environmental biology, Faculty of Science, University of Port Harcourt Nigeria; 1Department of Animal and Environmental Biology, Faculty of Science, Abia State University Uturu, Nigeria.
BACKGROUND: South-eastern Nigeria is malaria endemic and pregnant women are exposed to severe consequences of malaria. Government policies and program strategies for the control of malaria in pregnancy seem to end up in government health facilities which are not the only health care facilities available to the women. This work was designed to identify the health seeking behavior of pregnant women in the area, its effect on malaria parasitemia and the factors responsible for the observed behavior pattern.

METHODS: Three locations out of three states in South-east Nigeria were selected. After due ethical clearance, 844 pregnant women were randomly selected from both public and private health facilities. Peripheral blood smears and placental histology were used to determine infection rate, but placental histology with higher sensitivity was used in all comparisons. Questionnaires were used to capture information on the attitude and choice of pregnant women.

RESULTS: Private Maternities/Delivery homes were most preferred and patronized (41.4%), followed by Maternal/Child health centers (26.4%) and private hospitals with 20.1% by pregnant women. The least preferred was public hospitals (12.1%). Similar trend was found in all locations except in Afikpo where Maternal/Child health centers were most preferred (37.6%) followed by Private Maternity/Delivery homes (33.5%), Private hospitals (17.8%), and Public hospitals (11.2%). Differences were significant ($X^2$=43.9514, $p<0.05$). Levels of parasitemia was high among pregnant women who patronized Private Maternity/Delivery homes (70.8%) and reduced to the lowest (47.1%) among women who patronized Public hospitals. Differences were significant ($X^2$=30.0009, $p<0.05$). Personal and good health attention was the most important factor determining the choice of women (42.3%) followed by financial status of the family (30%) with husbands decision being the least (10.6%).

CONCLUSION: Public hospitals with full investment of resources and expertise to fight malaria in pregnancy are losing patronage to Private Maternities/Delivery homes due to poor attitude to patients. This calls for urgent attention and re-orientation of health care providers in public facilities. Empowering women could improve the financial status of the family.

P556: Malaria case-management following change of policy to confirmed diagnosis and targeted ACT treatment in Kenya

Andrew Nyandigisi1, Dorothy Memusi1, Samwel Kigen1, Beatrice Machini1, Alex Muturi1, David Soti1, Gabriel Otieno1, Sophie Githinji1, Dejan Zurovac2

1Division of Malaria Control, Ministry of Public Health & Sanitation, Nairobi, Kenya; 2Management for Sciences of Health, Nairobi, Kenya; 3Malaria Public Health Department, KEMRI-Wellcome Trust–University of Oxford Collaborative Programme, Nairobi, Kenya; 4Centre for Tropical Medicine, Nuffield Department of Clinical Medicine, University of Oxford, UK; 5Center for Global Health and Development, Boston University, Boston, Massachusetts, US

BACKGROUND: In 2010, the major change in malaria case-management policy in Kenya was a shift from presumptive treatment of fevers to universal parasitological diagnosis and targeted treatment with artemether-lumefantrine (AL). Between 2010 and 2012, a series of activities were undertaken to support implementation of the new policy. Regular monitoring of the quality of malaria case-management is critical to inform policy makers, implementers and donors on the implementation progress.

METHODS: Five national, cross-sectional surveys using range of quality-of-care assessment methods were undertaken at public health facilities. The changes in national health systems and case-management indicators between the baseline survey undertaken prior to the implementation of the new policy and four follow up surveys are measured.

RESULTS: The number of assessed facilities ranged between surveys from 172 to 176, interviewed health workers from 216 to 237 and evaluated outpatient consultations for febrile patients from 1,208 to 2,405. Compared to baseline results, the health systems indicators showed improvements by the end of 2012: availability of malaria diagnostics increased from 55% to 76%, AL stock-out declined from 27% to 22%, access to new guidelines increased from 0 to 57%, trained health workers from 0 to 26% and malaria supervision increased from 19% to 48%. In the same period, malaria testing increased from 24% to 47% while patients with fever who were both tested and treated according to the test result improved from 16% to 39%. At facilities with AL and malaria diagnostics, malaria testing increased from 43% to 58% while those patients who were both tested and treated according to the test result increased from 28% to 48%. Treatment with AL for test positive patients improved from 83% to 93% while antimarial treatment of test negative patients declined from 53% to 22%. The performance results for other health systems and case-management indicators and trends over five survey rounds will be presented during the meeting.

CONCLUSIONS: By the end of 2012, most of the key indicators have shown improvements however the changes were smaller than expected and for most indicators are still below the targets aiming at universal intervention coverage and adherence practices.

P557: A PCR Method for Estimating Clonal Dynamics of Plasmodium falciparum

Josaphat Nyataya, Beth K Mutai, and John N Waitumbi

Walter Reed Project, Kenya Medical Research Institute, Kisumu, Kenya

BACKGROUND: P. falciparum clonal assessment is traditionally done by polymerase chain reaction of MSP-1, MSP-2 and glurp genes of P. falciparum followed by allelic discrimination by gel electrophoresis. This method has limitations such as subjectivity of size scoring and inability to quantify the alleles. Due to its high resolution, capillary electrophoresis (CE) can resolve PCR fragments to one base pair and when the amplicons carry a fluorescent label, alleles have two other parameters: height and area. In this report, we present an optimized method for estimating the relative abundance of P. falciparum alleles using peak height and area.

METHODS: Five national, cross-sectional surveys using range of quality-of-care assessment methods were undertaken at public health facilities. The changes in national health systems and case-management indicators between the baseline survey undertaken prior to the implementation of the new policy and four follow up surveys are measured.

RESULTS: The number of assessed facilities ranged between surveys from 172 to 176, interviewed health workers from 216 to 237 and evaluated outpatient consultations for febrile patients from 1,208 to 2,405. Compared to baseline results, the health systems indicators showed improvements by the end of 2012: availability of malaria diagnostics increased from 55% to 76%, AL stock-out declined from 27% to 22%, access to new guidelines increased from 0 to 57%, trained health workers from 0 to 26% and malaria supervision increased from 19% to 48%. In the same period, malaria testing increased from 24% to 47% while patients with fever who were both tested and treated according to the test result improved from 16% to 39%. At facilities with AL and malaria diagnostics, malaria testing increased from 43% to 58% while those patients who were both tested and treated according to the test result increased from 28% to 48%. Treatment with AL for test positive patients improved from 83% to 93% while antimarial treatment of test negative patients declined from 53% to 22%. The performance results for other health systems and case-management indicators and trends over five survey rounds will be presented during the meeting.

CONCLUSIONS: By the end of 2012, most of the key indicators have shown improvements however the changes were smaller than expected and for most indicators are still below the targets aiming at universal intervention coverage and adherence practices.
P558: Antimalarial isomeric sesquiterpenes from Scleria striatonux and in silico evaluation of their drug metabolism and pharmacokinetic profiles


1 Pharmacochemistry Research Group, Department of Chemistry, University of Buea, BP 63 Cameroon; 2 Centre for Drug Candidate Optimization, Victorian College of Pharmacy, Monash University; 3 Department of Chemistry, University of Minnesota, 207 Pleasant Street, SE, Minneapolis, Minnesota.

BACKGROUND: Many lead compounds often fail to enter the market as a result of poor metabolism and pharmacokinetic profiles. It is therefore imperative to check lead compounds for their ADME (absorption, distribution, metabolism and excretion) or ADMET (when toxicity issues are considered) properties in the early stages of drug discovery/development paradigm. One consideration rapidly gaining support is the in silico evaluation of the drug metabolism and pharmacokinetic profiles. In this study we assess the drug metabolism and pharmacokinetic profiles of novel isomeric sesquiterpenes isolated from the Cameroonian spice Scleria striatonux De Wild (Cyperaceae) that exhibited various levels of antimalarial activity in vitro.

METHODS: S. striatonux rhizomes were harvested in Oku, North West Region of Cameroon and identified by the collaboration of botanists at the Limbe Botanic Garden and the National Herbarium in Yaoundé, Cameroon. Extraction with CHCl3/MeOH (1:1) afforded 450 g of crude extract that was subjected to bioassay-guided fractionation on silica gel and sephadex LH-20. Analytical HPLC of the sub fractions was performed using H2O (0.1% formic acid) / CH3CN (0.1% formic acid). Six isomeric sesquiterpenes were collected after Semi-preparative HPLC. The 3D structures of the six compounds including one tautomer were subjected to a set of ADMET-related properties using a mixture of in silico and experimental techniques. A total of 46 descriptors were calculated using the QikProp program running in normal mode. Drug metabolism was done on human liver microsomes.

RESULTS: The compounds exhibited minimal degradation in incubations with human liver microsomes and based on the microsomal data, they would be considered likely to show low-to-intermediate hepatic clearance in vivo. Overall, the test compounds were found to have acceptable physicochemical properties and fall within the ranges associated with “drug-like” molecules. There were no significant differences between the experimental and predicted values for the compounds.

CONCLUSION: Modern drug discovery techniques incorporate both in silico and experimental techniques to predict “drug-likeness” of molecules. Our results show that theoretical models could be used to assess the ADMET profiles of a set of isomeric sesquiterpene compounds fairly accurately.

*P559: Potential of a Khaya ivorensis – Alstonia boonei extract combination as antimalarial prophylactic remedy*

Roselyne Nzangue Tepongning 1, Leonardo Lucantoni 2, Gene Urge Dori 3, Serge Rakiswende Yerbanga 4, Annette Habletzi 5.

1 University of Ngaoundere, Department of Biomedical Science, Ngaoundere, Cameroon; 2 University of Camerino, School of Pharmacy, Piazza dei Costanti, 62032 Camerino (MC), Italy; 3 Direction de la Promotion de la Médecine et de la Pharmacopée Traditionnelle, Burkina Faso.

BACKGROUND: The decoction of the combined stem barks of Khaya ivorensis A. Chev. (Meliaceae) and Alstonia boonei De Wild (Apocynaceae) has a history of use in traditional medicine of central Cameroon for malaria treatment but also for the prevention of the disease.

AIM OF THE STUDY: The purpose of this investigation was to determine the antiplasmodial activity of Khaya ivorensis (K) and Alstonia boonei (A) preparations in the murine malaria model Plasmodium berghei/Anopheles stephensi and to estimate their prophylactic potential of the formulations prepared according to the traditional recipes.

RESEARCH METHODOLOGY: Aqueous extracts from the stem-bark of the two plants were prepared and tested separately and in combination according to the long prophylactic protocol. Parasitemia reduction in treated animals was calculated from Giemsa smear counts, of two replicate experiments. Toxicity and locomotor activity were also monitored.

RESULTS: The combination KA was found to exhibit antiplasmodial activity in the murine malaria model. In mice treated with the combination remedy at a dosage of 200 mg/kg/day, parasitemia values of 6.2% ± 1.7 and 6.5% ± 0.8 were recorded, compared to 10.8% ± 1.3 and 12.0% ± 4.0 in controls (p < 0.01). Doubling the dosage of the extracts did not significantly increase parasite suppression. When extracts of K and A were administered separately at a dosage of 400 mg/kg, a reduction in parasitemia was still obtained, but it did not reach statistical significance. Toxicity studies yielded comforting results: LD50 equal to 2779.5 mg/kg. Moreover, mice did not display morphological modifications, significant alterations in locomotor activity or any other sign of illness.

CONCLUSION: The antiplasmodial activity and the wide dose interval between the therapeutic dosage and the toxic dosage exhibited by the KA herbal combination in the murine malaria model argue in favor of its use as an antimalarial prophylactic remedy. It remains to be demonstrated by human clinical trials whether the combination remedy, when taken by inhabitants during malaria transmission season, can reduce parasite density and lead to a reduction of malaria episodes in the community.

P560: Malaria parasitaemia impregnant women attending ante natal care at University of Port Harcourt Primary health care centre aluu, port harcourt, rivers state, Nigeria

Sidney O. Nzeako, Florence O. Nduka and Obilete A. Origie

Department of Animal and Environmental Biology, Faculty of Biological Science, College of Natural and Applied Science, University of Port Harcourt, Rivers State, Nigeria.

BACKGROUND: Malaria in pregnancy is a major public health problem in Nigeria. This problem is further aggravated by the paucity in Primary health facilities and preference of alternative health care providers especially in the rural areas. This study is aimed at determining the malaria parasitaemia in primigravidae and multigravidae pregnant women on anti-natal care programme at University of Port Harcourt Primary Health Care Centre, Aluu, Rivers State, Nigeria.

METHODS: Venule blood samples were randomly collected from eighty pregnant women receiving ante natal care in the University of Port Harcourt Primary Health Care Centre were collected after obtaining ethical clearance. These blood samples were put in EDTA properly designated bottles and taken to the Department of Animal and Environmental Biology Parasitology Laboratory for examination. The standard thick and thin smears were used to examine the blood specimens. Data were analysed with Excel Anova.

RESULT: Overall prevalence showed that 48 (60%) of the pregnant women were infected with malaria. Specific Plasmodium prevalence amongst the infected showed that 27(33.8%) were infected with P. falciparum; 11(13.8%) for P. vivax; 9(11.25%) for F. malariae and 1(1.25%) for P. ovale. There was a significantly (P<0.05) increased Plasmodium species prevalence in the primigravidae (84.8%) than in the multigravidae (42%).

CONCLUSION: The high prevalence recorded in the primigravidae indicated that the multigravidae were relatively immune-efficient to malaria than the primigravidae. This could be due to delayed antibody expression and/or lack of awareness of the primigravidae on necessary preventive measures in pregnancy and the depressed immunity due the pregnancy.
P561: Effects of Artemether-Lumefantrine and Artesunate-Amodiaquine on Electrocardiographic Qtc Interval in Nigerian Children with Uncomplicated Plasmodium falciparum malaria

O.S. Michael, R.I. Funwei, O. Abiodun, and C.O. Falade
Department of Pharmacology and Therapeutics, Faculty of Basic Medical Sciences, University of Ibadan, Nigeria

BACKGROUND: Certain antimalarials have been associated with significant cardiac toxicity. Artemisinin based combination therapy (ACT) is now the global standard for the treatment of acute uncomplicated malaria. We evaluated the effects of the two most widely prescribed ACTs on QTC interval prolongation which has been associated with drug related cardiac toxicity.

METHODS: As part of a larger study evaluating the efficacy and safety of artemether-lumefantrine (AL) and artesunate-amodiaquine (ASAQ) 32 children (16 in each group) with acute uncomplicated falciparum malaria were enrolled in a tertiary hospital in southwest Nigeria where malaria transmission is intense. Enrollees received standard doses of AL and ASAQ supervised. Electrocardiographic monitoring was done at Days 0, 3, 7, 14, 21 and 28 using a standard 12-lead electrocardiogram at a paper speed of 25 mm/sec, followed by a tracing at 12.5 mm/s for 10 seconds for interval and rhythm evaluation.

RESULTS: There was a sustained shortening of QTC which was significant on days 3,7,14 and 21 in children that received ASAQ. While QTC changes after treatment were not significant among those that received AL. There was no record of QTc prolongation in any of the enrollees. No adverse clinical cardiac effects were recorded throughout the duration of the study.

CONCLUSION: Artemether-lumefantrine and Artesunate-amodiaquine did not prolong QTc and were not associated with any significant cardiac adverse drug effects. The recorded QTc effect may be associated with resolution of the malaria associated pyrexia.

P562: Dry season malaria entomological survey in a South-Western Nigerian State shows sympatric occurrence of two major vector species

Adedayo O Oduola 1,2, Abiodun Obembe 3, Judith B Olojede 1, Taiwo S Awolola 1
1Molecular Entomology and Vector Control Research Laboratory, Nigerian Institute of Medical Research; 2Department of Zoology, University of Ilorin, Nigeria; 3Department of Biosciences & Biotechnology, Kwara State University, Maitete, Nigeria.

BACKGROUND: Baseline entomological studies provide needful information for the choice and implementation of malaria vector control interventions. In a bid to present the first malaria entomological survey report and determine the appropriateness of indoor residual spray (IRS), a proven malaria vector control strategy, in Osun State Nigeria, a need assessment of baseline entomological indices was conducted.

METHODS: Pyrethrum Spray Collection of anthropophilic mosquito samples was carried out in three local government areas in Osun State. Identified Anopheles gambiae s.s samples were subjected to species specific Polymerase Chain Reaction (PCR) assay for sibling species composition. Sporozoite infection and origin of the blood meal in the vectors were probed by Enzyme Linked Immunosorbent Assay (ELISA) while the numbers of people who slept overnight in the rooms considered were noted for indirect man-biting rate estimations.

RESULTS: Ninety-six percent of the Anopheles mosquitoes collected were An. gambiae s.l. Indoor resting density (IRD) and man-biting rate (MBR) of 0.79 and 0.37 was found in Irewole Local Government compared with the lowest (Average IRD 0.29, MBR 0.13) observed in Boripe Local Government area. All the female Anopheles mosquitoes fed on human blood and tested negative to Plasmodium falciparum circumsporozoite antigen. The PCR analysis showed a predominance of An. gambiae s.s. (95%) over An. gambiae arabiensis (5%).

CONCLUSIONS: Sympatric occurrence of An. gambiae s.s and An. arabiensis, the major malaria vectors in Nigeria, is a necessary precondition for implementing malaria vector control intervention in Osun State. Besides, the predominance of indoor resting An. gambiae s.s and the incidence of exophlic An. arabiensis suggest a combination of IRS and LLINs for this region.

P563: Comparative study of malaria diagnosis by microscopy and polymerase chain reaction (PCR) using outpatients from the University of Cape Coast Hospital

Dorotheah Obiril 1,2, Margaret Sarpong-Nsiah 1, Daniel Boakye 1
1Department Animal Biology, University of Ghana, Accra, Ghana; 2Human Biology Department, University of Cape Coast, Ghana; 3Noguchi Memorial Institute for Medical Research, Accra, Ghana

BACKGROUND: Malaria is a major cause of disease in children and pregnant women with P. falciparum as the main infecting species in Ghana. Routine microscopic diagnosis is very specific and requires expertise. This study determined the specificity and sensitivity of microscopic diagnosis of malaria against PCR using outpatients from the University of Cape Coast Hospital.

METHODS: Peripheral blood samples of 112 patients who manifested clinical signs of malaria were recruited for the study. A pair of thick and thin blood films per patient was prepared on a single slide for microscopy. DNA was extracted from dried blood spots on filter paper and amplified by a nested PCR. Gel electrophoresis was performed to identify specific band sizes for species identification.

RESULTS: A total of 18/112 patients representing 16% were positive for malaria by microscopy while 51/112 patients representing 46% were positive by PCR. P. falciparum formed the most dominant species with 15 out of the 18 positive microscopic cases against P. malariae with only 3 cases. PCR further confirmed the dominance of P. falciparum by detecting 47 against 4 for P. falciparum and P. malariae in a total of 51 positive cases respectively.

CONCLUSION: PCR showed a high specificity and sensitivity compared to microscopy in malaria diagnosis due to increased number of cases diagnosed for PCR. P. falciparum infections dominated the malaria cases reported at the University of Cape Coast in the Cape Coast Municipality is dominated by P. falciparum.

P564: The efficacy of clindamycin plus quinine in the treatment of uncomplicated falciparum malaria: a meta-analysis

Charles O. Obonyo, Elizabeth A. Juma
Centre for Global Health Research, Kenya Medical Research Institute, Kisumu, Kenya

BACKGROUND: Artemisinin-based combinations are the recommended treatment for uncomplicated falciparum malaria, but are costly and in limited supply. Clindamycin plus quinine is an alternative non-artemisinin-based combination, recommended by WHO as a second-line drug. The objective of this review was to compare the efficacy of clindamycin plus quinine versus other antimalarial drugs for the treatment of uncomplicated falciparum malaria.

METHODS: We searched the Cochrane Infectious Diseases specialized register, Cochrane Central Register of Controlled Trials (CENTRAL), MEDLINE, EMBASE, and LilACS. Two authors independently assessed study eligibility, extracted data and assessed the risk of bias. The primary outcome measure was treatment failure by day 28. We compared dichotomous data using relative risk (RR) in a fixed effects model.

RESULTS: Seven randomized controlled trials (929 participants) were included. The risk of day 28 parasitological failure was significantly reduced by clindamycin plus quinine compared with quinine (RR 0.14...
P565: Field Assessment and Comparison between the disposal rate of 8002E Nozzles fitted with Ceramic and Metal Orifice

Obu Buete

OBJECTIVE: Several interventions have been employed in the fight against malaria since ages. This includes aerial spraying, fogging, and in recent times the Indoor Residual Spraying (IRS). This is the application of a potent predetermined residual insecticide capable of killing the vector, when it sits on the treated surface. The WHO has certified and approved the use of several handheld compression pumps which use nozzles of diverse materials. For the best results in the Indoor Residual Spray operations, the recommended nozzle is the ‘8002 E’ flat fan nozzle. (The figure 80 refers to the 80° spray angle of the nozzle, and the spray angle must always be used for IRS operations.) This was recommended by the WHO for use in all IRS operations globally. This nozzle is also used on porous surfaces (e.g. mud walls, rough stone walls, raw brick and cement block walls etc.) as one needs more water/insecticide mixture volume on a porous surface (such as mud walls of huts) and very rough cement block walls etc.) as one needs more water/insecticide mixture. An 8002 E nozzle delivers 757 ml mixture per minute (0.2 US gallons). For easy calculation purposes one can use 760 ml. It is a fact that most of the Hudson Xpert pumps supplied to IRS programs come equipped with 8002 E nozzles. The serviceability of the nozzle depends on various factors, since the dosage rate at every point in time can have cost implications on the programme. It is therefore very much necessary to periodically carry out nozzle calibration. This activity which serves as a quality control mechanism also helps programme managers to know whether nozzles are delivering the right dosage. It is an operational requirement therefore for Field Supervisors to ensure routine nozzle calibration sessions during the spray season. This will enable them make informed decisions on nozzles in use before discarding them.

P566: Stability of antigen-specific cytokine levels to Plasmodium falciparum antigens in a malaria endemic area during a prolonged absence of malaria transmission

Lytticia Ochola

INTRODUCTION: Malaria-specific cytokine responses have been implicated in protective immunity and pathogenesis of clinical malaria. Naturally exposed populations acquire clinical immunity to malaria after repeated Plasmodium falciparum infection. The longevity of antigen-specific T-cell responses in the absence of transmission has not been well characterized. Furthermore, studies on multiple cytokine responses to P. falciparum antigens that may be involved in protection from infection or disease are limited.

METHODS: We assessed levels of pro-inflammatory (interleukin-(IL)-6, interferon-γ (IFN-γ), RANTES and tumor necrosis factor-α (TNF-α)) and anti-inflammatory (IL-5, IL-10) cytokines and chemokines in response to six P. falciparum pre-erythrocytic or blood-stage antigens in 100 individuals from a highland area of Kenya with a recent interruption of transmission (14 months with no clinical malaria episodes in the entire area, April 2007- May 2008). Cytokine and chemokine responses were assessed by cytometric bead assay. Responses were assessed in April 2008, October 2008 and April 2009. Individuals in the cohort had no episodes of clinical P. falciparum malaria over the one-year study period and no asymptomatic parasitemia at any of the three collection time points.

RESULTS: Three patterns of immune responses emerged, with some variation by antigen: a decrease in immune response during the first 6 months that was sustained at 12 month follow-up (IL-5, IFN-γ and TNF-α), a decrease during the first 6 months, followed by an increase at 12 month follow-up (IL-10), and no change over time (IL-6 and RANTES). Other than IL-10 responses to the pre-erythrocytic stage antigens, cytokine patterns did not differ between pre-erythrocytic and blood-stage antigens by age.

CONCLUSIONS: In the absence of malaria transmission, IFN-γ, TNF-α, IL-5 and IL-10 responses to P. falciparum antigens decrease, while IL-6 and RANTES responses remain stable. Future studies will assess whether loss of specific immune responses is associated with loss of clinical immunity to malaria.

P567: Whole-genome sequencing of Malawi Plasmodium falciparum paediatric isolates reveal selective pressure on putative drug and vaccine genes

Harold Ocholla

1Malawi-Liverpool-Wellcome Trust Clinical Research Programme, Blantyre, Malawi; 2College of Medicine, University of Malawi, Blantyre, Malawi; 3Liverpool School of Tropical Medicine, Pembroke Place, Liverpool L3 9QA, UK; 4Centre for Medical Parasitology, Department of International Health, Immunology and Microbiology, Faculty of Health Sciences, University of Copenhagen and Department of Infectious Diseases, Copenhagen University Hospital (Righospitalet); 5Wellcome Trust Sanger Institute, Hinxton, Cambridge CB10 1SA; 6Institut de Recherche en Sciences de la Santé, Bobo-Dioulasso, Burkina Faso; 7Malaria Research and Training Centre, Faculty of Medicine, Pharmacy and Dentistry, University of Bamako, Bamako, Mali; 8KEMRI-Wellcome Trust Research Programme, Kilifi, Kenya; 9Department of Infectious Diseases, Heidelberg University School of Medicine, Germany; 10Shoilo Malaria Research Unit, Mae Sot 63110, Thailand; 11Texas Biomedical Research Institute, San Antonio, Texas, USA; 12Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford OX3 7BN; 13Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT; 14King Fahd University of Petroleum and Minerals, Department of Biology P. O. Box 468 Dharkan, 31261 Saudi Arabia; 15Mahidol-Oxford Tropical Medicine Research Unit, Bangkok 10400, Thailand; 16Centre for Tropical Medicine, University of Oxford, Oxford OX3 7LJ, United Kingdom

In order to provide a map of genetic variation and selection in Malawi, we obtained whole genome sequences of 93 uncultured paediatric P. falciparum isolates collected from a region of high malaria transmission. We have catalogued 115,965 high quality SNPs and using this SNP variation, we provide a comprehensive analysis of selection processes at work in this population. Several invasion antigens were identified as under balancing selection including various families of merozoite surface proteins (MSP, MSP3, DBL-MSP), EBA175, AMA1 and SURFIN4.2. Comparisons of the Malawian P. falciparum population to geographically disperse others (Burkina Faso, Mali, Kenya, Cambodia and Thailand) identified signals of recent selective sweeps at key genes including pfdhps, amo1 and trap. F* analysis between these populations provides evidence of population divergence largely driven by drug selection on pfcr, pfmdhps and pfmdr-1 and reflects the parasite’s local adaptation to drug pressure. We also report previously unidentified regions under positive selection and containing selective sweep in P. falciparum, particularly a region on chromosome 12 containing transcription factors and a putative phospholipid-transporting ATPase protein. Further investigation will determine whether these insights provide a basis for malaria transmission surveillance.
**P568: Fitness effects of dihydropteroate synthase mutations in Plasmodium falciparum transfecants**

Ochong

**INTRODUCTION:** The anti-folates sulfadoxine-pyrimethamine (SP) and trimethoprim-sulfamethoxazole (TMP/SMX) maintain partial protective efficacy against malaria when used for intermittent preventive therapy or daily prophylaxis, respectively, despite resistance-mediating mutations that are common in Africa. The high prevalence of antfo late-resistant parasites might be enhanced by increased fitness over the wild type parasites, in addition to the selective effects of drug pressure. Resistance to antifolates is mediated by mutations in dihydrofolate reductase (DHFR) and dihydropteroate synthase (DHPS). This study explored the impact of various resistance-mediating mutations in DHPS on the relative fitness of P. falciparum.

**METHODS:** D10 strain-derived P. falciparum transfecant clones in which the Pf dhps codons 436, 437, 540 and 581 were modified through allelic exchange at the endogenous genomic locus (Triglia et al) were obtained from MR4. Equal proportions of different clones were inoculated into single cultures and maintained under standard culture conditions for 60 days. Every 6 days, parasite DNA was isolated and PCR followed by pyrosequencing was carried out to determine the proportion of parasites in the cultures. The genotypes of each sample were analyzed using PSQ software and reported as a fraction of the alleles present at each SNP site of interest.

**RESULTS:** In co-culture experiments, the D10-triple mutant (436A + 437G + 540E) outcompeted D10-wild type, D10-single mutant (437G) and D10-double mutant (437G + 581G or 436A + 437G) parasites. The D10-wild type outcompeted D10-single mutant (437G) and double mutant (437G + 581G or 436A + 437G) parasites. The D10-single mutant (437G) outcompeted all the double mutants. There was no change in parasite composition when the D10-double mutants (437G + A581G) and (436A + 437G) were co-cultured.

**DISCUSSION AND CONCLUSION:** Our results suggest complex fitness effects of different mutations in Pf dhps. Surprisingly, in the presence of other mutations, the resistance-mediating 540E mutation, which is associated with clinically relevant resistance to SP, offers a fitness advantage compared to other genotypes. Polymorphisms that alter drug sensitivity may have unexpected effects on parasite fitness, and fitness advantages of some polymorphisms may enhance the establishment and spread of drug resistance.

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**P569: Comparative study of malaria diagnosis by microscopy and polymerase chain reaction (PCR) using outpatients from the University of Cape Coast Hospital**

Dorotheah Obiri1,3, Margaret Sarpong-Nsiah2, Daniel Boakye1

1Department Animal Biology, University of Ghana, Accra, Ghana; 2Human Biology Department, University of Cape Coast, Ghana; 3Noguchi Memorial Institute for Medical Research, Accra, Ghana

**BACKGROUND:** Malaria is a major cause of disease in children and pregnant women with P. falciparum as the main infecting species in Ghana. Routine microscopic diagnosis is very specific and requires expertise. This study determined the specificity and sensitivity of microscopic diagnosis of malaria against PCR using outpatients from the University of Cape Coast Hospital.

**METHODS:** Peripheral blood samples of 112 patients who manifested clinical signs of malaria were recruited for the study. A pair of thick and thin blood films per patient was prepared on a single slide for microscopy. DNA was extracted from dried blood spots on filter paper and amplified by a nested PCR. Gel electrophoresis was performed to identify specific band sizes for species identification.

**RESULTS:** A total of 18/112 patients representing 16% were positive for malaria by microscopy while 51/112 patients representing 46% were positive by PCR. P. falciparum formed the most dominant species with 15 out of the 18 positive microscopic cases against P. malariae with only 3 cases. PCR further confirmed the dominance of P. falciparum by detecting 47 against 4 for P. falciparum and P. malariae in a total of 51 positive cases respectively.

**CONCLUSION:** PCR showed a high specificity and sensitivity compared to microscopy in malaria diagnosis due to increased number of cases diagnosed for PCR. P. falciparum infections dominated the malaria cases reported at the University of Cape Coast in the Cape Coast Municipality is dominated by P. falciparum.

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**P570: Immune Profiles of Children Resistant to P. falciparum Infection in a Holoendemic Region of Tanzania**

Michael Odera1, 2, Rose Odhiamb0,1, Bronner Goncalves2, Kun-Lin Lee2, Yogendra Pal Khasa2,1, Edward Kabyemela2, Robert Morrison2, Jerome Chibwana,1, Janet Aebig1, Michael Fried1, 2 and Patrick Duffy3, 4

1Mother-Offspring Malaria Studies Project, Muheza Designated District Hospital, Tanzania/ Seattle Biomedical Research Institute, Seattle, WA, USA; 2Egerton University, Njoro, Kenya; 3Laboratory of Malaria Immunology and Vaccinology, National Institute of Allergy and Infectious Diseases, NIH, MD, USA

**INTRODUCTION:** The conventional understanding of natural acquired immunity to Plasmodium falciparum malaria is that it is slow to develop and does not sterilize. In a longitudinal birth cohort study between 2002-2006 in Muheza, Tanzania, where malaria is holoendemic, we observed that a subset of children (~10%) never developed patent parasitemia on bloodsmears collected every 2-4 weeks.

**HYPOTHESIS:** In malaria endemic region there are children who remain resistant to blood stage malaria infection and these children display a distinct humoral and cellular immune profile.

**RESULTS AND DISCUSSION:** In ELSA studies, these “resistant” children developed no or only low levels of antibody to P. falciparum apical merozoite antigen (AMA-1) and merozoite surface protein 1 (MSP-1) compared to the susceptible children (i.e., those with positive blood smear on at least one occasion), who as a group developed substantial responses to these antigens. Conversely, the resistant children acquired equal or higher levels of seroreactivity to novel preerythrocytic antigens under investigation in our laboratory as potential vaccine candidates. Seroreactivity to CSP was low in both groups and did not discriminate between resistant and susceptible children. From these results, we surmised that some children in the cohort may have controlled preerythrocytic malaria through unknown mechanisms. We re-enrolled a subset of these children for follow-up during 2011-2012 to assess their humoral and cellular immune profiles. A subset of the resistant children were identified, matched with a group of susceptible children on the basis of several baseline parameters. As observed during early childhood, the resistant children continued to have lower reactivity to AMA-1 and MSP-1 during late childhood; CSP seroreactivity continued to be low in both groups of children. Data analysis for cellular immune responses is ongoing.

**CONCLUSION:** Based on these data, we suggest that a subset of children living in areas of stable malaria transmission may develop a high degree of resistance to malaria that prevents patent parasitemia, and these children, who we call “elite controllers”, maintain a distinct seroreactivity profile throughout childhood.
**P571: Evaluation of malaria control programme in two rural communities in South Eastern Nigeria**

**Friday Odey** 1, **Angela Oyo-Ita** 2, **Austin Ihesie** 3, **Christopher Iklaki** 4, **Martin Meremikwu** 4

1 Departments of Paediatrics, Faculty of Clinical Sciences, University of Calabar, Nigeria; 2 Department of Community Medicine, Faculty of Clinical Sciences, University of Calabar, Nigeria; 3 Department of Community Medicine, University of Uyo teaching Hospital, Uyo, Akwa Ibom State, Nigeria; 4 Department of Obstetrics and Gynaecology, Faculty of Clinical Sciences, University of Calabar, Nigeria

**BACKGROUND:** Malaria control targets were set by the Nigerian health authorities for attainment by 2013. The extent of progress and whether these targets will be achieved is not yet known. This study was undertaken to assess the extent of attainment of malaria control goals in two communities in south-eastern Nigeria.

**METHODS:** Cross-sectional, household survey conducted in 20 communities in Eket and 10 communities in Ibeno local government areas (LGAs) respectively in Akwa Ibom state, Nigeria in November 2011. Public secondary and primary health facilities in the two LGAs were assessed. The household survey and facility tools were checklists adapted from the WHO toolkit for monitoring and evaluation of malaria programmes. Data collected was entered into EPI-INFO, double checked and analysed.

**RESULTS:** One thousand and forty five participants in 266 households were assessed. Only 19.1% of those surveyed and 41.1% of under-five children were treated appropriately with the recommended artemisinin combined (ACT) drugs. Also, 10.1% of the population and 1.8% of under-five children had finger prick presumably for malaria test before treatment. Ownership of insecticide treated bed nets was 73% while usage was 48% (58.1% in pregnant women and 66.1% in under-five children). Thirty-two (26.7%) pregnant women received two doses of sulphadoxine-pyrimethamine as malaria prophylaxis. Two facilities did not use the recommended ACTs for treating malaria. Only the secondary health facilities offer laboratory diagnosis and treatment of complicated malaria.

**CONCLUSION:** The figures for these malaria control parameters fall below the 80% target for the year 2010. This survey has shown that the Nigeria may not meet the malaria control targets for the year 2013 unless more interventions are implemented.

**P572: Does insecticide resistance hinder the use of LLINs?**

**Adedayo Oduola** 1, 2, **Adeniyi Adeneye** 1, **Judith Olojede** 1, **Isaac Oyewole** 1 and **Taiwo Awolola** 1

1 Molecular Entomology and Vector Control Laboratory, Public Health Division, Nigerian Institute of Medical Research, Lagos, Nigeria. 2 Department of Zoology, University of Ilorin, Ilorin,Kwara, Nigeria. 3 Department of Biosciences and Biotechnology, Babcock University, Ogun State, Nigeria.

**BACKGROUND:** The use of long-lasting insecticidal nets (LLINs) remains the mainstay for malaria prevention. Although there are different human behaviors and misconceptions hindering the use of LLINs, the evolution of insecticide resistance is a major threat facing malaria control. Here we investigate perceived efficacy of LLIN by communities’ in areas with and without insecticide resistance.

**HYPOTHESIS:** Insecticide resistance hinders the utilization of LLIN

**METHODOLOGY:** Four villages where insecticide resistance had been established were selected for the study. Four other villages without resistance were used as control. Prior to LLIN distribution, focus group discussions were held to obtain descriptive information on people’s knowledge and beliefs about malaria control. 12 months later, structure questionnaire was developed and pretested and used to explore issues brought forward during focus group discussion. A total of 800 adults (20-60 yrs) randomly selected from the eight villages (100 per village) were interviewed. Specific issues investigated include cause of malaria, preferred mosquito control interventions, perceived efficacy, beneficial and side effects of using LLINs and other methods of malaria control.

**RESULTS:** Regular field survey six months following nets distribution showed that, about 50% of households from insecticide resistance villages had removed the LLINs from their beds. The main reason given is that the nets provided no protection against mosquitoes bites with only 25% of them having the nets after the 12th month. In contrast, >80% households from villages without resistance had the nets in use 12 months after distribution. There was no significant difference in the side effect reported by both groups. A significant proportion (> 80%) of respondents from both groups associated malaria to mosquito bites. However, 80 - 90% respondents in villages without resistance perceived the use of LLIN beneficial and effective in controlling mosquito, < 50% in villages with resistance found LLIN effective with a significant number still preferring the use of aerosol to LLIN.

**CONCLUSION:** Although the respondents have little or no knowledge of insecticide resistance, the presence of resistance correlated to net usage in the villages. To maintain the highest level of net usage, resistance tests should be an integral component of LLINs campaign.

**P573: In vivo Therapeutic Effects of Selected Nigerian Medicinal Plants in Model Malaria Infection in Mice**

**Emmanuel O. Ogbadoyo, Ani N. Agwu, Maimuna U. Bello, Raheem A. Rufai, Adamu Y. Kabir**

Global Institute for Bioexploration/Department of Biochemistry, Federal University of Technology, Minna, Nigeria

**BACKGROUND:** Nigeria and the Democratic Republic of the Congo accounts for over 40% of malaria deaths globally. No vaccine is yet available. Therefore chemotherapy remains a major control measure. Increasing resistance to insecticides and parasite resistance to existing drugs make the search for new antimalarials very compelling. The huge chemical diversity of plants and recorded successes in antimalarial drug development from plants make turning to plants for new antimalarial drugs very attractive. This study aimed to evaluate extracts of two Nigerian medicinal plants for their therapeutic effects in model malaria infection.

**METHODS:** Leaves of Newbouldia laevis were extracted in methanol and partially purified to obtain different fractions, and Crateava andansoni leaves extracted in ethanol. Extracts and fractions were each administered to mice infected with Plasmodium berghei at various concentrations for four consecutive days in a curative test and parasitemia monitored. The mean survival period and LD50 (Newbouldia laevis) only were also determined.

**RESULTS:** crude methanol extract of Newbouldia laevis leaves at 600mg/kg body weight reduced parasitemia by 45.3% on day 4 post treatment compared with 67.4% of the standard drug, artesunate at 50mg/kg body weight, while the ethylacetate fraction reduced parasitemia by 46.4% at 200mg/kg body weight. Vacumm Liquid Chromatography (VLC) of the ethylacetate fraction gave seven fractions with fractions 3, 5 and 7 producing percentage reduction in parasitemia of 47.78, 50.03 and 52.55 respectively at 100mg/kg body weight each. The survival periods in days were 22.33, 16.67, 20, 36, and 28.67 for the crude methanol extract, ethyl acetate fraction, and VLC fractions 3, 5, and 7 respectively. Preliminary toxicity studies gave an LD50 of greater than 5000mg/kg body weight of the crude methanol extract of Newbouldia laevis leaves. Crude ethanol extract of Crateava andansoni leaves at 100, 200 and 400mg/kg body weight gave percentage reduction of parasitemia of 48, 60 and 62 respectively compared to 64% by the standard drug chloroquine at 5mg/kg body weight. The corresponding survival periods in days were 18.67, 22.33, 26.67, and 28.00 respectively.

**CONCLUSION:** It is concluded that Newbouldia laevis and Crateava andansoni are potentially useful as sources of antimalarial drug(s).
P574: Biodegradable Vector Control Agents; Larvicidal activity of Indigenous Plant Extracts against Anopheles gambiae

Omonike Ogbole

OBJECTIVE: In recent years, use of environment friendly and biodegradable natural insecticides of plant origin have received renewed attention as vector control agents due to their rich in bioactive chemicals, active against a limited number of species including specific target insects. As a part of a programme on possible utilization of indigenous plant extracts in pest control practices, twelve medicinal plants selected based on their ethnobotanical use as insect anti-feedant anti-malarial, insecticidal, and mosquito repellent were tested for their larvicidal activity against the fourth instars larva of Anopheles gambiae the primary vector of malaria in sub-Saharan Africa.

METHODS: Dried powdered leaves of the eight medicinal plants were extracted by maceration in redistilled methanol for 72 h. Most active extracts from Argemone mexicana and Hyptis suaveolens were fractionated into hexane, chloroform and ethyl acetate solvents by liquid-liquid partitioning. Larvae of An. gambiae were collected and reared in well water. Toxicity was evaluated by exposing 4th instar larvae to different concentrations (6.25-1000 µg/mL) of extracts, larval mortality was recorded after 24 h of exposure and LC95 values determined using the non-linear regression analysis.

RESULTS: Results were compared to those of larvae exposed to N,N-diethyl-3-methylbenzamide, the reference insecticide and untreated groups. The chloroform and hexane fractions of Argemone mexicana leaf were the most active with EC95 value of 0.2096 µg/mL and 0.2202 µg/mL, respectively. All results were significant (p < 0.05) when compared to the control.

Though several plants from different families have been reported for larvicidal activity, only a few botanicals have moved from the laboratory to field use.

CONCLUSION: The two of the plant extracts; Argemone mexicana and Hyptis suaveolens show promising activity in mosquito control, commercial utilization should be feasible, this would generate local employment and stimulate local efforts to enhance public health.

P575: Divergence of genes encoding fertilisation proteins in P. ovale curtisi and P. ovale wallikeri: evidence for a biological barrier between these sympatric species?

Mary Chiaka Ogukwe and Colin J. Sutherland

1Department of Immunology and *HPA Malaria Reference Laboratory (MRL), London School of Hygiene & Tropical Medicine, London, United Kingdom
2Department of Immunology and *HPA Malaria Reference Laboratory (MRL), London School of Hygiene & Tropical Medicine, London, United Kingdom
3Department of Pharmacology and Therapeutics, 1 Institute for Advanced Medical Research and Training, College of Medicine, University of Ibadan, Ibadan, Nigeria

INTRODUCTION: Plasmodium ovale accounts for ~6% of imported malaria cases in the UK and accounts for a significant proportion of malaria infections in recent surveys of asymptomatic children in Ghana and Uganda. Povole consists of two different species. Plasmodium ovale curtisi and Plasmodium ovale wallikeri co-exist in natural populations but do not recombine. An understanding of the biology of both species is therefore crucial to malaria elimination in countries where they exist, and an understanding of the mechanisms preventing recombination between these very similar but distinct taxa may provide novel insights into the genetic structure of Plasmodium species populations in general.

METHODS: In order to investigate possible barriers to inter-species mating and recombination between the two species, we used bioinformatic tools to identify homologues of genes encoding fertilisation-related proteins in other species. Using database searches on NCBI and PlasmoDB, homologue sequences of an LCLL-domain containing complement control protein (CCp) were obtained with closest hits of Puvix as and PKNkowlesi. BLASTX searches with CCp protein, 6-cysteine gene family members and Pfg377 were performed in the P. o. curtisi genome fragment database (Sanger Institute) to find Povale-specific homologues. Sequence fragments obtained were assembled into contigs and PCR primers designed for amplification and direct sequencing of these genes from both P. o. curtisi and P. o. wallikeri.

RESULTS AND CONCLUSION: Our data reveal dimorphism in all 3 loci between P. o. curtisi and P. o. wallikeri from these fertilisation genes.

P576: Evaluation of the comparative efficacy and effectiveness of artemether-lumefantrine, artemesunate-amodiaquine and artesunate-amodiaquine plus chlorpheniramine in Nigerian children with acute uncomplicated malaria

Mary C. Oguki1, Catherine O. Falade1,4, George O. Ademowo1,4, Colin J. Sutherland2,3

1Department of Immunology and *HPA Malaria Reference Laboratory (MRL), London School of Hygiene & Tropical Medicine, London, United Kingdom
2Department of Pharmacology and Therapeutics, 1 Institute for Advanced Medical Research and Training, College of Medicine, University of Ibadan, Ibadan, Nigeria

INTRODUCTION: Drug resistance has posed a serious threat to malaria chemotherapy in the past two decades. In order to curb this problem, WHO has recommended the use of Artemisinin-based combination therapy (ACT) for the treatment of falciparum malaria. In Nigeria, artemether-lumefantrine (AL) and artesunate-amodiaquine (ASAQ) have been adopted as preferred options. However, ASAQ was only available as separate drugs that were co-administered until recently. To encourage compliance, fixed dose combinations are recommended. Artemoclo(TM) (AQC), a fixed dose formulation of artesunate plus amodiaquine plus chlorpheniramine was evaluated for its comparative efficacy and effectiveness with AL and ASAQ for acute uncomplicated malaria in Nigerian children. Little is known about the therapeutic efficacy of these artemisinin combinations and the prevalence of molecular markers associated with antimalarial drug resistance.

METHODS: A total of 160 children with P. falciparum infection were recruited and randomized into three study groups (AL, ASAQ, and AQC). All patients were followed up for 42 days to study the clinical and parasitological response according to the WHO protocol (2009). We assessed the polymorphisms of the pfcr and pfdmr1 genes by direct sequencing method.

RESULTS: 144 of 160 patients completed the study. At day 14, adequate clinical and parasitological response (ACPR) was 100% for AL and AQC while that for ASAQ was 98% (p=0.39). Day 28 ACPR were 91.1%, 92% and 95.9% for AL, ASAQ and AQC (p=0.62). Pfcrt haplotype CVIF was more common prior to ACT treatment compared to the day of failure. On the other hand, prior to treatment, 34% of children harboured parasites with the pfdmr1D haplotype NFD at codons 86, 184 and 1246. In the 13 children evaluable after treatment, 62% carried the NFD haplotype. This is weak evidence of selection for the NFD genotype by ACT (p=0.069).

CONCLUSION: All three regimens were highly efficacious. There was evidence for selection against CQ-resistant P. falciparum genotypes at pfdmr1 and pfcrt by ACT treatment. This work provides useful baseline estimates of the prevalence of markers of resistance as ACT becomes widely used. Future evidence of parasite selection by continuing ACT use may be revealed as changes in occurrence of these markers.
P577: Evaluation of the quality of antimalarial drugs towards eliminating malaria.

Grace Gbotosho PhD1, Abayomi Sijude 2 PhD and Blessing Ogunlade 1
1Department of Pharmacology and Therapeutics, Basic medical Science,University of Ibadan, Nigeria; 2:Department of Pharmacology and Therapeutics, College of Medicine, Ekiti State University, Nigeria.

BACKGROUND: Quality of antimalarial drugs is an important factor to be considered for the effective elimination of antimalarial drug resistant. It determines the efficacy and safety of drugs. Chemotherapy remains the only best intervention for the treatment of malaria but there could still be treatment failure if the drug is not of standard quality. Unfortunately there is an alarming increase in substandard and counterfeit antimalarial drug including artemisinin combination therapy in many endemic areas. Efforts in this study was devoted to evaluate the quality of commonly used brands of amodiaquine (AQ) tablets including brands of drug containing Amodiaquine co-formulated with artesunate in the South West Nigeria.

METHOD: A total of 39 drug samples from 8 different brands of amodiaquine tablets and amodiaquine co-formulated with artesunate were obtained from different location in South west Nigeria. Standard quality control test were performed on all the brands. The percentage of active drug ingredient in solution was determined using High Performance Liquid Chromatography (HPLC) techniques.

RESULTS: A total of 20 drugs passed the dissolution test (51.28%) using the United State Pharmacopoeia (USP) standard while 19 samples (48.72%) failed the dissolution rate test. The result of the assay of the actual content of the active ingredient (amodiaquine) showed that 61.54% of all the AQ brands analysed failed the assay test using the USP standard. In some of the drugs that failed the assay test, 43.59% contained AQ above the USP range while 30.77% contained AQ below the USP range.

CONCLUSION: The result of this study revealed a high dissolution and content failure in the Amodiaquine tablets or amodiaquine containing antimalarials preparations indicating the presence of substandard (poor quality) antimalarial drugs in south west, Nigeria. This study recommends a more frequent monitoring of the quality of antimalarial products to prevent occurrence of treatment failure.

P578: Antiplasmodial compound from young twigs and leaves of Caesalpinia bonduc (Linn) Roxb

Oluwanke O. Ogunlana1, Hye-Sook Kim2, Yusuke Wataya2, Joseph O. Olanuj3, Afobli A. Akindahunsi4, Ning H. Tan5
1Department of Biological Sciences, College of Science and Technology, Covenant University, PMB 1023, Ota, Ogun State, Nigeria; 2Faculty of Pharmaceutical Sciences, Okayama University; Tushima, Okayama, Okayama 700-8530, Japan; 3Department of Medical Biochemistry, Faculty of Basic Medical Sciences, College of Medicine, Lagos State University, Ikeja, Lagos State, Nigeria; 4Department of Biochemistry, Federal University of Technology, Akure, Nigeria; 5State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, Yunnan, China.

Malaria remains one of the major parasitic diseases in many tropical and subtropical regions of the world. It appears to be the most prevalent disease in these areas despite the many intervention programmes. As part of the effort towards discovery of new antimalarial drugs, screening of the young twigs and leaves of C. bonduc (Linn) Roxb showed moderate antiplasmodial activity with IC50 values of 16 and 18 μg/mL and selectivity index (SI) of 0.69 and 0.29 respectively. Phytochemical investigation of the bioactive extracts led to the isolation of two new cassane diterpenes, 1a,7a-diacetoxy-5a,6β-dihydroxyl-cass-14(15)-epoxy-16,12-olide (1) and 12a-ethoxy-1,14β-diacetoxy-2a,5a-dihydroxy-cass-13(15)-en-16,12-olide (2); and others, bonducellin (3), 3,4′-dihydroxy-2′-methoxy-chalcone (4), 7,3′-dihydroxy-3,11-dehydrohomoisoflavane (5), Luteolin (6), queretin-3-methyl ether (7), Kaempferol-3-O-B-d xylopyranoside (8) and Kaempferol-3-O-a-L-rhamnopyranosyl(1’-2’)-B-d-xylopyranoside (9). Compound 4 exhibited moderate antiparasoidal activity with IC50 and SI values of 33 μM and 0.33 respectively while other isolated compounds showed insignificant antiparasomial activity when compared to existing aniplasmodials. This research helps to validate the traditional use of C. bonduc and propose the possibility of the derivatives of methoxy-chalcone as suitable antiplasmodial drug lead.

P579: Effect of Antioxidant Status on the Progression of Plasmodium berghei Infection in Albino Mice

Biochemistry Programme, Department of Biological Sciences, College of Natural and Applied Sciences, Crawford University, Faith City, Ijebu, Nigeria.

BACKGROUND: Modernization and the resulting lifestyle modifications have been implicated in the increase of oxidants and the reduced consumption of natural food substances rich in antioxidants. This work was done to assess the role of antioxidant status in the progression of P. berghei infection in mice.

METHODS: Albino mice were separated into three groups of fifteen (15) animals each : Normal Control (NC), Lead Acetate (PbAc) treated and Vitamin C (Vit C) treated, to which were orally administered 1ml distilled water, 50mg/kg BW Lead Acetate and 25mg/kg BW Vitamin C respectively for 14 days. SOD and Catalase activities, Thiobarbituric acid reactive substances (TBARS) and reduced glutathione levels were assessed in organs and plasma of five animals from each group to ascertain compromise or enhancement of antioxidant status of the animals. The remaining animals were inoculated with Plasmodium berghei (NK 65) and sub-divided into PbAc (+), PbAc (-), Vit C (+) and Vit C (-) and PbAc (-) (Parasitized Normal Control). (+) and (-) connote subgroups with continued or discontinued administration of pre-treatment agents respectively. Microscopic examination of thin blood films were used to assess parasitaemia daily for 28days.

RESULTS: Results of analysis done immediately after pre-treatment showed significantly raised TBARS level, lowered catalase activity, SOD activity and reduced glutathione levels in the organs and plasma of PRO group and a reverse trend in the Vit C group compared with NC, establishing comprised and enhanced antioxidant status in the PRO and Vit C groups respectively. Progression of parasitemia significantly increased in the PRO (+) and PRO (-) groups and reduced in the Vit C (+) and Vit C (-) groups compared to PNC from day 5. Continuation of pre-treatment agents in the (+) groups showed significant difference only in the Vit C groups when compared with the (-) groups.

CONCLUSION: The progression of P. berghei parasitaemia in mice may be delayed by enhanced antioxidant status.

P580: Coartem®: 20 years of Science and Innovation in Patient Care

Bernhard Gouy1, Christine Manyando2, Kamal Hamed2, Heiner Grueninger3, Michael Makanga4
1Walther Reed Project-Centre for Clinical Research, Kenya Medical Research Institute, Nairobi, Kenya; 2Tropical Diseases Research Centre, Ndola, Zambia; 3Novartis Pharmaceuticals Corporation, NJ, USA; 4Novartis Pharma AG, Basel, Switzerland; 5European and Developing Countries Clinical Trials Partnership, Cape Town, South Africa

BACKGROUND: Adoption and continued deployment of arteether–lumeфантрил (AL; Coartem®), together with other malaria control strategies, has played a significant role in reducing the malaria burden in many endemic countries.

METHODS: Efficacy and safety profile of AL has been evaluated in a...
clini cal development program spanning over 14 years and enrolling about 5,000 patients. Best practice sharing programs and initiatives for supply chain management have been developed. 

**FINDINGS:** AL has consistently demonstrated 28-day PCR-corrected cure rates of over 95%; rapid parasitemia, gametocytemia and fever clearance; and a good safety profile in Plasmodium falciparum malaria. Flavored dispersible tablet formulation was developed jointly by Novartis and Medicines for Malaria Venture to meet specific needs of children. AL treatment of pregnant women in second and third trimesters has been efficacious and well tolerated. To enhance adherence and reduce pill burden for adults and children weighing ≥35kg, a novel fixed-dose formulation, 80/480mg tablet (80mg arteether/480mg lumefantrine), has been developed with proven bioequivalence to 4 standard tablets (20mg arteether/120mg lumefantrine). About 600 million AL treatments to date have been provided without profit to control disease in sub-Saharan Africa in an effort to eradicate malaria. New strategies like SMS for Life, a mobile based supply chain management tool initially deployed to expand access to ACTs is now being utilized in other settings. Novartis has an ongoing commitment to train and educate healthcare workers and communities through best practice sharing workshops for Public Health officials from National Malaria Control Programs in malaria endemic countries, and development of patient information and training materials. Innovative strategies including elimination strategies in low malaria transmission areas and re-innovation of product to improve patient adherence will be of further interest. 

**INTERPRETATION:** These initiatives go beyond mere deployment of drugs and thus support in maintaining and further evolving a patient-centric approach. They are essential for achieving a sustained health benefit in developing countries. 

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**P581: Scaling up home-base Management of Malaria in Delta state, South-South Nigeria.**

Mirabel Ohwo1, Dr Patrick Adah2, Oteju Kehinde1 Aghama Gabriella1, Amaka Igbeke3, Ralph Enushai3.  

1Promoting Initiative for Malaria Eradication Project, Delta state, Africare-Nigeria; 2Society for Family Health, Edo Territory, Nigeria. 

**BACKGROUND:** Malaria remains a major challenge in the community and the role of Patent Medicine Vendors (PMV) cannot be overemphasized due to their number and proximity to the people at the grass root level. They are often the first point of call for majority of community members thus; the need to educate them on malaria diagnosis, current treatment, prevention and appropriate counseling, recognition of danger signs and prompt referral is very important. 

**METHODOLOGY:** Malaria case management training was conducted in Delta state, covering 22 LGAs and 61 participants were trained. The training was divided into Three (3) clusters to ensure effective coverage. The participants were taken through Quality service, Malaria Overview, Epidemiology of Malaria, Malaria treatment, appropriate ACTs storage, malaria prevention, M & E Record keeping and Pharmacovigilance. 

**RESULT:** Of all 61 participants trained 67% were male, 32% female. 73.4% Baseline of knowledge was accessed through a pre-test, while at the end of the training a post test was done 83.1% showing 10% increase in knowledge. 60% of PMVMs report use of chloroquine and SP in treating malaria. 40% were not aware of the National guide line for treatment and diagnosis of Malaria. 

**RECOMMENDATION:** Recommended trainings should be done at regular intervals. Publicity on the reliability of RDT should be encouraged. Availability of ACTs and the use of ACT should always be used to treat confirmed cases of malaria. Sustainability and ownership of the programme is important. 

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**P582: Perceptions of Caretakers of Sick Children on a Possible Malaria Vaccine in Kenya**

David Ojaka, Sylia Thiam, Emmanuel Yamo, Hellen Gakuru, 1AMREF Kenya, Nairobi Kenya; 2AMREF Headquarters, Nairobi Kenya. 

**BACKGROUND:** Development of a malaria vaccine is envisaged to provide a cost-effective strategy to reduce malaria. Stage three trials of the RTS,S malaria vaccine are currently on-going in a total of 11 sites in sub-Saharan Africa, with three of them being located in Kenya. There is need for nationwide quantitative data; these have been provided by the Kenya Service Provision Assessment Survey (KSPA) for 2010. The objective of this further analysis of the KSPA 2010 data was to determine the attitude of caretakers towards a malaria vaccine by selected determinants. 

**METHODS:** In the KSPA survey, a standard questionnaire was used for the interview. Data collected from each selected facility were carefully reviewed to ensure quality before being double entered into database developed using CSPro software. In this further analysis of the KSPA data, descriptive statistics and multinomial regression were applied using STATA 11 to analyze the determinants of accepting a child to be immunized with the malaria vaccine. 

**RESULTS:** Many of the respondents (97%) took their sick children to health facilities at the level of the district hospital or below, with the dispensary receiving 22% of the caretakers. The majority (77%) of the caretakers were in their early and mid-reproductive careers (between 20 and 34 years of age) with over 90% of the caretakers being mothers. The majority (around 88%) indicated that they would accept a child in their community or their own child to be vaccinated with the possible malaria vaccine. A regression analysis between acceptance of one’s own child to be vaccinated with the identified background variables revealed a significant relationship with region, satisfaction with services, age of the caretaker, and schooling. 

**CONCLUSIONS:** The overwhelming support for the vaccine suggests high expectation which needs to be carefully managed as the vaccine is released in the future. The results also show he need for targeting specific segments of child caretakers with relevant messages. Some of these segments include residents of regions where acceptance is low; service providers in health facilities; the older caretakers; those who have not been to school. 

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**P583: Accuracy of malaria microscopy tests in malaria case management in Nigeria: A case for urgent system strengthening.**

Abiodun Ojig, Uwem Inyang, Bolattoi Aiyenigba, James Tibenderana, Elizabeth Streat. 1; Sylla thiam2, Bolatito Aiyenigba,1, James Tibenderana1 3PMI/USARDS, Nigeria; 2PMI/USAID, Nigeria; 3Malaria Consortium, Africa Regional Office, Uganda. 

**BACKGROUND:** Treatment of malaria in Nigeria is largely based on clinical diagnosis despite the recommendations in the 2011 National Treatment Guidelines that all suspected malaria cases should be confirmed by either microscopy or RDTs. PMI in Nigeria is supporting the strengthening of malaria diagnosis in selected states. Laboratory Scientists were trained on malaria microscopy and followed up. This abstract presents the findings of the evaluation of the quality of routine microscopy performed by the trained laboratory scientists in Zamfara and Oyo States. 

**METHOD:** An adapted WHO malaria diagnosis quality assurance guideline was used. Blind rechecking of randomly selected 15 reported (10 positive and 5 negative) routine malaria smear slides was carried out by expert microscopist (WHO certified) from health facilities where personnel had been trained (basic 10-day malaria microscopy course) and where functional microscopes and reagents were available. All
P585: Malaria Prevalence in children under five Years and Pregnant women attending selected hospitals in Ihitte Uboma LGA, Imo state, Nigeria

OBI, ROBERT,1 NWANEBU, F.C.,2 OKANGBA, C.C., and NWANEBU, C.K.
1Department of Microbiology, Federal University of Technology, Owerri, PMB 1526, Owerri, Imo State, Nigeria; 2Department of Medical Microbiology and Parasitology, College of Medicine, University of Lagos, Nigeria.

INTRODUCTION: Malaria illness impose great burden on the society as it has adverse effects on the physical, mental and social well being of the people as well as on the economic development of countries endemic for the disease. Malaria is a serious disease affecting children and adults but its consequences are more severe among children and pregnant women. Nigeria is known for high prevalence of malaria which is the leading cause of morbidity and mortality in the country. Records show that at least 50% of the population of Nigeria suffers from at least one episode of malaria yearly. Henceforth, the aim of this study is to determine the prevalence of malaria among children under five years and pregnant women in Ihitte Uboma, LGA, Imo state, Nigeria.

METHODS: The study included 200 indigenes of Amakohia community selected randomly. These were comprised of 100 infants (50 males and 50 females) and 100 pregnant women. The purpose of this study was fully explained to them and their community leaders, and their informed consents were obtained before blood samples were collected from them. The samples were screened for presence of malaria parasites using the thick and thin malaria blood films, stained with Giemsa stain. The stained films were examined using the x100 oil immersion objective.

RESULTS: The study shows that all the blood samples obtained from the pregnant women were positive for malaria parasite with the age groups of 19-21 and 28-30 producing the highest significant clinical signs. Similarly, all the samples obtained from the under five were positive.

CONCLUSION: Malaria infection indeed showed a high level of endemicity in Ihitte Uboma thus calling for adequate measures to be put in place to monitor its negative impact on these most vulnerable members of the community.

P586: Emerging determinants of risk of uncomplicated malaria in Gambian children: a case-control study

Joseph Okebe, Julia Mwesigwa, Eugene Lama, Fanta Njie, Kalifa Bojang
Medical Research Council Unit, The Gambia

BACKGROUND: The burden of malaria has decreased in many parts of Africa following the scaling up of interventions for prevention and control. This decline is associated with changes in the risk of disease by age and geographical location of populations. Thus, studies to reassess the importance of known risk factors and to identify new determinants of malaria risk in the population are needed.

STUDY OBJECTIVE: To identify factors associated with the risk of uncomplicated malaria in children aged 6 months to 12 years in The Gambia.

METHODOLOGY: This was a case-control study and cases were children with slide-confirmed malaria infection recruited from the paediatric outpatient clinics of the Edward Francis Small Teaching Hospital and the Clinical Services Department of the Medical Research Council Unit, The Gambia. Controls were age-matched children residing within a 400m radius of the case with a negative rapid diagnostic test for malaria. We collected data on factors associated with risk: recent antimarial drug use, use of treated bednets,
house structure, knowledge of malaria and socio-demographic factors using a structured questionnaire.

RESULTS: We recruited 136 case-control pairs; 54.2% of cases and 47.4% of controls were male. In univariate analyses, maternal marital status, father’s education level, observed bednet in the house, wall material, window type, number of people sleeping in a room and a history of indoor residual spray of the house were risk factors for malaria. In a bivariate analysis with conditional logistic regression, mother’s occupation (OR 1.36, 95% CI 1.08 - 1.71), source of funding for illness (OR 0.39, 95% CI 0.19-0.79) and type of windows in the residence (OR 0.62, 95% CI 0.39 - 0.98) were significant determinants of the risk of malaria during the wet season.

CONCLUSION: The risk of malaria in The Gambia with high coverage of control interventions is associated with indices of socio-economic status, occupation and source of income.

P587: In-vitro antimalarial drug susceptibility studies of Plasmodium falciparum isolates from The Gambia using SYBR Green I cytometry

Fatima El-Fitouri, Joseph Okebe, Fatou Joof, Alfred Amambua-Ngwa, Abdullahi Ahmad, Davis Nwakanma, Umberto D’Alessandro
Medical Research Council Unit, The Gambia

BACKGROUND: Following reports of Artemisinin resistance in Southeast Asia, the WHO recommends regular monitoring of therapeutic efficacy (TE) ideally in conjunction with in vitro tests for susceptibility of Plasmodium falciparum to components of Artemisinin combination therapies. By accurately determining the effect of individual drug components on the progression of parasite stages, in vitro tests circumvent some of the confounding factors that characterise in vivo studies. Compared to other methods, SYBR green staining and cytometry allows for distinct quantification of different parasite developmental stages, thereby permitting better resolution on the effect of drugs on P. falciparum development. This project aimed at determining the in vitro susceptibilities of P. falciparum isolated from the Gambia to currently used antimalarial compounds.

METHODS: We analysed whole blood samples from children with confirmed uncomplicated malaria enrolled in a TE study in The Gambia during the 2012 transmission season. We determined the IC50 (50% inhibitory concentration) of 36 isolates using SYBR Green drug assay against 4 drugs; Artemisinin, Chloroquine, Lumeatranfine and Mefloquine. Parasites were incubated for 48 hours, stained with SYBR Green and counted on a FACSCount flow cytometer. Results were analysed using Flowjo and Flowing softwares. IC50s were generated using Graphpad prism software.

RESULTS: Gambian isolates had a median IC50 of 28nM and 541nM for Artemisinin and Lumeatranfine respectively. We found a wide variance of susceptibility to ART (interquartile range (IQR, 17nM- 40nM).

CONCLUSION: P. falciparum isolates from the Gambia showed IC50 range for Artemisinin higher than previously reported for the region. SYBR Green assay has potential to classify resistant phenotypes and broaden our knowledge of parasite biology. Further analysis comparing isolates with high IC50’s to the clinical data and mutations in regions of strong selective sweep is in progress. Rigorous surveillance is vital to sustain the useful lifespan of currently employed ACTs in Africa.


BACKGROUND: Reports of emerging resistance to artemether-lumefantrine have reinforced the need for regular monitoring of antimalarial drug efficacy in health facilities where the drug is regularly administered. In 2010, an efficacy study conducted in three geographical regions in The Gambia showed an overall efficacy of 95.5% with variations across sites: 88.1% (west), 100% (central) and 98.4% (east) respectively.

In 2012, we repeated the study in the same sites and in addition, gave the study drugs under direct observation to exclude the effect of compliance on observed efficacy.

METHODS: We enrolled children aged between 6 months and 12 years with a fever and parasitologically confirmed P. falciparum mono-infection. They were admitted on the ward for drug administration, clinical and laboratory (blood slide, filter paper) monitoring during the first three days and subsequently as outpatients until 28 days post enrolment or reappearance of infection and/ or symptoms. Slides were double-read with resolved by a third microscopist and filter samples analysed to determine parasite genotypes in cases of documented treatment failure. The study endpoint was adequate clinical and parasitological response (ACPR) on day 28.

PRELIMINARY RESULTS: The study was conducted between September 2012 and January 2013. Overall, 248 children; 52% males with a mean age of seven (±2.7) years were enrolled. The median baseline parasitemia for each site was 29,478/μl, 23,746 and 40,880 respectively. Uncorrected ACPR was 96.6%; 94%, 100% and 96% across the sites. Three cases of late clinical failure and five with late parasitological failures were reported. There were no serious adverse events. PCR correction for ACPR is in progress.

DISCUSSION: Artemisin-lumefantrine remains efficacious and well tolerated for the treatment of uncomplicated P. falciparum malaria in children.

P589: Detecting changes in mean of malaria time series using change point analysis

Okello O Gabrielii, Ottieno, A. M. Joseph1
1University of Nairobi, Kenya; 2KEMRI-University of Oxford-Wellcome Trust Research Programme, Kenya

BACKGROUND: A sequence of observation usually undergoes sudden changes at unknown times. Hence, there is need to find out if a shift has occurred in time series data by identifying set of change points. Change point detection is the identification of abrupt changes in time series (sequential) data. Change point detection can be performed using Statistical Process Control or statistical change point detection methods. The objective of the study was to detect points of change in mean of malaria time series using Cumulative Sum (CUSUM) Change Point Analysis.

METHODS: The study used the monthly reassembled data of discharge diagnosis of malaria for all age groups from Eldoret region for the period
January 2010 to December 2011. CUSUM Change Point Analysis method was used to detect changes in the time series data by (1) calculating cumulative sums then (2) using bootstrapping to make inferences.

RESULTS: Most important changes in mean of time series for malaria cases occurred between May 2010 and December 2011 where the trends of malaria cases have reduced. It is shown that statistically detected changes in the mean of a time series coincide with identifiable period when the interventions were put in place and when there were epidemics, which might have caused these change points.

CONCLUSION: It is suggested that CUSUM Change Point Analysis is a very promising appropriate and elegant method for time series analysis in epidemiological studies for detecting changes in longitudinal time series data and should be employed alongside other time-series tools. The method reaches far beyond traditional methods of statistical analysis that are not well suited to detect changes in long historical time series data. Future studies may involve wider exploration of change point analysis techniques and further testing and refinement is needed to fully develop its promising capabilities.

P590: Local perceptions of intermittent screening and treatment for malaria in schoolchildren on the south coast of Kenya

George Okello1, Sarah Ndegwa2, Katherine E Halliday3, Kara Hanson4, Simon I Broker2,3, Caroline Jones1,6,7
1Social and Behavioural Research Group, Kenya Medical Research Institute-Wellcome Trust Collaborative Programme, Kilifi, Kenya; 2Malaria Public Health & Epidemiology Group, Kenya Medical Research Institute-Wellcome Trust Collaborative Programme, Nairobi, Kenya; 3Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, UK; 4Faculty of Policy and Public Health, London School of Hygiene and Tropical Medicine, UK; 5Centre for Tropical Medicine, Nuffield Department of Clinical Medicine, University of Oxford, Oxford UK; 6Department of Public Health & Primary Care, University of Oxford, Oxford, UK

BACKGROUND: The intermittent screening and treatment (IST) of school children for malaria is one possible intervention strategy that could help reduce the burden of malaria among school children. Future implementation of IST will not only depend on its efficacy and cost-effectiveness but also on its acceptability to parents of the children who receive IST as well as those responsible for its delivery. This study was conducted alongside a cluster randomized trial to investigate local perceptions of school-based IST among parents and other stakeholders on the Kenyan south coast.

METHODS: Six out of the 51 schools receiving the IST intervention were purposively sampled, based on the prevalence of Plasmodium infection, to participate in the qualitative study. Twenty two focus group discussions and 17 in-depth interviews were conducted with parents and other key stakeholders involved in the implementation of school health programmes in the district. Data analysis was guided by the framework analysis method.

RESULTS: High knowledge of the burden of clinical malaria on school children, the perceived benefits of preventing clinical disease through IST and previous positive experiences and interactions with other school health programmes facilitated the acceptability of IST. However, lack of understanding of the consequences of asymptomatic parasitaemia for apparently healthy school children could potentially contribute to non-adherence to treatment, and use of alternative antimalarial drugs with simpler regimens was generally preferred. The general consensus of stakeholders was that health workers were best placed to undertake the screening and provide treatment, and although teachers’ involvement in the programme is critical, most participants were opposed to teachers taking finger prick blood samples from children. There was also a strong demand for the distribution of mosquito nets to augment IST.

CONCLUSION: School-based malaria control through IST was acceptable to most parents and other stakeholders, but careful consideration of the various roles of teachers, community health workers, and health workers, and the use of antimalarial drugs with simpler regimens are critical to its future implementation.

P591: Gastro intestinal smooth muscle cholinergic effects of herbal ‘antimalarial’ concoction of Lawsonia inermis, Cymbopogon citratus, Citrus aurantifolia and Kuburat Okeowo

BACKGROUND: The effect of drug resistance to malarial infection has led communities in Africa, and elsewhere, which have limited capacity to differentiate between fever and malaria, to seek alternative cure from their local herbs. We investigate the gastrointestinal smooth muscle cholinergic effects of concoctions from leaves of Lawsonia inermis, Cymbopogon citratus, Citrus aurantifolia and Citrus paradise; used to treat presumptive malaria by Sabo inhabitants in Sagamu, Nigeria.

METHODS: The rat stomach fundus strip was exposed to graded doses of pilocarpine and the herbal mixture (HM) in tyrode solution and effects recorded using transducer connected to computerized 17400 data capsule recorder. Atropine was used as blocking agent to compare the effects pilocarpine and HM on the cholinergic receptors.

RESULTS: The patterns of contractility of the fundus of the rat stomach strip in normal and atropinized tyrode solution were dissimilar when exposed to HM and pilocarpine. The inhibition potential of atropine on pilocarpine effect on the cholinergic receptor was consistent (range, 30.5 - 40.7%) relative to HM effects on cholinergic receptor (range, -38.8 - 77.7%).

CONCLUSION: The findings from this study showed this HM with biphasic activities, a seeming unpredictable agonistic-antagonistic behaviour on smooth muscle cholinergic receptor. This provide basis to explore these herbs for potential gastroprotective properties alongside anecdotal report of relieving self-reported malarial symptoms in users in the study community. In addition, provide a screening method to understand the effects of local herbs used in different conditions on GI smooth muscle activity to protect the human subject.

P592: Polymorphisms within the Interleukin-23 receptor (IL-23R) exon are associated with susceptibility to pediatric severe malarial anaemia in Western Kenya

Winnie A. Okeyo1, Elly O. Munde1, Wilson Okumu2, Evans Rabballah3, Samuel B. Anyona4, John M. Vulule1, John M. Ong’e’cha1, Douglas J. Perkins1,4, Collins Ouma1,3
1Maseno University, Maseno, Kenya; 2Masinde Muliro University of Science and Technology, Kakamega, Kenya; 3Kenya Medical Research Institute (KEMRI), Kisumu, Kenya; 4Centre for Global Health, Department of Internal Medicine, University of New Mexico School of Medicine, NM, USA

Plasmodium falciparum malaria remains a major cause of morbidity and mortality in sub-Saharan Africa. The most common manifestation of severe malaria in pediatric populations resident in holoendemic P. falciparum transmission areas, such as western Kenya, is severe malarial anaemia [SMA, hemoglobin (Hb)<6.0g/dL, any density parasitaemia], as defined in this region. However, globally, SMA is defined by WHO as Hb<5.0g/dL, and any density parasitaemia. The immunogenetic basis of selective protection against SMA is still poorly defined, as much as this is a necessary prerequisite in the design of an effective malaria vaccine. IL-23 is a cytokine that has been associated with differential susceptibility to SMA in western Kenya. Immunologic functions of the IL-23 cytokine are dependent on its interaction with the IL-23 receptor (IL-23R). So far, no studies have reported the association between polymorphic variants in the IL-23R gene and susceptibility to SMA. Therefore, the role of two IL-23 receptor exon variants, 1644G/T (rs1884444) and 53219C/T (rs7530511), in conditioning susceptibility to SMA (both regional and global definition) was investigated in a pediatric population (n=268) resident in western Kenya and presenting in a rural hospital with malaria. Haematological parameters including Hb levels were determined to stratify the population into non-SMA and SMA. The IL-23R genotypic profiles were determined using TaqMan 5'
allelic discrimination assay, while haplotypes were constructed using the HPLC software. Chi-square analyses revealed that proportions of heterozygous individuals at 6644G/T were significantly higher in the SMA group [SMA, Hb<6.0g/dL, any density parasitemia, P=0.044]. Further multivariate regression analyses controlling for covariates showed that relative to GG, heterozygous 6644GT individuals had an increased risk to this regional definition of SMA (OR; 2.30, 95% CI, 1.26-4.21; P=0.007). When the population was stratified according to WHO definition of SMA, chi-square analyses revealed that individuals with the CC genotype at 53219 (53219CC) were significantly higher in the SMA group [Hb<6.0g/dL, any density parasitemia] (P=0.021) relative to non-SMA. Additional multivariate analyses demonstrated that individuals with 53219CC genotype had increased susceptibility to the WHO definition of SMA (OR; 5.62, 95% CI, 1.40-22.58; P=0.044). Further regression analyses also revealed that the carriage of the 1644T/53219C (TC) haplotype was associated with increased risk of susceptibility to SMA, [(Hb<6.0g/dL, any density parasitemia) (OR; 1.88, 95% CI; 1.09-3.27, P=0.024)] relative to non-carriager. Preliminary data presented here demonstrate that the IL-23 receptor exon variants (6644G/T and 53219C/T) are associated with susceptibility to SMA in this paediatric population of western Kenya.

P593: Insecticide susceptibility of Anopheles gambiae s.s. mosquitoes in Ibadan, South-West Nigeria.

Patricia N. Okorie1, CS Wondji2, LA Kelly-Hope2, Olusegun G Ademowo1.
1Institute for Advanced Medical Research and Training, College of Medicine, University of Ibadan, Nigeria; 2Liverpool School of Tropical Medicine and Hygiene, Liverpool, United Kingdom

BACKGROUND: The emergence of insecticide resistance in Anopheles mosquitoes has great implications for the Nigeria Malaria Control Programme as it scales up distribution of Long Lasting Insecticide Nets (LLINS) and increased Indoor Residual Spraying (IRS) across the country. The aim of this study was to determine the insecticide susceptibility levels of malaria vectors in Ibadan, South-West, Nigeria.

METHODS: Larval mosquitoes were collected from two sites, Ojoo and Bodija, in Ibadan. Two-three day old adult female Anopheles mosquitoes reared from these larval collections were exposed to test papers impregnated with three classes of insecticides: pyrethroids (1.0% permethrin, 0.05% lambda-cyhalothrin), organophosphates (1.0% fenitrothion) and organochlorines (4% DDT). At least hundred mosquitoes were used per insecticide. In addition, mosquitoes from Bodija were exposed to 0.15% cithinur, 0.05% deltamethrin and 0.5% etofenprox. Twenty female An. gambiae were randomly selected from the mosquitoes exposed to pyrethroids and used for species and molecular form identification and their genotype at the kdr loci was also determined.

RESULTS: Mosquitoes from the two sites were resistant to pyrethroids and DDT. The mortality rates in Bodija and Ojoo respectively were: DDT (41.8% and 57.8%); 0.05% lambda-cyhalothrin (60.7% and 87.7%); and 1.0% permethrin (73.1% and 69.8%), respectively. In Ojoo, the mortality rates to the additional insecticides were 69.5% (0.5% etofenprox), 80.8% (0.05% deltamethrin) and 87.7% (0.15% cyfluthrin). For 1.0% fenitrothion, 100% susceptibility was recorded at Bodija and Ojoo respectively. Molecular analyses identified all samples from both sites as An. gambiae s.s. In Ojoo, all samples were further identified as S molecular form, while in Bodija S molecular form was more predominant (88.8%) compared with the M form (11.2%). The 1014F kdr mutation was not high with allele frequencies of 5.7% and 18% in Ojoo and Bodija, respectively.

CONCLUSION: The low frequency of this target site mutation even in resistant mosquitoes indicates that metabolic resistance probably plays an important role in the resistance observed to pyrethroids and DDT in these locations. It is imperative to implement insecticide resistance management strategies in this region to halt the development resistance in order to maintain the effectiveness of current and future control interventions.

P594: Comparative field evaluation of combinations of long-lasting insecticide treated nets and indoor residual spraying for malaria prevention

Fredros O Okumu1,2, Edgar Mbeleyi1, Godfrey Lingamba1, Jason Moore1,2, Alex J Ntamutungiro1,2, Deo R Kavishe1, Michael G Kenward3, Elizabeth Turner1, Lena M Lorenz2 and Sarah J Moore1,2

Environmental Health and Ecological Sciences Thematic Group, Ifakara Health Institute, Ifakara, Tanzania; 2Department of Disease Control, London School of Hygiene and Tropical Medicine, London, UK; 3Department of Medical Statistics, London School of Hygiene and Tropical Medicine, London, UK

BACKGROUND: Long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) are commonly used together in the same households to improve malaria control despite inconsistent evidence on whether such combinations actually offer better protection than nets alone or IRS alone.

METHODS: Comparative tests were conducted using experimental huts fitted with LLINs, untreated nets, IRS plus untreated nets, or combinations of LLINs and IRS, in an area where Anopheles arabiensis is the predominant malaria vector species. Three LLIN types, Olyset®, PermaNet 2.0® and Icon Life® nets and three IRS treatments, pirimiphos-methyl, DDT, and lambda cyhalothrin, were used singly or in combinations. We compared, number of mosquitoes entering huts, proportion and number killed, proportions prevented from blood-feeding, time when mosquitoes exited the huts, and proportions caught exiting. The tests were done for four months in dry season and another six months in wet season, each time using new intact nets.

RESULTS: All the net types, used with or without IRS, prevented >99% of indoor mosquito bites. Adding PermaNet 2.0® and Icon Life®, but not Olyset® nets into huts with any IRS increased mortality of malaria vectors relative to IRS alone. However, all IRS treatments, only pirimiphos-methyl significantly increased vector mortality relative to LLINs alone, though this increase was modest. Overall, median mortality of An. arabiensis caught in huts with any of the treatments did not exceed 29%. No treatment reduced entry of the vectors into huts, except for marginal reductions due to PermaNet 2.0® nets and DDT. More than 95% of all mosquitoes were caught in exit traps rather than inside huts.

CONCLUSIONS: Where the main malaria vector is An. arabiensis, adding IRS into houses with intact pyrethroid LLINs does not enhance household level protection except where the IRS employs non-pyrethroid insecticides such as pirimiphos-methyl, which can confer modest enhancements. In contrast, adding intact bednets onto IRS enhances protection by preventing mosquito blood-feeding (even if the nets are non-insecticidal) and by slightly increasing mosquito mortality (in case of LLINs). The primary mode of action of intact LLINs against An. arabiensis is clearly bite prevention rather than insecticidal activity. Therefore, where resources are limited, priority should be to ensure that everyone at risk consistently uses LLINs and that the nets are regularly replaced before being excessively torn. Measures that maximize bite prevention (e.g. proper net sizes to effectively cover sleeping spaces, stronger net fibres that resist tears and burns and net use practices that preserve net longevity), should be emphasized.
P595: Evaluation of Saliva-derived Genomic DNA using OMNIgene Kit in Detection of Malaria and Human Single Nucleotide Polymorphisms Genotyping

Wilson Okumu

In malaria holoendemic areas such as western Kenya, *Plasmodium falciparum* malaria is detected through microscopic examination of blood films obtained through an invasive procedure from a suspected patient. The use of invasive procedure to obtain blood as the primary source suffers from many challenges, which include; the pain associated with pricking that has turned away many would-be-volunteers in clinical studies and the risk of inoculating the study participants with pathogenic microbes if proper sterilization is not performed at the point of prick. Therefore, an immediate need to develop a non-invasive, specific and sensitive method of detecting malaria and other diseases in routine health care services is sought. Genomic DNA has been an important source of specimen in genetic variants and disease association studies. A cross-sectional study was designed to evaluate the quality of saliva-derived DNA relative to traditional blood derived DNA of the same individuals with clinical symptoms of malaria (aged 24 years; n=100) and resident in *Plasmodium falciparum* holoendemic transmission region of western Kenya. They provided matched blood and saliva samples for DNA extraction using OMNIgene protocols. The separated DNA was subsequently used to genotype parasite Merozoite Surface Protein (MSP-2) and human FcyRIIA-176F/V polymorphisms. The MSP-2 parasite genotyping was carried out through nested PCR while FcyRIIA -176F/V genotyping was performed on Applied Biosystems StepOnePlus™ Real-Time PCR system through a TaqMan Allelic Discrimination Assay. DNA obtained from the OMNIgene extraction performs the same as DNA from blood in detection of circulating *P. falciparum* parasites and in successfully genotyping human FcyRIIA -176F/V through high-throughput SNP detection. The results demonstrate that OMNIgene approaches to extracting DNA from saliva is a potential procedure to detecting diseases such as malaria through non-invasive procedure with a significant contribution to diagnosis and medical research.

P596: Reduction of malaria prevalence in children less than 5 years of age in a high malaria transmission area in Uganda following continuous use of Indoor Residual Spraying (IRS).

Dr. Edwidah Tukahebwa1, Dr. Vincent Owiny2, Mr.Thomas Okwiir3

1Vector Control Division, Ministry of Health, Kampala Uganda; 2Oyam District Local Government, Uganda.

BACKGROUND: Preventing malaria transmission in areas of high transmission is a big challenge facing national malaria control managers. Provisions and promotion of ITNs in Uganda, for many years, have failed to control and eliminate malaria in high malaria transmission areas. None the less, the intervention which currently has proved more effective is IRS using Bendiocarb WP (Carbamate). This study aimed to evaluate the effectiveness of IRS in prevention of malaria transmission in children less than 5 years of age in a high malaria transmission area of northern Uganda, a case for Oyam District.

METHODS: A finger prick blood sample was taken from the second finger of the left hand side of 1008 children using blood lancets and thick blood smears were made. Giemsa stain was used with a mixing ratio of 1:9 (10%) to stain the thick blood smears and the malaria parasites were read against 200WBC using compound microscope lens magnification X100. One year malaria prevalence data before any IRS was implemented and malaria prevalence data 6 months after the 6th round of IRS exercise were obtained from district health management information system. Vector data was obtained using pyrethrum spray catch.

RESULTS: Out of 1008 children involved in the study, 58 (5.8%) were confirmed having *Plasmodium falciparum* parasites. There was very good reduction in malaria case numbers in health units after the sixth round of IRS exercise. An average of two vectors, *Anopheles gambiae* s.l, were caught and identified in each residential home.

CONCLUSIONS: The continuous use of IRS reduced the malaria prevalence rate from 63% to 5.8% in December 2012. This trend is encouraging when pursuing malaria elimination. However, the malaria control managers must embark on a large scale IRS implementation covering both low and high transmission areas to achieve stability of elimination.

P597: Do co existing co morbidities further drive up household and health system cost of malaria treatment?

Chinenyen Okwuosa

INTRODUCTION: More than 3 billion people live in malarious areas and the disease causes between 1 million and 3 million deaths each year. One of the major health issues facing most developing countries is the challenge being posed by the disease malaria. This study seeks to explore the impact of co morbidities on the treatment cost of malaria.

METHODS: The study was carried out in two malaria holo endemic areas Achi and Oji river communities both in Oji-River Local Government Area of Enugu State, Southeast Nigeria. It was a hospital based retrospective and prospective study. Information was also extracted from 125 in patient and out patient records. These were used to establish resource utilization by patients, direct medical treatment cost including the cost of drugs and laboratory tests. Consent was sought from the hospital administration before the data was collected.

RESULT: From data abstracted from 125 outpatient and in-patient case notes each, 84 and 97 cases of co morbidity were observed respectively. The most common co-morbidity associated with malaria was upper respiratory tract disease (70.2% and 57.7%) followed by diarrhea (17.8% and 22.7% respectively). The co morbidities cost associated with OPD and IPD per case are 293.4 and 663.4 naira respectively. The cost associated with malaria in OPD cases is 4.18 naira and the IPD cost is 17.93 naira.

DISCUSSION: From our study we found out that the combined cost of treating malaria and an associated co morbidity on the out-patient level puts an additional almost two dollar strain on the individual. This cost is almost tripled at the inpatient level. The direct cost to households of the disease was quite high and could constitute an impediment to control of the disease, especially with most expenditure paid out-of-pocket by consumers. Interventions are needed that will significantly decrease the economic burden of malaria to both households and the health system.

P598: Subtle morbidities associated with malaria co-infection with schistosomiasis among children in South-west Nigeria.

Oladele S Victoria1, Awobode O Henrietta, Onile S Gbenga,2 Anumudu I Chikae3

1Department of Zoology, University of Ibadan, Ibadan, Oyo State, Nigeria

BACKGROUND: Malaria and schistosomiasis are the most prevalent tropical diseases in sub-Saharan Africa and together exert a huge burden of mortality and morbidity. Under-recognized, ‘subtle’ morbidities such as caloric malnutrition, growth stunting, anaemia, and poor school performance are all significant correlates of both parasitic infections. This study aimed to determine the relationship between subtle morbidities and co-infection with malaria and schistosomiasis among children in South-western Nigeria.

METHODS: A cross-sectional study was conducted among primary and secondary school children, simple random sampling was used to select 240 children. Blood and urine samples were collected from the children and analysed by microscopy for *Plasmodium falciparum* and *Schistosoma haematobium*. Packed cell volume (PCV) and some anthropometric indices (height, weight) were measured as indicator of subtle morbidities
of infection with the two parasites.

RESULTS: The overall prevalence of malaria was 27.9%, schistosomiasis prevalence was 24.6% and co-infection prevalence with the two parasites was 8.3%. The highest prevalence occurred among children between ages 11-15years (Risk ratio=0.766; 95% CI: 0.497-1.181; p<0.05). The average malaria parasite density was 195.67/μl blood.

Values for packed cell volume (PCV) were normal in most of study subjects with an overall frequency rate of 88.3% while 11.7% were anaemic ($\chi^2$=0.957). From the study subjects only 1(0.4%) had splenomegaly, all others had normal spleen.

CONCLUSIONS: The effect of malaria co-infection with schistosomiasis has on subtle morbidity in the study subjects is not clear but useful information on the endemiaity of both parasites has been obtained. The high prevalence of infection among children between ages 11-15years showed their frequent contact with water bodies (recreation or farming) harbouring the snail intermediate host.

P599: Prevalence of malarial infections in sickle cell patients attending a tertiary health institution in Ile –ife, Nigeria

Adegboyega O. Oladipo, Josephine A Osewe, Olarinde O Olaniran, Saturday J Udoh

BACKGROUND: This is a prospective study which includes 150 clinically diagnosed sickle cell patients that are not on antimalarial prophylaxis visiting the sickle cell clinic. The aim to is determine the prevalence of malarial parasites in known sickle cell patients and its distribution among different age groups and sex in Obafemi Awolowo University Teaching Hospitals Complex (OAUTHC), Ile-ife, south western Nigeria.

METHODS: Thick and Thin blood films technique for laboratory diagnosis of malaria parasite and estimation of malaria parasite density was carried out according to WHO standards.

RESULTS: The study showed that out of 150 subjects used, 114(76%) were positive for malaria parasites while 36(24%) were found to be negative. Out of the 50 control samples used, 8(16.0%) were positive with insignificant parasitaemia of 124cm/ml, while 42(84.0%) were negative confirming the high prevalence and parasitaemia. The infection was high in male patients 69(60.5%) than the females 45(39.5%) with P value less than 0.5 (P<0.5) showing that there is no association between males and females with or without parasitaemia. Patients within the age groups 11-20 years had a higher prevalence, 40(78.4%) followed 21-30 years 27(77.2%), 0-10 years 26(70.3%), 31-40years 20(80.0%) while age groups 41-50 years had 1(50.0%). Parasitic density was higher for children between ages 11-20years (38,500µl/cm) and patients within the reproductive age group 21-30years(29,000µl/cm) followed by 0-10years(22,300µl/cm), and 31-40years(18,500µl/cm) while the least is seen within ages 41-50(7,500µl/cm). Plasmodium falciparum had a higher occurrence of 68(61%), followed by Plasmodium malariae, 21(7.5%) while Plasmodium vivax and Plasmodium ovale had no occurrences.

CONCLUSIONS: According to this study, malarial infection is known to escalate sickle cell crisis, therefore, preventing malaria in people with sickle cell disease may help to reduce crisis and all problem associated with it.

P601: Evaluation of host humoral antibody production against Plasmodium falciparum recombinant circumsporozoite antigen in Nigerian children

Olusawosap A Oalubla, Oluseyi E Ogulanla, Chiaka Anumudu

BACKGROUND: The challenge of malaria and efforts targeted at developing malaria vaccines triggered this study on the reactivity of IgG and its subclasses in the test serum specific to CSP. This work was directed at assessing the influence of age and gender on host humoral antibody against Plasmodium falciparum recombinant circumsporozoite antigen in Nigerian children.

METHODS: In all, 67 serum samples (>10,000 parasites/μl of blood) collected from malaria-infected children at the University College Hospital, Ibadan during the transmission season were analyzed by ELSA.

RESULTS: The mean absorbance values of IgG subclasses reactive against P. falciparum CSP appeared to be age-dependent and ranged from 0.01 for IgG4 in younger children to 0.95 for IgG3 in older children. The sixty-seven subjects investigated in this study had significantly higher mean IgG1 and IgG3 than the uninfected controls (p<0.01). This follows the order IgG3 > IgG1 > IgG2 > IgG4 which confirmed the prevalence of the cytotoxic antibodies (IgG1 and IgG3) in 65% of the malaria infected children over the non-cytotoxic subclasses (IgG2 and IgG4). Similarly, there was low production of IgG4 and IgG2 levels in 35% of the subjects compared with control. IgG was detected in the serum of North American Subjects (NAS) which served as negative control for CSP-specific IgG subclasses. Although the NAS titre was lower than that of the malaria subjects in Nigeria, its IgG2 was, however, higher (0.16) than that of other subclasses. The mean absorbance values of the total serum IgG subclass were higher than those of IgG subclasses specific to P. falciparum circumsporozoite antigen. The mean absorbance values of the
total serum IgG subclass follows the order IgG2>IgG1>IgG4>IgG3.

CONCLUSION: Antibody levels increases with age in both male and female children. Antibody levels are higher in male than female children of the same age group. Age and gender-dependent correlations of results suggest that acquired immunity could play a significant role in protection from malaria.

P602: Malaria parasitaemia and CD4+ T cell count in HIV patients attending Tertiary Medical Center, Nigeria.

Olaniran Olarinde, Udoh Saturday Jack, Ogiogwa Irube Joseph.
Department of Medical Microbiology and Parasitology Obafemi Awolowo University Ile-Ife, Nigeria.

CD4+ T Cell count is an important immunological marker of disease progression in HIV seropositive patients. This study was carried out to determine the effect of Malaria on the population of CD4+ T Lymphocytes, white blood cell, and pack cell volume of HIV sero positive patients attending active antiretroviral therapy clinic of the federal medical centre Abeeokuta. 122 subjects, 20 control subject were selected for this study. Clinical diagnosis was used as a case definition or malaria and malaria was confirmed from microscopic examination of thick film of blood sample obtained from the subject of study during presentation. The CD4+ count was evaluated during presentation by using flow cytometry. There was a significant decrease of CD4+ count of the subjects (P<0.05). However of the 122 subjects, 73 (59.8%) and 49(40.2%) where female and male respectively. Based on the presence of malaria Parasite, there is a significant decrease in CD4+ count in HIV and malaria co-infected subjects compared to the control subjects. (P<0.05), white blood cells was similar in both groups (P>0.1). There was significant difference in age paired test between the two groups (P<0.05), this result led to the conclusion that there is progressive depletion of CD4+ cells, PCV in HIV co-infection with malaria. A larger prospection study is needed.

P603: Association between Killer Immunoglobulin-like receptor genes and falciparum malaria in Ibadan southwest Nigeria.

Olukemi K Amodu1, Subulade A Olaniyan2, Adekunle A. Bakare2, Louis-Marie Yindom3, David J Conway4, Adebowale A Adeyemo5, Olayemi O Omotade6.
1Institute of Child Health, College of Medicine, University of Ibadan, Ibadan, Nigeria; 2Cell Biology & Genetics Unit, Department of Zoology, University of Ibadan, Ibadan, Oyo, Nigeria; 3University of Oxford. Weatherall Institute of Molecular Medicine, John Radcliffe Hospital, Oxford; 4London School of Hygiene and Tropical Medicine, London; 5National Institutes of Health, Bethesda, Maryland, USA

BACKGROUND: The relevance of innate immunity in malaria has been drawing more attention as protective immunity against the early stage of malaria infection. Killer Immunoglobulin-like Receptors (KIRs) are a group of Natural Killer Cell Receptors (NKR) that have been recently implicated in malaria pathogenesis and the outcome of malaria. The frequencies of KIR genes have been known to vary among different populations because of their different selective pressure imposed by host-pathogen interactions. In order to investigate the role of innate immune responses in the outcome of malaria infection, KIR genes were investigated for a possible association with malaria by comparing across 3 clinical groups; asymptomatic malaria, severe malaria and Uncomplicated Malaria groups in Ibadan south-west Nigeria.

METHODS: Five hundred and sixty children (205 controls with asymptomatic malaria, 154 with uncomplicated malaria and 201 with severe malaria) were enrolled in this study. PCR-SSP (Sequence Specific Priming) technique was used to type 15 functional KIR genes. Differences in the frequency of activating and inhibitory KIR genes across clinical groups were determined using one-way analysis of variance with a post test for linear trend. Comparison of KIR gene frequencies between asymptomatic and other clinical groups was done using binary logistic regression adjusting for age, sex, tribe and log-transformed parasite density. P values of < 0.05 were regarded as statistically significant.

RESULTS: The KIR genes KIR2DL5, KIR2DS3 and KIR2DS5 were significantly higher among the malaria cases than in the asymptomatic control group when measured by Chi square values. After further analyses using logistic regression modeling, only the activating KIR genes, KIR2DS3 and KIR2DS5 remained significantly associated with malaria (p=0.001, OR= 2.54, CI= 1.49-4.32; p=0.01, OR=1.64, CI=1.11-2.40 respectively).

CONCLUSIONS: In conclusion, KIR2DS3 and KIR2DS5 were associated with susceptibility to malaria infection in children in Ibadan, Nigeria. Activating KIR genes apparently play a role in determining susceptibility to malaria infection.

P604: Reduction in Prevalence of Plasmodium falciparum Chloroquine/ Amodiaquine Resistance (Pfcr) and Multidrug Resistance (Pfmdr) genes in Southwestern Nigeria

1Olashehinde G.J., 1Ajayi A.A., 1Adediji M.O., 1Akinjobunola O 1Department of Biological sciences, College of Science and Technology, Covenant University, PMB 1023, Ota, Ogun State, Nigeria. 2 Department of Computer and Information Sciences, College of Science and Technology, Covenant University, Ota, Ogun State, Nigeria. 3Department of Microbiology, Faculty of Science, University of Uyo, P.M.B. 1017, Uyo, Akwa Ibom State, Nigeria.

BACKGROUND: Molecular methods that detect genetic markers of drug resistance are potentially powerful tools for tracking drug-resistant malaria and providing advance information on the emergence of drug resistance patterns in the field. Such can be used to design malarial control strategies in regions where malaria is highly endemic. In this study, the combination of Pfcr and Pfmdr1 mutations in isolates associated with chloroquine and amodiaquin resistance was observed in Southwestern Nigeria.

METHODOLOGY: DNA was extracted from 140 Plasmodium falciparum positive blood samples using the QiaAmp DNA Blood Mini kit extraction method. Nested Polymerase Chain Reaction followed by Restriction Fragment Length Polymorphisms (PCR/RFLP) were used for the detection of P. falciparum chloroquine resistance transporter (Pfcr) and P. falciparum multidrug resistance 1 (pfmdr1) genes.

RESULT: Out of the 140 Plasmodium falciparum positive samples, 5.7% harbored the chloroquine/amodiaquine resistant gene (Pfcr) while 7.1% harbored the multidrug resistant gene (Pfmdr1).

CONCLUSION: A remarkable reduction in the prevalence of crt and mdr 1 genes was noticed when compared with earlier findings from southwestern Nigeria and other regions of the world. The observed reduction in chloroquine/amodiaquine resistant markers suggests that there is a decline in the prevalence of resistant parasites as well as drug pressure in this region.

P605: Impact of PermaNet 3.0 on malaria vector in an area of pyrethroid resistant Anopheles gambiae s.s. in south western Nigeria

James Olojede1, AO Adeogun2, 'M Tola3, AO Oduola1, IO Oyewole1, and CN Amajoh3, TS Awolola4.
1Department of Zoology, University of Ibadan, Ibadan, Nigeria; 2Department of Zoology, University of Ibadan, Ibadan, Nigeria; 3Department of Zoology, University of Ilorin, Ilorin, Nigeria; 4Department of Biological Sciences, Babcock University, Ilisan Remo ; 5National Malaria Control Program, Federal Ministry of Health, Abuja, Nigeria; 6Department of Medical Microbiology and Parasitology, College of Medicine University of Lagos

BACKGROUND: PermaNet® 3.0 a combination long-lasting insecticidal...
net (LLIN) is designed to have increased efficacy against pyrethroid-resistant malaria vectors. This study reports the impact of this improved tool on entomological indices in an area with pyrethroid resistant malaria vectors in south western Nigeria.

METHODS: We compared the efficacy of PermaNet® 3.0 with PermaNet® 2.0 and untreated polyester net as control (UTC) in three villages; Ilara, Irolu and Ijesa selected based on baseline entomological indices in Remo North LGA of Ogun State. In all, 137 PN 3.0, 147 PN 2.0 and 150 untreated nets were distributed at Iroru, Ijesa and Ilara respectively. Nets were distributed to cover all sleeping spaces and were evaluated for insecticidal activity. Mosquitoes were collected monthly for 12 months and analysed. Arithmetic means of mosquito catches per house, entomological inoculation rates (EIR), mean mosquito blood feeding rate, mean mortality and mean parity rates amongst PN3.0, PN 2.0 and the UTC villages before and during the intervention were compared.

RESULTS: Anopheles gambiae s.l. accounted for >98% of the Anopheles population in the three villages and was abundant for 6-7 months. Deltamethrin, permethrin, lambdacyhalothrin and DDT resistance were confirmed in the 3 villages. kdr mutation was the sole resistance found at Ilara and kdr plus p450-based metabolic mechanisms at Irolu and Ijesa. Esterase and GST mechanisms were absent. Bioassays repeated on domestically used PN 2.0 and PN 3.0 had (100%) bio-efficacy. Efficacy remained (100%) for both net types after the 3rd, 6th, 9th and 12th month following nets distribution. The use of PN 3.0 showed significant reduction in mosquito densities with a ‘mass killing’ effect. It also induced changes in endophilic and anthropophilic tendencies, reduced blood feeding, lowered mosquito parity rates and sporozoite rates compared to PN 2.0 and UTC villages. EIR was significantly reduced in PN 2.0 village (75%) and PN 3.0 village (97%) post LLIN-distribution but remained same for UTC village.

CONCLUSION: The study confirms the effectiveness of PN 3.0 in reducing malaria transmission compared to pyrethroid-only LLINs in the presence of malaria vectors with kdr plus metabolic-based resistance mechanisms.

P606: Effect of Crude Ethanolic Extract of Medicinal Mushroom, Ganoderma lucidum (W. Curt.:Fr.) P. Karst. on Triglyceride Metabolism in Plasmodium berghei Malarial Infection

Olarewaju M. Olubas1 and E. Chukuwu Onyeneke2
1Department of Biochemistry, College of Natural Sciences, Joseph Ayo Babalola University, Ikiji-Arakeji, Osun State, Nigeria; 2Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin-City, Edo State, Nigeria.

BACKGROUND: The malarial parasite has been observed to have a tremendous requirement for lipids during the replicative stages that take place in the mammalian host. An early and consistent metabolic alteration during infection is increased serum triglyceride levels characterized by an increase in VLDL levels. Elucidating stage-specific requirements for import, synthesis, and utilization of lipids in malarial parasite infection could be of considerable promise for developing novel anti-malarial intervention strategies. Thus, this study was designed with the aim of monitoring changes associated with triglyceride metabolism in Plasmodium berghei-infected mice treated with crude aqueous extract of Ganoderma lucidum (GLE).

RESULTS: Parasitemia was suppressed to about 85% in mice treated with GLE while CQ achieved 100% parasite clearance 3 days after treatment. Serum and liver TG, TC, VLDL concentrations and LPL activities were observed to be significantly higher in GLE and IFC mice compared to CQ at day 1 (post-treatment) compared to day 0 (before treatment). Considerable time-dependent significant decreases were observed in the lipid levels and LPL activities in GLE and CQ mice compared to IFC.

CONCLUSION: Data generated in this study suggest that the antimalarial activity of Ganoderma lucidum and chloroquine may involve mechanism relating to down-regulation of triglyceride metabolism, an important cellular event for parasite replication.

P607: Molecular characterization of the cytochrome b gene and in vitro atovaquone susceptibility of Kenyan Plasmodium falciparum isolates

Luise Ingasia Hosea Akala, Agnes Cheruiyot, Redemptah Yeda, Angela Omondi, Dennis Juma, Charles Okudo, Wallace Bulimo, Ben Andagalu, Jacob Johnson, Edwin Kamau
Global Emerging Infections Surveillance program, United States Army Medical Research Unit (USAMRU-K)

BACKGROUND: Atovaquone (ATQ) is an anti-malarial drug used in combination with proguanil for the curative and prophylactic treatment of malaria. ATQ drug failure is associated with point mutations at position 268 in cytochrome b gene (cytb), exchanging tyrosine for serine (Y268S) or, less frequently, asparagine (Y268N). The resultant ATQ-resistant growth IC50 phenotype of these mutants is some 1000-fold higher than sensitive strains. Though antimalarial resistance has not been reported in Kenya, the status of these polymorphisms and their implications to AP treatment outcome is undetermined. This study established the in vitro susceptibility of Kenyan P. falciparum field isolates to this drug and correlated them with mutations at codon 268 in cytochrome b gene (cytb).

METHODS: One hundred and seventy three P. falciparum field isolates collected during 2008 to 2011 from Kenya were analyzed for the in vitro atovaquone susceptibility using malaria SYBR Green I based assay. Mutations in the cytb gene were determined by PCR-RFLP and sequence analysis.

RESULTS: Median (IC50) was 3.168N M, depicting atovaquone susceptibility. Based on the resistance cut-off, all but 4 Kenyan isolates were atovaquone sensitive. Of the 173 isolates genotyped 48% had the wild type Y at amino acid position 268 while 33%, 22% and 5% had mixed genotypes Y/S, Y/N/S and Y/N respectively. There was no correlation between these polymorphisms and in vitro antimalarial response (P=0.0752).

CONCLUSION: The presence of diverse polymorphisms was somewhat unanticipated given that atovaquone has never been a prescription drug in Kenya. Further studies on the mutations within this gene in Kenya are important to better understand the natural polymorphism of this gene. With continued use of AP for prophylaxis, efficacy studies to follow-up the in vitro data would be warranted.

P608: Toxicological effects of prolonged and intense use of mosquito coil emission in rats the implications on malaria control

Olabunmi Otubanjo

BACKGROUND: Mosquito coil is a vector control option used amongst individuals to prevent malaria in endemic low income countries. While certain studies have addressed this issue, additional studies are required to increase knowledge on the adverse health effects caused by the prolonged use of mosquito coils.

METHODS: Toxicological effects of fumes from two locally manufactured mosquito coil insecticides (containing pyrethroids: transfluthrin and...
d-allethrin as active ingredients) on male albino rats was investigated. The haematological, biochemical indices, histopathology and mutagenicity evaluations in rats exposed to mosquito coil fumes during 2, 4, 8, 12 and 16 week periods were recorded. Haematological determination was performed using automated hematology analyzer to determine White Blood Cell (WBC), Packed Cell Volume (PCV), Red Blood Cell (RBC) and Platelet (PLT) counts, while biochemical evaluations were determined using available commercial kits. Gross histopathological changes were studied for the kidney, liver and lungs in sacrificed rats. The rat sperm head abnormalities assessment was used to evaluate mutagenicity.

RESULTS: Mosquito coil fumes produced significant increase (P< 0.05) in the levels of total protein, total albumin and bilirubin, when animals were exposed from two weeks to 16 weeks with transfluthrin. Similarly, elevation in the activities of aspartate amino transferase, alanine amino transferase and alanine phosphatase increased significantly in both insecticides. Increase in WBC, RBC and PCV were recorded for all the exposure periods, however PLT count showed no significant increase (P>0.05). Mutagenicity assessment revealed sperm abnormality was statistically significant (P<0.05) compared with the control at 8, 12 and 16 weeks post exposure to transfluthrin. Histological studies revealed severe lung damage evidenced by interstitial accumulations, pulmonary oedema and emphysema in exposed rats. Intracellular accumulations and severe sinusoidal congestion of liver cells were observed from 12 weeks exposure, indicating liver damage

CONCLUSION: The long term and indoor usage of mosquito coil must take into cognizance the pathological consequences.

P609: Malaria and dengue co-infection among febrile cases in Ibadan, Nigeria

Olufunmilayo Oyero (Phd)
Institute for advanced Medical Research and Training (IAMRAT) College of Medicine, University of Ibadan, Nigeria

INTRODUCTION: Malaria and dengue are two common mosquito infections that are very important and can cause high morbidity and mortality particularly in the tropical countries where factors favoring the massive breeding and maintenance of the vectors exist. In Nigeria and many sub-Saharan African countries, febrile cases are often presumed and treated as malaria infection and it is only when there is a treatment failure (following the administration of antimalarials) that other diagnosis are sought. However this does not include diagnosis for endemic viral agents. This study therefore sought to determine a possible co-infection of malaria and dengue fever virus among cases.

METHODOLOGY: One hundred and eighty-eight sera and whole blood specimen obtained from consenting individuals were screened for concurrent dengue and malaria infection. Indirect Elisa and rapid card insta test developed by Cortez diagnostics were employed.

RESULT: Dengue specific IgM and Plasmodium falciparum specific histidine rich protein-2 (PfHRP-2) were detected in 15 (8.0%) of the 188 cases.

CONCLUSION: Though rarely reported, this study confirmed malaria and dengue co-infection among febrile cases in Ibadan. This may have contributed to the very high morbidity rate of malaria in Nigeria. It is therefore noteworthy that complications such as bleeding, shock, liver failure and fluid in lungs often experienced with severe malaria are also associated with dengue infection. Therefore such presentations may be due to a multi organism infection and an inclusion of dengue in the differential diagnosis of febrile illnesses is hereby advocated.

This is because an early and specific diagnosis of dengue may lead to the institution of early supportive treatment with an overall reduction in mortality.

P610: Asymptomatic malaria parasitaemia in sickle-cell disease patients: how effective is chemoprophylaxis?

Rachel Kotila1, Abiola Okeola1 & Olufunmilola Makanjuola2
1Department of Haematology, College of Medicine, University of Ibadan, Nigeria; 2Department of Medical Microbiology & Parasitology, College of Medicine, University of Ibadan, Nigeria

BACKGROUND: Sickle-cell trait confers protection against malaria while homozygote sickle-cell disease (SCD) patients are at greater risk of malaria infection, hence the use of malaria chemoprophylaxis in SCD patients. The use of malaria chemoprophylaxis and asymptomatic parasitaemia were studied in SCD and non-SCD patients.

METHODS: A semi-structured questionnaire was administered to both patients and controls; a thick blood film was also examined in both the groups.

RESULTS: Sixty-nine percent of patients use proguanil, 22% do not use any form of chemoprophylaxis, while 9% use pyrimethamine. There was no significant difference between level of parasitaemia in patients and controls (p = 0.1), a positive smear was found in equal numbers of patients on chemoprophylaxis and those not on chemoprophylaxis (p = 0.3). In the month preceding the study, 31% of patients vs 18% of controls had received treatment for malaria. There were no significant differences between patients and controls in frequency of malaria attacks (p = 0.06), last episode of malaria (p = 0.2). Ten percent of patients and 2% of controls use bed-nets.

CONCLUSION: This study did not find any advantage in the use of malaria chemoprophylaxis in SCD patients over controls or SCD patients not on chemoprophylaxis. Vector control should also be considered in the fight against malaria. There is a need to look into why both patients and controls fail to use bed-nets in a malaria endemic country.

P611: Partial Outcome Appraisal of an Ongoing Efficacy Study Trial to Reduce Vulnerability to Malaria Mortality in Children

By Pharm. Vincent Olughor
University College Hospital Ibadan Nigeria

BACKGROUND: Plasmodium is one of three apicomplexan parasites of significance to man, with Plasmodium falciparum responsible for over 75% of Malaria-related Mortality among black children in sub-Saharan Africa and other places where Malaria is stable and transmission occurs all year round and affects mainly Children. Classical regions where stable malaria infections can be found are West Africa and Low Lands of Papua New Guinea.

Malaria continues to kill children made vulnerable by the presence of I-GATA (immature Glutamine-to-Alanine Transaminase Apparatus in children <72 months of age), ‘Heparan’ gene expression exclusively by parasites, and the ‘heparin like’ like factor 9 in sickle-cell disease patients: how effective is chemoprophylaxis?

RESEARCH PROBLEM/HYPOTHESIS: The emergence of resistance to Artemeter-Lumefantrine, reported by WHO, along the Laos/Cambodia border, is an indication that the development of resistance by the parasite to antimalarials over the years is an inherent property. The need for new approaches is beginning to seem relevant. The hypothesis for testing assessed by this study is as follows: “there are surrogate markers in
human blood for assessing vulnerability to malaria mortality which may respond to intervention manipulation that can reduce such vulnerability.”

**METHODOLOGY:** Healthy black children aged 3-9 years living in Ibadan who showed low values for one or more values at baseline of the surrogate markers of vulnerability to malaria-mortality were recruited for the trial and a standard polymeric nutraceutical therapy benchmarked as “SPNT-23” was used for the manipulation for a duration of 28 days. The outcome data were collected thereafter.

**SUMMARY OF RESULTS:** Analysis of data collected so far showed statistical significance P-values for three of the surrogate markers used including serum albumin.

**CONCLUSION:** It is possible to reduce vulnerability to malaria mortality in the future.

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**P612: Submicroscopic Parasitemia in Rural and Urban Lagos, South-Western Nigeria**

OlukosiYA1, Ajabye OG2, Okoh HI3, Oyebola K4, Iwalokun BA5, Agomo CD6, Oyebode TA7, Oyebode TA7, Okolosola AO8, Adetola3

**INTRODUCTION:** Submicroscopic parasitemia is increasingly becoming a diagnostic challenge at estimating the true burden of malaria, with the increasing effectiveness of malaria control efforts and urbanization. This study evaluates the performance of microscopy and qPCR in diagnosing and quantitating malaria parasites in an urban and a rural clinical trial site.

**METHODOLOGY:** Cross-sectional surveys were conducted in hospital laboratories in rural Ijede and urban Lekki sites of Lagos SW Nigeria, spanning the period 2008-2010. Malaria parasite detection and quantitation using microscopy and qPCR were compared for these two sites.

**RESULTS:** Prevalence by PCR at the urban site was 29% (330/1158) and 11% (111/997) by microscopy while at rural Ijede corresponding values were 30.2% (197/652) and 25% (168/658) respectively. The differences between the prevalence values at both sites were not significant by PCR ($\chi^2=0.52, P=0.47$) but very significant by microscopy ($\chi^2=57.6, P<0.0001$). Composite sensitivity for microscopy had lower sensitivity compared to PCR with a value of 41% and a specificity of 90%. At individual study sites, sensitivity of microscopy was better at the rural site with 61% than at the urban site 30% and specificities at both sites were 99% and 98% respectively. Sub-microscopic parasitaemia at the rural site was less, 74/197 (37.5%), compared with the urban 211/336 (62.2%), ($\chi^2=30.34, P<0.0001$). This was reflected in the variance in the geometric mean parasite density (GMPD) observed-6999p/µL at the rural, and 2701p/µL at the urban sites. In children 5years or less, the parasitaemia was significantly more than in the rest of the sample population at the rural and urban sites with GMPD 13579p/µL and 13797p/µL, compared to 4289µLand 3357µL in participants over the age of 5years at the rural and urban sites respectively. Quantitation by microscopy and qPCR agreed reasonably(Cohen’s Kappa agreement 0.48, Spearman’s rank’s correlation coefficient was 0.5, CI 0.37-0.59, mean difference of log units in the Bland Altman plot of 0.87; and 95% limits of agreement(mean ± 2SDs) between the two methods, 1.5 to 3.1).

**CONCLUSION:** Result from this study highlights the possible role for qPCR in diagnosing and quantitating malaria parasites in urban centers of Lagos, and other apparent regions of diminishing prevalence.

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**P613: Evaluation of microscopy, RDT and PCR methods in the reduction of over diagnosis of malaria by clinical method among Nigerian children**

Ojurongbe Olusola1, Adegbosin Olanike1, Taiwo Sunday Samuel2, Alli Oyebode Terry Armstrong2, Olowe Adekunle Olugbenga1, Ojurongbe Taiwo Adetola3

1Department of Medical Microbiology & Parasitology, Ladoke Akintola University of Technology, Osogbo; 2Department of Biomedical Science, Ladoke Akintola University of Technology, Osogbo; 3Department of Mathematical and Physical Sciences, Osun State University, Osogbo

**BACKGROUND:** Current malaria control goals include elimination and ultimately eradication. These ambitious goals will require, among other things, the use of effective diagnostic tools for accurate detection and monitoring of all malaria cases, including submicroscopic parasitemia.

**METHODS:** Children presenting at the outpatient department of two tertiary hospital were clinically diagnosed for malaria based on malaria presumptive diagnosis. Blood samples was collected from all the children and the blood was used to prepare thick and thin blood smear for microscopic detection. Also an RDT, Paracheck, which detects P. falciparum HRP2 was performed immediately. Blood was spotted on filter paper from where DNA was extracted for stevor PCR.

**RESULTS:** In All 217 patients that were clinically diagnosed for malaria. When this was subjected to laboratory methods 108 (49.8%), 83 (38.2%) and 123 (56.7%) were positive by microscopy, RDT and PCR respectively. Using a composite reference for Microscopy, RDT and PCR, 71 children were infected with P. falciparum and 90 uninfected. PCR had a sensitivity of 97.3% and specificity of 62.5%, Microscopy 77.2% sensitivity and 72% specificity while RDT has 63.2% sensitivity and 87.4% specificity. The negative predictive value of PCR, Microscopy and RDT was 97.8%, 81.1% and 67.7% respectively while the positive predictive value were 56.8%, 66.9% and 84.5% respectively. PCR was the most sensitive method while RDT was the least sensitive particularly for cases with low parasitaemia.

**CONCLUSION:** The study revealed the need for complete shift from symptom-based-diagnosis to parasite-based management. This will bring significant improvement to tropical fever management and reduce over diagnosis, drug wastage and also help to curtail development of malaria drug resistance.

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**P614: AnoSpEx: A stochastic spatially-explicit computational model to simulate and validate Anopheles metapopulation dynamics towards malaria control and eradication.**

Oyewole Adekunle Olugbenga1, Ezekiel Adebiyi1, Jason Rasgon2

1Department of Computer and Information Sciences, Department of Computer and Information Sciences, Covenant University, Nigeria, West Africa

**BACKGROUND TO THE STUDY:** Malaria incidence has increased in Africa due to climate change, insecticide and drug resistance, and social/ economic issues. It is most commonly transmitted through the bite of infected female Anopheles mosquitoes. Anopheles mosquitoes transmit
malaria, a major public health problem among many African countries. One of the most effective methods to control malaria is by controlling the Anopheles mosquito vectors that transmit the parasites. ANOSPEX model will be useful in a predictive and exploratory manner to develop, evaluate and implement novel strategies to control malaria and possible eradication.

STATEMENT OF THE RESEARCH PROBLEM: Malaria constitutes a public health problem in some African countries, mostly affected are countries within Sub-Saharan Africa. It has been a major source of concern to the health of infants and pregnant women.

RESEARCH METHODOLOGY EMPLOYED: Mathematical models have both predictive and explorative utility to investigate the advantages and disadvantages of different malaria control strategies. We have developed a Visual C++ based, stochastic spatially-explicit model (ANOSPEX: Anopheles Spatially-Explicit) to simulate Anopheles metapopulation dynamics. The model is biologically rich, parameterized by field data, and driven by field-collected 8-year weather data from Macha, Zambia. Simulation results were validated using (CDC, CBT and HLC) mosquito collection data from Macha, Zambia.

RESULTS: We were able to build a model that could simulate the metapopulation dynamics of Anopheles species over a period of 1095 days.

DISCUSSION AND CONCLUSION: Computational models as tools for malaria control are steps in the right direction. AnoSpEx model was instrumental to study the metapopulation dynamics of Anopheles species towards malaria control. Validation of the AnoSpEx model revealed that it was able to predict a similar trend to the pattern of real-life Anopheles CDC, CBT, and HLC trap collection data from Macha, Zambia. We hope to integrate novel genetic control methods into AnoSpEx in the future towards developing an advanced model for eradicating malaria. Such genetic control methods as SIT, lethal densovirus and biological weapon like entomopathogenic fungi will be integrated into an advanced version of AnoSpEx (AnoSpEx++) for malaria eradication campaign.

P616: Placental malaria and its effect on pregnancy outcome in the Blue Nile State, Sudan

Samia Omeri, Eltahir Khalil, Abdalla Sharief, Galal Yosif and Muatsim A/Rahim

A/rahim

BACKGROUND: Malaria is a major public health problem in Sudan. Each year, more than 1 million women become pregnant in Sudan, of those 750,000 are in areas of intense perennial, high seasonal transmission or in areas of irrigation. Maternal mortality in Sudan is 509/100,000. Malaria during pregnancy also impacts the infant’s health, as a result of infection of the placenta and malaria-caused maternal anaemia, which both contribute to low birth weight (LBW), the single biggest risk factor for neonatal mortality. A Safe motherhood survey in 1999 in Sudan found that 46% of infant mortality occurred during the neonatal period.

METHODS: Between January 2012 and February 2013, 717 women delivering at Damazeen Hospital and Rusiers Hospital the two largest health centers located in the Blue Nile State, Sudan were recruited. Peripheral malaria slides were taken prior to delivery; placental smears were taken at the time of delivery. Information on the newborn and placenta were recorded soon after birth including infant birth weight and placental weight determination of the outcome of pregnancy. Length of gestation was estimated based on date of last menstrual period, and assessment of the newborn at delivery. The majority of pregnant women were between 21-30 years of age and 18.5% of whom were primigravidae.

RESULTS: Based on detection of parasites in either peripheral or placental blood smears primigravidae women were more infected (39.2%) compared to multigravida women (22.9%) at delivery time (p=0.01). Thirty five percent of newborns weighed less than 2.5 Kg at delivery time. Generally, multigravidae women had babies with mean birth weight that is significantly higher than that of primigravidae (p=0.03), the same was true for the mean placental weights (p=0.017). Placental malaria was significantly associated with pre-term delivery and intrauterine growth retardation (p<0.01). Moreover, birth weight was significantly lower in mothers infected deliveries compared to non infected deliveries in both gravidity groups (p=0.03).

CONCLUSION: Placental infection in Blue Nile State is prevalent and infections were associated with poor pregnancy outcome: pre-term, still birth and low birth weight.

P617: Anti-protozoan activities of Harungana madagascariensis stem bark extract on trichomonads and malaria.

Omotayo omisore

BACKGROUND: Plasmodium and Trichomonas are pathogenic protozoans and cause more sickness and debilitation than other members of the group. Malaria is one of the world’s greatest killers ranking in this respect with cancer and heart disease. H. madagascariensis is traditionally used to treat haemorrhages, anaemia, fever and headaches among others.
This study investigated the antimalarial and anti-trichomonal properties of the plant stem bark.

**METHOD:** The ethanolic stem bark extract of Harungana madagascariensis (Hypericaceae), (Choisy) Poir were evaluated for their activities on Trichomonas gallinaceae (Rivolta) Stabler isolated from the pigeon (Columba livia). It was also tested for their anti-malarial activity on N67 Plasmodium yoelii nigeriensis (in vivo) in mice and on Plasmodium falciparum isolates in vitro. The anti-trichomonal screening was performed in vitro using Trichomonas gallinaceae culture. The minimum lethal concentration (MLC) is the lowest concentration of the test extract in which no motile organisms were observed. The anti-malarial effects were determined in-vivo for suppressive, curative and prophylactic activities in mice receiving a standard inoculum size of 1 x 10^7 (0.2 ml) infected erythrocytes of Plasmodium yoelii nigeriensis intraperitoneally, and the in vitro was performed against 3 isolates of Plasmodium falciparum in a candle jar procedures.

**RESULTS:** The IC(50) of the extract, metronidazole (MDZ) (Flagyl) on Trichomonas gallinaceae at 48 h are 187 and 1.56 microg/ml. The IC(50) of the extract, chloroquine (CQ) and arteether (ART) on Plasmodium falciparum are between 0.052 and 0.517 microg/ml for the extract and 0.021 and 0.0412 microg/ml for ART and CQ, respectively. The actions of the extract in vivo study on Plasmodium yoelii nigeriensis showed that in both suppressive and prophylactic tests the percentages chemotherapy suppressive were between 28.6-44.8% and 30.2-78.2% respectively, while only 80 mg/kg of the extract reduced the parasitaemia level when compared to the control and the standard drugs in curative test.

**CONCLUSION:** Harungana madagascariensis stem bark extract therefore exhibited significant anti-protozoan effects against Trichomonas and Plasmodium both in vivo and in vitro.

P618: Relationship between Antioxidant and Antiplasmodial Activities of Compounds from *Donodanae angustifolia* and *Senecio roseiflorus*.

**Kerubo, L. O., Midiwo, J. O., Akala, H. M.**

1 Department of Chemistry, Nairobi University, Nairobi, Kenya; 2 Institut für Chemie, Potsdam Universitä, Potsdam, Germany; 3 United States Army Medical Research Unit-Kenya, Walter Reed Project, Kisumu.

**BACKGROUND:** There is generation of Reactive oxygen species (ROS) during malarial infection and antioxidants would contribute towards limiting these oxidative damage. Flavonoids which are the main phytochemical feature of *Donodanae* species, display antiplasmodial activities which could be attributed to their radical scavenging activities (RSA). There is speculation that the antiplasmodial activities pure compounds could be due to the ability of the phenolic compounds to wipe out reactive oxygen species (ROS) generated in different stages of malaria infection. In this study the RSA and antiplasmodial activity of pure compounds from *Donodanae angustifolia* and *Senecio roseiflorus* was compared. This was to ascertain whether there is a correlation between antiplasmodial activity and RSA.

**METHODS:** The crude extract and the pure compounds were assayed using an automated micro-dilution technique to determine 50 % growth inhibition of cultured parasites. Two strains (chloroquine sensitive Sierra Leone 1 (D6) and chloroquine-resistant Indo-china 1 (W2)) of *Plasmodium falciparum* parasites were used. Radical scavenging test was done using 1,1-diphenyl-2-picrylhydrazyl (DPPH) where the scavenging activities of the samples were measured as the percentage decrease in absorbance (at 517 nm) of DPPH radical after mixing the sample with this reagent.

**RESULTS:** The compounds tested showed antiplasmodial activities between 7.6 and 23.6 mg/ml. The diterpenoids tested showed no RSA activity at all as expected. The flavonol, kaempferol was found to be the most active with % RSA of 96.8 at 50 µM followed by 3,5,4'-tri-hydroxy-7-methoxyflavone. The structure-activity relationship showed that flavonols had appreciable activity as compared to 3-methoxy flavones indicating the importance of the hydroxy group at the C-3 position.

**CONCLUSION:** Some flavonoids exhibited both antiplasmodial and radical scavenging activities although there was no clear relationship between the activities. There seems to be additional factors other than antioxidant activities that would be contribute to antiplasmodial activity of compounds.

P619: Larvicidal activity, qualitative and quantitative components of *Parquestina nigrescens* leaf extracts on different larval stages of an African malaria vector.

**Omuya Funmilola and Oladipupo Kolawole**

Department of Microbiology, School of Sciences, Federal University of Technology, Akure, Nigeria

**BACKGROUND:** Hemoparasites are parasites of significant health importance considering the morbidity and mortality rates recorded globally as a result of the diseases they causes. Malaria is one of such diseases which affect both humans and other animals. *Plasmodium* species responsible for human malaria are vectored by female *Anopheles* mosquitoes. Drug resistance displayed by *Plasmodium* contributed to the difficulty in combating malaria which has necessitated the need to search for alternative control measures. Traditionally, plants and herbs play crucial roles in controlling diseases. *Parquestina nigrescens* is one of these plants. In this study the larvicidal activity of different extracts of *Parquestina nigrescens* were investigated on *Anopheles* mosquito larvae.

**METHODS:** The leaf part of *Parquestina nigrescens* was collected, air dried and blended in preparation for extraction processes using different solvents namely methanol, ethanol and aqueous solution. Qualitative and quantitative components of the leaf extracts were assessed. The bioactivity of these extracts were conducted on laboratory reared second and fourth instars of *Anopheles* mosquito larvae for 48 hour and the mortality recorded at 12 hours intervals. Each treatment was carried out in triplicates. The LC 

**RESULTS:** In ethanol extract treatment, the highest percentage mortality of 95% was recorded in the fourth instars while 84% mortality was recorded in the methanol extract. The highest impact of the plant bioactivity was recorded on the second instars where 100% mortality was recorded in the ethanol treatments. The treatments were significantly different when P<0.05. The qualitative screening of the plant revealed the presence of compounds such as Saponin, Alkaloid and Flavonoid while quantitative assessment showed that the alkaloid has the highest value of 1.81.

**CONCLUSION:** This study revealed that the leaf extract of *Parquestina nigrescens* has larvicidal property on *Anopheles*. Hence it might be a potential biological control agent for malaria vector.

P620: A review of current status of insecticide resistance of malaria vectors in Kenya

**Benyl Ondeto1,2, 3 (MSc), Horace Ochanda4 (PhD), Joseph Mwangangi4 (PhD), Evan Mathenge2 (PhD), Kiambo Njagi2 (PhD), Charles Mbogo1 (PhD)**

1 Kenya Medical Research Institute, Center for Geographic Medicine Research - Coast, Kenya; 2 School of Biological Sciences, University of Nairobi, Nairobi, Kenya, 3 Division of Malaria Control, Ministry of Public Health and Sanitation, Kenya, 4 Mount Kenya University, Thika, Kenya.

**BACKGROUND / INTRODUCTION:** The presence of a well coordinated malaria insecticide resistance database in Kenya will help in planning malaria vector interventions. Such information is important in developing strategies that facilitate improved vector control resulting in significant reduction of morbidity and mortality of malaria. The main goal of this study was to assemble a database on the current status of insecticide resistance of *Anopheles gambiae* s.s., *An. arabiensis* and *An. funestus* in Kenya that are competent vectors of human malaria.

**METHODOLOGY:** Data was obtained from published literature through Pubmed and Hinari searches. Each data source was assigned a specific identification code and entered into Microsoft Excel. Base maps were then
**P621: Age-specific half-lives of antibodies to Plasmodium falciparum antigens in the absence of significant malaria transmission**

Bartholomew N. Ondigo1,2, Jim S. Hodges1, Kathleen F. Ireland2, Gideon M. Ngwena3, David E. Larar4, David L. Narum5, Ayub V. Ofulla6, Chandy C. John1*

1Department of Biomedical Science and Technology, Maseno University, Maseno, Kenya. 2Center for Global Health Research, Kenya Medical Research Institute, Kisumu, Kenya. 3Division of Biostatistics, School of Public Health, University of Minnesota, MN, USA. 4Department of Pediatrics, University of Minnesota, MN, USA. 5Department of Medical Physiology, Moi University, Eldoret, Kenya. 6Division of Malaria Vaccine Development, Walter Reed Army Institute for Research, MA, USA.

**BACKGROUND:** Measurement of antibodies to Plasmodium falciparum antigens provide a method of assessing prior exposure and could assist in prediction of future susceptibility to clinical malaria. This study aimed to assess changes in antibodies to multiple antigens, rates of age specific antibody seroreversion and half-lives of antibodies during a period when malaria transmission was low in an unstable area.

**METHODS:** Antibodies to eleven P. falciparum antigens were measured in a matching pair of plasma samples (n=1000) from individuals in this area in May 2007 and July 2008. Measurements were done by multiplex cytometric bead assay (CBA) and ELISA. A linear/spline regression model was used to determine the age specific proportions of seroreversion and logistic regression used to estimate age specific half-lives of antibodies to P. falciparum antigens.

**RESULTS:** Acquisition of antibodies with age differed according to antigen. Three patterns of acquisition were noted: rapid (acquired >80% of individuals by 20 years of age: apical membrane antigen-1 (AMA-1), erythrocyte-binding antigen-175 (EBA-175), glutamate-rich protein (GLURP)-R2, merozoite-surface protein-1 (MSP-1)α, and MSP-1β), moderate (acquired by >40% of individuals by 20 years of age: liver-stage antigen-1 (LSA-1), GLURP-R0, and merozoite surface protein-3 (MSP-3) and slow (acquired by ≤40% of individuals by 20 years of age: circumsporozoite protein (CSP), thrombospondin-related adhesive protein (TRAP), and schizont extract (SE). Antibody seroreversion rates over the 14 month period were bimodal (frequent seroreversion in children <1 year of age, decreasing with age to minimal seroreversion over the 14 month period were biphasic (frequent seroreversion in children <1 year of age, decreasing with age to minimal seroreversion from ~10 years of age on) for all antigens except CSP, GLURP-R0 and TRAP which had a slow, monophasic decrease in the frequency of seroreversion with age across the full spectrum of ages, and SE, which demonstrated which had a slow, monophasic decrease in the frequency of seroreversion with age across the full spectrum of ages, and SE, which demonstrated with age.

**CONCLUSION:** Age-specific half-lives of antibodies with age differed according to antigen. These results, though still alarming are better than in the past where a fatal case was recorded every 30 seconds. However, the elimination of this endemic disease remains a major challenge in the tropics particularly in Cameroon. Therefore, identification of community factors especially in rural areas in order to strengthen prevention strategies in the country would be of great asset.

**P622: Assessment of attitudes and practices of rural women towards malaria in western region, Cameroon: Strategic implications for prevention programs**

Sanou Sobze Martin1, Onohiol James-Francis1, Fokam Josep2, Kamedjie Pete Patrick Martial1, Guëtiya Wadoum Raoul Emeric2, Djeunong Dongho Ghyslaine Bruna1, Tenoh Guedougou Alain3, Temgue Gaët4, Roseline Tepogning Nzangue5, Vittorio Colizzi˄, Annette Habluetzel˄˄, Gianluca Russo***

1Faculty of Sciences, University of Dschang, Dschang, Cameroon. 2Chantal Biya International Reference Centre (CIRCB) for research on HIV/AIDS prevention and management, Yaoundé, Cameroon. 3University of Ngaoundéré, Adamawa, Cameroon. 4University of Rome Tor Vergata, Rome, Italy. 5University of Cameroon, Italo. 6University of Rome La Sapienza, Rome, Italy.

**BACKGROUND:** In sub-Saharan Africa, a child under 5 years dies of malaria every minute. These results, though still alarming are better than in the past where a fatal case was recorded every 30 seconds. However, the elimination of this endemic disease remains a major challenge in the tropics particularly in Cameroon. Therefore, identification of community factors especially in rural areas in order to strengthen prevention strategies in the country would be of great asset.

**Methodology:** A cross-sectional and analytical study was conducted from June to August 2011 among rural women in the health district of Dschang, West Cameroon. Data were collected by interview by the use of a questionnaire. Epi Info Version 3.5.3 was used for the analysis and Chi squared test (at α = 0.05) for the comparison of proportions.

**RESULTS:** 517 women, with median age 40 years were enrolled. On average, there were seven people per household with a child ratio (<5 years) / household 3/2. 4.9% of women were pregnant, with 32% having attended no antenatal care. 80% of women (93% of pregnant women), did not sleep under an insecticide impregnated bed net, the main reasons being: non-availability (46%), and non-acceptance (19%). Furthermore, among women complaining of fever (<64%), no significant difference (p > 0.9) was observed between those who used the insecticide impregnated bed net and those who did not make use of it. Finally, the presence of the ceiling in some homes (45.2%) proved important as there was a highly significant difference (p < 0.0001) among women complaining of fever who dwelled in houses with a ceiling and those whose homes did not have.

**CONCLUSION:** The non-use of insecticide impregnated bed nets in rural areas resulted primarily from the non-availability and its non-acceptance. Therefore, a wide distribution of these insecticide impregnated bed nets combined with awareness campaigns, will not only promote accessibility and acceptability, but also their proper use, which will surely aim at achieving the Millennium Development Goal (MDG) number 6.

**P623: KAP of ITNs among pregnant women in ALUU community, Rivers state, South South, Nigeria.**

Onwubiko

**BACKGROUND:** Malaria infection during pregnancy is a major public health challenge especially in the tropical and subtropical regions of the world. This study was conducted between May and June 2013, and sought to know the Knowledge, Attitude, and the Use of Insecticide
treated nets (ITNs) among pregnant women in ALUU community, South South, Nigeria.  

METHODS: A descriptive cross-sectional survey was undertaken in the antenatal clinics of the primary health centers located within ALUU community. A structured self-administered questionnaire was used to collect data on the KAP of ITNs, from the 210 respondents.  

RESULTS: Majority of the respondents showed reasonable knowledge of malaria, including correct association between malaria and mosquito bites. There was also substantial knowledge of the prevention of malaria, through the use of ITNs. It was observed that 89.05% (n=187), have heard about ITNs and 90% (n=189) of the respondents agree to the necessity of ITNs use to prevent malaria in pregnancy. It was also observed that 56.67% (n=119) of the sampled women have ITNs in their homes and have used it before. Among the people that have the ITNs, 66.39% (n=79), 17.65% (n=21), 14.29% (n=17), and 1.68% (n=2) of the women got their ITNs from the health centers/ health workers, the church, friends/relatives and market, respectively. However, of this number, 18.57% (n=39) women affirmed to have used the ITNs the night preceding the survey, 8.5% (n=18) did not use it regularly, 10% (n=21) and 38.57% (n=81) stopped using the ITNs several weeks and months respectively, prior to the time of the survey.  

CONCLUSION: Despite tremendous efforts, made by various agencies, and strategies developed to control malaria in endemic regions as ALUU community, malaria is still one of the highest causes of morbidity and mortality in the high risk group. High knowledge of ITNs, positive attitude with poor practice was observed among these women attending ante-natal clinics. Furthermore, health workers and individuals should continually enlighten the public on the proper use of ITNs to reduce morbidity and mortality among this vulnerable group.

P624: Comparative Evaluation of Mosquito Repellent Effectiveness and Acceptability of Ocimum Gratissimum Volatile Oil and Advanced Odomos in Umunhua North Local Government Area of Abia State, Nigeria

Opolot-Ahia E.T. and Chibueze E. A.  
1Dept of Public Health Technology Owerri, Imo State, Nigeria

BACKGROUND: In spite of multiplicity of measures targeted towards prevention and control of malaria in Nigeria, it has remained the most significant public health problem, accounting for 30% childhood morbidity (WHO, 2010). The use of mosquito-repellents (natural and synthetic) has formed part of the prevention tool since early 18th century. At the moment, repellents sold in Nigerian markets are all synthetic, with prizes too exorbitant for common people. This study compared the repellency effectiveness and the acceptability of the volatile oil of Ocimum gratissimum, in an olive oil base (assigned Treatment A), with Advanced Odomos (N,N-diethylbenzamide), a synthetic cream (Treatment B). The aim is to find an alternative natural repellent that is cheap, safe and abundant.  

METHODS: Of the 200 subjects randomly selected from an adult population of 600 people, 100 each were allocated to each treatment group by simple balloting. For each of the 10 days of study, (with 10 subjects from each group), subjects were given 3 ml of the appropriate test cream to apply on the exposed part of their skin and observe any mosquito bite. Acceptability for Treatments A and B were high; 65% and 83% respectively.  

CONCLUSION: Ocimum gratissimum plants are very abundant in Nigeria. The leaves are popularly used for spices, medicine and to repel mosquitoes. An improved technology for extraction and formulation will make it more attractive and hence, enhance the fight against malaria.

P625: Risk of readmission or death within 6 months after initial discharge among Ugandan children with severe malarial anemia and cerebral malaria

Robert O. Oyoka1, Nathan Brand1, Karen Hamre1, Paul Bangirana2, Richard Idro2, Chandy C. John3  
1Department of Paediatrics, Makerere University, Kampala, Uganda, 2Department of Psychiatry, Makerere University, Kampala, Uganda, 3Division of Global Pediatrics, University of Minnesota, Minneapolis, USA.

INTRODUCTION: Severe malarial anemia (SMA) is a leading cause of morbidity and mortality among young children in sub-Saharan Africa. Children with SMA appear to have an elevated risk for re-hospitalization and death during the first 6 months following discharge, but the risk of hospital readmission and death for children with cerebral malaria (CM) has not been assessed.  

METHODOLOGY: We followed up a cohort of children aged 18 mo - 12 y who were successfully treated for CM and SMA for six months. These two cohorts were then compared to a similar cohort of healthy community children (CC) followed up for a similar period of time. The risk of readmission to hospital or death within 6 months of admission was then determined.  

RESULTS: A total of 167 children with CM, 144 with SMA and 160 community children were included. The primary endpoint, readmission or death within 6 months, was infrequent in CC (5%), but more frequent in children with SMA (25.7%, P<0.0001) or CM (16.8%, P=0.0007). Readmission or death was higher in children with SMA than children with CM (P=0.07). Assessing individual outcomes, frequency of readmission within 6 months of discharge was higher in children with SMA (21.5 %) or CM (16.2 %) as compared to CC (5%), P<0.0001 for SMA, P=0.001 for CM), but did not differ significantly between children with CM and children with SMA (P=0.22). Most hospitalizations were for malaria, though on many hospitalizations no blood smear confirmation was obtained. Frequency of death was also higher in children with SMA (3.4%) as compared to CC (0%, P=0.02) or children with CM (0.6%, P=0.07). Most deaths were reported as due to a febrile illness. Conclusion: Children with SMA and CM have a higher risk of readmission within 6 months after discharge than community children, and children with SMA have a greater risk of death within 6 months of discharge than children with CM or community children. Further study is needed to assess causes of readmission or death in children with severe malaria.

P626: Clinical predictors of hospital readmission in Ugandan children with cerebral malaria

Nathan Brand1, Robert O. Oyoka1, Karen Hamre1, Paul Bangirana2, Richard Idro2, Chandy C. John3  
1Department of Paediatrics, Makerere University, Kampala, Uganda, 2Department of Psychiatry, Makerere University, Kampala, Uganda, 3Division of Global Pediatrics, University of Minnesota, Minneapolis, USA.

BACKGROUND: Cerebral malaria (CM) is the most severe form of malaria affecting more than 800,000 children each year in sub-Saharan Africa. Survivors of CM are known to suffer a number of neurological and cognitive sequelae. Determination of clinical symptoms associated with hospital re-admission for children treated for cerebral malaria could help identify those CM children at greatest risk for severe morbidity and mortality.  

METHODOLOGY: We prospectively followed up a cohort of children aged 18 mo - 12 years who had survived an episode of CM from Mulago Hospital. Clinical factors at admission were related by logistic regression to the risk of admission to the hospital in the first 6 months post-discharge.
RESULTS: A total of 165 children completed the follow-up. Twenty children (12.1%) were readmitted to the hospital for malaria during 6-month follow-up. Compared to children who were not readmitted, children who were readmitted had a higher frequency of measured fever (T> 37.5, 85.0% vs. 57.9%; P=0.01) and lactic acidosis (blood lactate > 5.5 mmol/L, 60% vs. 27%, P=0.008) on admission, and were less likely to have received antibiotics during their initial stay at the hospital (55% vs. 80%, P=0.02).

CONCLUSION: Measured fever and lactic acidosis on admission and lack of antibiotics during hospital stay predict risk of readmission in children with CM.


Samuel Oppong1, Felicia Amoo-Sakyi2, Keziah Malm1, Constance Bart-Plange1
1 National Malaria Control Programme - Ghana

BACKGROUND: In spite of the fact that proven control interventions such as use of Long Lasting Insecticide Nets, Indoor Residual Spraying and treatment with ACTs, are being implemented in Ghana, the northern part of the country still has the highest burden of malaria. The northern zone includes the Upper East, Upper West and Northern regions with marked seasonal transmission of malaria. This study seeks to assess the impact of malaria control interventions on disease burden (morbidity and mortality) in northern Ghana since 1998.

METHOD: Findings from major nationwide surveys conducted from 1998 were analyzed and compared with similar results in 2011. These include the MARA/ARMA Collaboration technical report 1998, the Ghana Demographic and Health Survey (GDHS) – 1998, 2003, 2008 and the Multiple Indicator Cluster Survey (MICS) report – 2011.

RESULTS: Parasite prevalence levels reduced from between 51-75% in 1998 to 27.5% in 2011 in Ghana. From 75% to 48% in the Northern Region; between 60%-70% to 44% in the Upper East and fairly stable around 51% in the Upper West region. Prevalence of fever in children under five years has decreased from 27% in 1998 to 19% in 2011 nationally. Results for the three northern regions were as follows; 38% to 32%, 34% to 25% and 30% to 29% in Northern, Upper East and Upper West regions respectively. The under-five mortality rate also decreased from 110 to 82 deaths per 1,000 live births in Ghana. In Northern Region, it decreased marginally from 171 to 124; from 155 to 98 in Upper East and from 156 to 108 deaths per 1,000 live births in Upper West region.

CONCLUSION: There have been tremendous gains from malaria control interventions instituted since 1998 in Ghana. Despite these successes, the burden remains high in the savanna zone (northern part) of the country. There is the need to target proven interventions towards these areas to reduce the burden of disease in the northern zone below the national averages.

P628: Evaluating the Prevalence of Drug Resistance in Intermittent Preventive Treatment for Malaria during Pregnancy

Oraka

BACKGROUND/OBJECTIVE: Due to the poor patient compliance with prophylaxis and increasing resistance of parasite strains to chloroquine, administration of intermittent preventive treatment in pregnancy (IPTp) with sulfadoxine/pyrimethamine is now recommended for all pregnant women living in areas with stable malaria transmission. However, resistance to sulfadoxine/pyrimethamine is on the increase which risks the drug being compromised. Thus, an urgent need exists to assess alternative drug regimens for IPTp.

DESIGN/METHOD: Numerous molecular epidemiologic studies showed that resistance to pyrimethamine is associated with the acquisition of mutations in Plasmodium spp. dihydrofolate reductase (dhfr) genes while resistance to sulfadoxine is associated with 3 mutations in dihydropteroate synthase (dhps) gene. Each mutation leads to a decrease in sensitivity to pyrimethamine (dhfr gene) and sulfadoxine (dhps gene).

RESULTS: On a systematic review, results indicated that 2 doses of IPTp with sulfadoxine/pyrimethamine retained activity to reduce placental malaria and low birthweight amongst pregnant that visited the clinic. About >72% of the pregnant women that visited the clinic benefited with 2 doses of IPTp in the proportional reduction of peripheral parasitaemia at delivery compared with that at enrolment while the rate of resistance was at <30%; and the proportion of placental infection was reduced by 75% compared with the efficacy of chloroquine prophylaxis administered the previous year.

CONCLUSION: An alternative approach involves systematic detection of placental infection at delivery by using blood smear, rapid diagnostic test, or PCR with placental blood. Conversely, placental infection prevalence may change with time because of changes in sulfadoxine/pyrimethamine efficacy (likely to decrease) and quality of IPTp implementation (likely to increase). Such an approach would also provide baseline data to assess efficacy of all preventive measures against pregnancy-associated malaria, including IPTp and use of insecticide-impregnated bed nets, and will enable assessment of these effects in a specific population.
P630: The apicoplast genome of *Plasmodium falciparum* provides novel target for malaria diagnosis using a field-adapted loop mediated isothermal amplification (LAMP) assay.

Eniyou C. Orieri, Jan Jacobs, Jean-Pierre Van geetruyden, Umberto D’Alessandro, Davis Nwakanma

1 Medical Research Council Unit, P.O.Box 273 Banjul, The Gambia. 2 Institute for Tropical Medicine, Antwerp, Belgium 3 International Health Unit, University of Antwerp, Belgium

**INTRODUCTION:** Current malaria diagnostic methods do not rapidly and accurately detect asymptomatic infections, though they contribute significantly to transmission. As malaria incidence declines and transmission becomes more heterogeneous, large numbers of samples need to be screened to target intervention measures appropriately. Polymerase chain reaction (PCR) methods accurately diagnose sub-microscopic infections but are not field-deployable. To overcome this handicap, this study reports the development and evaluation of an isothermal amplification method for malaria diagnosis targeting a conserved region of the malaria parasite apicoplast genome.

**METHODS:** Complete apicoplast genome sequences of *P. falciparum* from 15 Gambian isolates and 8 laboratory clones were aligned against the PlasmoDB reference sequence (ID: emb|X95275.2)). Primers were designed from a highly conserved region of approximately 1.5kb containing genes coding for several ribosomal proteins (including LSU rRNA and SufB). Primers were optimised for concentration, Mg²⁺ and annealing temperature. The detection limit was determined using ten-fold serial dilution of DNA from *P. falciparum* 3D7 clone. Assay sensitivity and specificity were determined by screening 60 archived DNA samples containing different parasite densities from both laboratory and field isolates.

**RESULTS:** Detection limit by both PCR and LAMP was ten-fold higher than the reference method, detecting <2 parasites/µl. Preliminary results using archived DNA samples indicated comparable diagnostic accuracy between the apicoplast genome-based LAMP assay and standard nested PCR with 100% sensitivity and specificity. End point detection by naked eye and agarose gel electrophoresis showed perfect agreement.

**CONCLUSIONS:** The novel apicoplast genome-based isothermal amplification technique (LAMP) showed comparable sensitivity and specificity to standard nested PCR. Being easily field-adaptable, without need for thermocycling equipment, this assay could facilitate targeted interventions towards malaria control and elimination.

P631: Hypoxaemia predicts death from *Plasmodium falciparum* malaria among children six to 59 months in Nigeria

Adebola Orimadegun

**BACKGROUND:** Previous studies have not considered assessment oxygen saturation in identifying individuals infected with *Plasmodium falciparum* at risk of deaths despite its association with hospital admission outcomes in other diseases.

**OBJECTIVE:** To investigate the prevalence and predictive value of hypoxaemia for deaths in Nigerian children less than five years with severe *falciparum* malaria.

**METHODS:** Oxygen saturation was prospectively measured alongside other indicators of disease severity in 369 under-5s admitted to a tertiary hospital in Nigeria. Oxygen saturation <90% was regarded as hypoxaemia. Cases of severe malaria were defined as those children in whom *falciparum* malaria parasitaemia was confirmed with blood film microscopy in the presence of any of the WHO-defined life-threatening features for malaria.

**RESULTS:** Overall mortality rate among children with severe malaria in this study was 8.1%. Of the 13 indicators of disease severity assessed, hypoxaemia (OR=7.54; 95% CI=2.80, 20.29), severe anaemia (OR=11.25; 95% CI=2.66, 47.63), co-morbidity with pneumonia (OR=19.27, 95% CI=2.87, 29.50), metabolic acidosis 6.21 (2.11, 17.47) and hypoglycaemia (OR=19.71; 95% CI=2.61, 25.47) were independently associated with death. Cerebral involvement, male gender, wasting, hypokalaemia, hypoponatremia, azotaemia and renal impairment were significantly associated with death in the univariate analysis but not in the logistic regression model.

**CONCLUSIONS:** Hypoxaemia independently predicts deaths in Nigerian children with severe malaria, irrespective other features. Efforts should always be made to measure oxygen saturation as part of the treatments for severe malaria in children.

P632: The effects of malaria and HIV co-infection on hemoglobin levels in pregnant women in Sekondi-Takoradi metropolis, Ghana

Verner N. Orish, Onyekachi S. Onyeabor, Richmond Afoakwah, Johnson N. Boampong, Ekeke Nwaejuna, Samuel Acquah, Adekunle O. Sanyaolu, Nnaemeka C. Iriemenam

1 Department of Internal Medicine, Effion-Kwanta Regional Hospital Sekondi-Takoradi, Sekondi P. O. Box 229, Western Region, Ghana; 2 The Satcher Health Leadership Institute, Department of Community Health and Preventive Medicine, Morehouse School of Medicine, Atlanta, USA; 3 Department of Human Biology, University of Cape Coast, Ghana; 4 Biotechnology and Nuclear Agriculture Research Institute, Atomic Energy Commission, Accra, Ghana; 4 Department of Medical Biochemistry, School of Medical Sciences, University of Cape Coast, Ghana; 4 Department of Medical Microbiology and Parasitology, College of Medicine of the University of Lagos, Ido-araba, PMB 12003 Lagos, Nigeria

**OBJECTIVE:** To assess the burden of malaria and human immunodeficiency virus (HIV) co-infection and to determine the risk of anemia among dually infected pregnant women in Sekondi-Takoradi, Ghana.

**METHODS:** A cross-sectional study was conducted at four hospitals in the Sekondi-Takoradi metropolis comprising 872 consenting pregnant women attending their antenatal care clinics, cross-checked with ultra sound or with clinical evidence of pregnancy.

**RESULTS:** The study showed that 34.4% of the pregnant women had anemia while 65.6% were non-anemic. Multivariable logistic regression analysis indicated that pregnant women with a single infection with either malaria or HIV were independently associated with increased odds of maternal anemia. In adjusted models, pregnant women co-infected with malaria and HIV doubled their risk of maternal anemia (adjusted OR, 2.67, 95% CI, 1.44-4.97, P = 0.002).

**CONCLUSION:** Dually infected pregnant women with malaria and HIV are twice likely to be anemic than those with a single or no infection. For all pregnant women in this region, it is imperative to control for malaria, HIV and anemia in other to improve birth outcomes.
P633: Indoor resting behaviour of anopheline vectors: implications for targeted application of entomopathogenic fungi in southern Ghana

Michael Osae

BACKGROUND: Continued success in the fight against malaria requires the development of novel malaria vector control tools to complement existing control strategies. Use of entomopathogenic fungi against adult mosquitoes is a promising vector control tool under investigation. One of the main prerequisites to use entomopathogenic fungi against a target insect species is a comprehensive understanding of its ecology and behaviour. With the aim of developing entomopathogenic fungi as a malaria vector control tool, this study investigated indoor resting behaviour and factors influencing choice of resting sites of anopheline mosquitoes from southern Ghana.

METHODS: Indoor resting anophelines were collected from six villages in southern Ghana. For each mosquito specimen we recorded the resting site, material, three dimensional location and microclimatic conditions at the collection point. Collected specimens were identified to species using morphological characteristics and PCR assays where appropriate. Distribution of the anophelines in relation to resting site and microclimatic conditions were analysed.

RESULTS: The resting positions of 431 female anophelines (132 An. gambiae, 176 An. coluzzii and 123 An. funestus) were recorded. Of these, the majority of An. gambiae s.s. (75.94%), An. funestus (73.60%) and An. coluzzii (58.19%) were found resting at heights above 200 cm, mainly on roof, ceilings and upper walls. The preferred resting materials included woody roofing components (timber, bamboo, palm and coconut fronds), walls (clay and cement) and cloth (mainly cotton). Light intensity and wind speed appeared to be the major microclimatic conditions influencing the choice of mosquito resting site. Over 90% and 95% of anophelines were found resting at locations with less than 20 Lux light and 0 ms^-1 wind respectively. Temperature and relative humidity also somewhat affected the choice of a resting site.

CONCLUSIONS: In southern Ghana, anophelines prefer to rest on dark coloured wooden, wall and cloth surfaces which are high above ground level. Based on this data, it can be concluded that dark wooden or cloth materials might be used as point source dissemination for entomopathogenic fungi or synthetic insecticides. These devices can be placed higher up roofs closer to walls in rooms to target anopheline vectors transmitting diseases in Africa.

P634: Efficacy, safety and tolerability of dihydroartemisinin-piperaquine for treatment of uncomplicated falciparum malaria in pregnancy in Ghana

Joseph Osarfo1, Pascal Magnussen2, Michael Alifrangis2, Annette Olsen2, Anthony Adusei2, Harry Tagbo1

1Department Of Community Health, Kwame Nkrumah University of Science and Technology, Ghana; 2Centre for Medical Parasitology, University of Copenhagen, Denmark; 3Unit for Parasitology and Aquatic Diseases, Institute for Veterinary Disease Biology, University of Copenhagen, Denmark.

BACKGROUND: Following review of malaria treatment policy in Ghana in 2008, dihydroartemisinin-piperaquine (DHA-PPQ) was added to the approved list of drugs for the treatment of malaria. DHA-PPQ is not indicated for use in pregnancy due to the paucity of data on its safety in pregnancy. However, we anticipate its use by self-medicating pregnant women. To fill important knowledge gaps, we assessed the safety, efficacy and tolerability of DHA-PPQ as compared to artesunate-amodiaquine (ASAQ) in a non-inferiority trial for treatment of uncomplicated falciparum malaria in second and third trimester pregnancies.

METHODS: Pregnant women who attended antenatal clinics in two districts in the Ashanti Region, a perennial transmission zone, were screened for falciparum malaria using both a rapid diagnostic test and microscopy. Those testing positive in both were recruited, individually randomized to DHA-PPQ or ASAQ, and followed up actively on days 1, 2, 3, 7, 14, 28 and 42 after the start of treatment, at delivery and 6 weeks post-partum. During this period, assessment of adverse events and study drug adherence and sampling blood for haematological and parasitological assessments and data collection on neonatal morbidity and mortality were performed.

RESULTS: The overall prevalence of peripheral parasitaemia was 12% (418/3506). Approximately 90% (344/384) of participants had parasite densities <1000/μl at baseline. Parasite density was associated with ownership of a treated net but independent of gravidity, gestational age or haemoglobin concentration at baseline. The DHA-PPQ arm had fewer adverse events than recorded in the ASAQ arm; vomiting (17% vs 29%; p=0.021), general weakness (40% vs 60%; p<0.001), dizziness (34% vs 66%; p=0.004). There were no differences in haematological indices between study arms.

Uncorrected cumulative parasitological efficacy by day 42 for DHA-PPQ was 93.7% and for ASAQ it was 93.4%.

CONCLUSION: DHA-PPQ appears safe and efficacious for treating uncomplicated falciparum malaria in the second and third trimesters of pregnancy. However, larger studies are needed to confirm the results of this preliminary study.

P635: The importance of human antibodies that mediate opsonic phagocytosis of whole merozoites in acquired immunity against malaria

Faith Osier1, Gaoqian Feng1, Michelle J. Boyle, Jack S. Richards2, Nadia Cross3, Fiona J. McCallum, James McCarthy1, Robin Anders3, Kevin Marsh1, James G. Beeson2

1KEMRI Centre for Geographic Medicine Research-Coast, Kenya; 2Centre for Immunology, The Burnet Institute, Melbourne, Australia; 3Queensland Institute of Medical Research (QIMR), University of Queensland, Brisbane, Australia; 4Department of Biochemistry, La Trobe University, Melbourne, Australia

*These authors contributed equally to this work.

BACKGROUND: The mechanisms underlying protective immunity against malaria are poorly understood but antibodies are thought to play a key role. Existing antibody-dependent functional assays have not consistently correlated acquired immunity in malaria endemic settings but are nevertheless used to assess the potential efficacy of sub-unit vaccines, and there is a need for new validated assays. We tested the ability of sera to opsonize whole merozoites for phagocytosis by monocytes as a potential correlate of naturally-acquired and vaccine-induced immunity.

METHODS: Individual sera were incubated with freshly isolated merozoites prior to phagocytosis using THP-1 cells, a promonocyte cell line. The assay was tested in two prospectively monitored cohorts of children experiencing high and low levels of malaria transmission intensity, as well as in samples from a human phase 1 vaccine trial of a major merozoite surface antigen, MSP-2.

RESULTS: Antibodies promoting phagocytosis of whole merozoites were acquired with increasing age and exposure to malaria. A high phagocytosis index was associated with a reduced risk of malaria episodes in the high transmission cohort, and with a reduced risk of multiple malaria episodes in the low transmission cohort. This protection was observed only in children with current or recent exposure to malaria parasites, suggesting that in nature such responses are boosted and may be maintained by frequent exposure. Furthermore, human immunization with MSP-2 induced antibodies with opsonic phagocytosis activity against merozoites.

CONCLUSION: Monocyte phagocytosis of whole merozoites appears to be an important mechanism in acquired immunity against malaria in humans. These findings represent a major advance in understanding protective immunity and may speed up the testing of potential malaria vaccine candidates.
P637: Is implementing full coverage of Long-Lasting Insecticidal Nets (LLINs) a good alternative strategy after Indoor Residual Spraying (IRS) with bendiocarb withdrawal in pyrethroid resistance areas?

Razaki Osse, Martin Akogbeto.
Centre de Recherche Entomologique de Cotonou, Cotonou, Benin.

BACKGROUND: From 2008 to 2010, IRS was implemented in the department of Ouémé in Benin. It was a large scale campaign highly successful with a drastic drop of 94% of the Entomological Inoculation Rate (EIR). But, considering the fact that the intervention was very expensive and burdensome, Benin National Malaria Control Program decided to shift IRS to LLINs in 2011. Olyset nets were distributed with a rate of one bednet for 1.9 people to the communities that were previously targeted by IRS. Did the LLINs strategy provide a better level of protection against malaria transmission than IRS? METHODS: This study was carried out in four districts of the department of Ouémé. Entomological surveillance carried out to assess indicators of transmission risk during the last year of IRS (2010) and the first year after the LLIN intervention (2011). Olyset nets were distributed with a subsample of the Anopheles gambiae s.l. were dissected to estimate the parity rates. A subsample of the An. gambiae s.l. collected was tested for presence of Plasmodium falciparum sporozoites. In addition, window exit traps and pyrethrum spray catches were performed to assess exophagic behavior of Anopheles vectors.

RESULTS: The spontaneous and widespread use of LLINs is a strategy as effective as IRS. In fact, Anopheline aggressiveness was the same during both periods (IRS and LLINs). Unlike, infectivity rates of An. gambiae for Plasmodium falciparum (CS+in = 0.02; CS+in = 0.029) (p=0.330) did not increase after the replacement of IRS by LLINs. This is the same for the daily inoculation rate: EIR=13 infective bites for a period of 9 months under IRS and 10.40 after IRS withdrawal for the same period. But, exophily decreased and parity rate increased after IRS cessation in all areas (p<0.001).

CONCLUSIONS: The large-scale use of LLINs is an effective alternative to the cessation of IRS.

P638: Establishing The Prevalence of SP Resistance Markers in Western Kenya

Maureen Otinga

The first-line anti-malarial treatment regimen for uncomplicated malaria in Kenya changed from Chloroquine (CQ) to Sulfadoxine-Pyrimethamine (SP) in 1998 and from SP to Artemether-Lumefantrine (AL) in 2004. In both instances, the changes were necessitated by widespread treatment failure. The baseline prevalence of SP resistance-associated genotypes and in vitro resistance was established between the period just after SP implementation (1999-2000) and another study was conducted as SP was being discontinued (2003-2005). According to the World Health Organisation, therapeutic efficacy studies remain the gold standard for guiding drug policy and should be undertaken at least every 2 years. Efficacy studies in regard to SP were last conducted more than seven years ago in Kenya. The goal of this study is to determine the trend in prevalence of the clinically relevant PfDHFR / PfDHPS quintuple mutant, which is associated with SP treatment failure in Africa, eight years after SP was discontinued as the first-line anti-malarial in Kenya.

P639: Toxicological effects of prolonged and intense use of mosquito coil emission in rats the implications on malaria control

Olabummi A Otabanjo, Emmanuel T Idowu, Oyenmwen J. Aimufua, Yomi-Onilude Ejowoke, Bamidele Akinsanya.
Department of Zoology, University of Lagos, Akoka, Lagos State, Nigeria.

BACKGROUND: Mosquito coil is a vector control option used amongst individuals to prevent malaria in endemic low income countries. While certain studies have addressed this issue, additional studies are required to increase knowledge on the adverse health effects caused by the prolonged use of mosquito coils.

METHODS: Toxicological effects of fumes from two locally manufactured mosquito coil insecticides (containing pyrethroids: transfluthrin and d-allethin as active ingredients) on male albino rats was investigated. The haematological, biochemical indices, histopathology and mutagenicity evaluations in rats exposed to mosquito coil fumes during 2, 4, 8, 12 and 16 week periods were recorded. Haematological determination was performed using automated hematology analyzer to determine White Blood Cell (WBC), Packed Cell Volume (PCV), Red Blood Cell (RBC) and Platelet (PLT) counts, while biochemical evaluations were determined using available commercial kits. Gross histopathological changes were studied for the kidney, liver and lungs in sacrificed rats. The rat sperm head abnormalities assessment was used to evaluate mutagenicity.

RESULTS: Mosquito coil fumes produced significant increase (P< 0.05) in the levels of total protein, total albumin and bilirubin, when animals were exposed from two weeks to 16 weeks with transfluthrin. Similarly, elevation in the activities of aspartate amino transferase, alanine amino transferase and alanine phosphatase increased significantly in both insecticides. Increase in WBC, RBC and PCV were recorded for all the exposure periods, however PLT count showed no significant increase (P>0.05). Mutagenicity assessment revealed sperm abnormality was statistically significant (P<0.05) compared with the control at 8, 12 and 16 weeks post exposure to transfluthrin. Histological studies revealed severe lung damage evidenced by interstitial accumulations, pulmonary oedema and emphysema in exposed rats. Intracellular accumulations and severe sinusoidal congestion of liver cells were observed from 12 weeks exposure, indicating liver damage.

CONCLUSION: The long term and indoor usage of mosquito coil must take into cognizance the pathological consequences.
P640: Comparative effects of administration of artemunate- amodiaquine and artemeter-lumefantrine on some biochemical indices in rats.

Chiagozie A Otuechere, Gloria Edewor, Oluwafemi Ezekiel Kale, Martins Ekor
Division of Biochemistry, Department of Chemical Sciences, Redeemer’s University, Mowe, Ogun State, Nigeria

BACKGROUND: Artemisinin-based combination therapies recommended by the World Health Organization has achieved excellent results in the fight against malarial scourge. However serious concern has been raised about uncontrolled use of these drugs especially in Nigeria where resort to self medication is rampant. Therefore, the present study was aimed at investigating the possible and comparative toxic potentials of artemeter-lumefantrine (AL) and artemesunate-amodiaquine (AA) using plasma and kidney biochemical indices in rats.

METHODS: Sixteen white albino rats were grouped into three. Group A (n=5) was given no treatment. Group B (n=6) and C (n=5) were administered twice daily oral therapeutic doses of artemeter-lumefantrine (1.14/6.86mg/kg/d) and artemesunate-amodiaquine (2.86/8.58mg/kg/d) respectively for seven days. Animals were sacrificed by cardiac puncture with serum, and kidney homogenate prepared and used for assays.

RESULTS: From our results, the two ACTs did not significantly (p>0.05) alter catalase, superoxide dismutase, glutathione-s-transferase, myeloperoxidase and reduced glutathione levels when compared with control. However, AL and AA elicited a 42% and 34% decrease in plasma total cholesterol levels. AL and AA administration did not significantly alter the absolute organ weights of the liver, kidney lungs and brain, although AA produced a marked increase in heart weight when compared with control. Artemesunate-amodiaquine but not artemeter-lumefantrine significantly increased (p<0.05) lactate dehydrogenase and heart weight respectively when compared with control. The drugs caused significant (p < 0.05) elevation of malondialdehyde (MDA) levels compared to the control group.

CONCLUSIONS: These results suggest that AA has the potential to increase the risks of renal and cardiac organ dysfunction in the users much more than AL. In addition, the drugs may also promote renal oxidative stress. However, caution is required above therapeutic indications or in chronic doses as this may predispose to oxidative damage or precipitate cardiac toxicity.

P641: Establishing The Prevalence of SP Resistance Markers in Western Kenya

Maureen Otinga

BACKGROUND: The spread of insecticide resistance among Anopheles mosquitoes raise the needs for new insecticidal malaria vector control strategies. For more than 2 decades, porphyrins have been suggested as photo-insecticidal tools. A suitable animal food fraction could be used as possible porphyrins carrier against Anopheles larvae. Neem extracts are known to possess insecticidal effects. Thus, neem (Azadirachta indica) plant derivatives could constitute candidate carriers for a meso-substituted cationic porphyrin (C12) against Anopheles mosquitoes. Based on these hypotheses, the possible larvicidal efficacy of C12 formulations using a cat food pellets fraction and neem derivatives as carriers for C12 porphyrin was assessed against larvae of wild-caught Anopheles gambiae s.l. from Vallée du Kou, a village of Burkina Faso where resistance have developed to chemicals in Anopheles population.

METHODS: In an outdoor trays experiment, the C12 porphyrin load and unload candidate carriers were exposed for 2 days to batches of 60 larvae of L2-L3 instars in water samples from potential Anopheles larvae breeding sites. Mortalities were estimated by counting dead and survival larvae within 44h postexposure.

RESULTS: Irrespective of breeding water type of larval treatment, all the tested C12 formulations induced larval mortality that was significantly different from the C12 unload carrier after 44h larval exposure. However, only the animal food based C12 formulation was able to induced about 100% mortality to the larvae within 44h postexposure. Among the tested porphyrin neem formulations, the porphyrin neem fruit combination (NF-C12) was able to induce about 82.7% larval mortality after 44h of exposure irrespective of breeding water type of larval treatment.

CONCLUSION: In the context of management of integrated vector control and resistance development in mosquito populations, the neem fruit used as C12 porphyrin carrier may constitute a promising larvicidal tool against Anopheles gambiae s.l.

P642: Evaluation of possible larvicidal activity of C12 porphyrin formulations using neem derivatives and animal food as carriers for C12 porphyrin against Anopheles gambiae s.l. larvae

Robert K. Ouedraogo

BACKGROUND: The spread of insecticide resistance among Anopheles mosquitoes raise the needs for new insecticidal malaria vector control strategies. For more than 2 decades, porphyrins have been suggested as photo-insecticidal tools. A suitable animal food fraction could be used as possible porphyrins carrier against Anopheles larvae. Neem extracts are known to possess insecticidal effects. Thus, neem (Azadirachta indica) plant derivatives could constitute candidate carriers for a meso-substituted cationic porphyrin (C12) against Anopheles mosquitoes. Based on these hypotheses, the possible larvicidal efficacy of C12 formulations using a cat food pellets fraction and neem derivatives as carriers for C12 porphyrin was assessed against larvae of wild-caught Anopheles gambiae s.l. from Vallée du Kou, a village of Burkina Faso where resistance have developed to chemicals in Anopheles population.

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CONCLUSION: In the context of management of integrated vector control and resistance development in mosquito populations, the neem fruit used as C12 porphyrin carrier may constitute a promising larvicidal tool against Anopheles gambiae s.l.
incidence of malaria.

**RESULTS:** Total IgG with subclasses levels against all antigens significantly increased with age. Furthermore, antibody levels were found higher in the high compared to low transmission season except for GLURP R2. The difference of IgG production between both transmission seasons were statistically significant for MSP3 (p = 0.0188), MSP2A (p = 0.0002) and GLURP R0 (p = 0.009). Our findings also showed that there was a correlation between the antibodies levels and concomitant *P. falciparum* asexual forms. Total IgG against MSP2B (p = 0.023), GLURP R0 (<0,001) and GLURP R2 (p = 0.001) were associated with reduction of malaria incidence.

**CONCLUSION:** Our results show that the evaluated antigens are immunogenic. Protection against clinical malaria is age dependent. Some antigen specific antibodies are associated with malaria incidence reduction in children. Age, season and concomitant parasitemia are found key determinants in the acquisition of natural immunity against clinical malaria and therefore should be considered in vaccine candidates’ development.

**P644:** Malaria and gravidity interact to modify maternal haemoglobin concentrations during pregnancy

Smiala Ouedraogo

**INTRODUCTION:** To determine the effect of malaria-focused preventive measures on anaemia in relation to gravidity, we analysed data from 3 studies carried out in nearby areas in south Benin between 2005 and 2012.

**MATERIAL AND METHODS:** On inclusion (first antenatal visit or ANV1) women’s age, area of residence, schooling, parity, gestational age, weight and height were recorded. Thick blood smears were performed at ANV1, second visit (ANV2) and at delivery. Women’s serum ferritin and CRP concentrations were also assessed.

The impact of gravidity on maternal haemoglobin (Hb) was analysed using a logistic or linear regression depending on the outcome.

**ETHICS:** Study approval was obtained from the Ethics Committee of the Faculty of Medicine of Cotonou in Benin.

**RESULTS:** In total, data from 3591 pregnant women were analysed. Both univariate and multivariate analyses showed a constant association between Hb concentrations and gravidity in the 3 periods of Hb assessment (ANV1, ANV2 and delivery). Mean Hb concentration was significantly lower in primigravidae than in multigravidae at ANV1 (mean difference = -2.4 g / L, P < 0.001). Afterwards, it increased importantly in primigravidae only, with a tendency to reversal between primigravidae and multigravidae which was confirmed at delivery (mean difference = -2.8 g / L, < 0.001). The prevalence of malaria was halved between ANV1 and delivery in primigravidae while it decreased only by 38% among multigravidae, who were less prone to be infected (malaria prevalence at ANV1, 20% and 10% respectively). Iron deficiency was more common in multigravidae, and it decreased slightly in this group between ANV1 and delivery.

**CONCLUSION:** In a context of IPTp, primigravidae were shown to improve progressively haemoglobin concentration throughout pregnancy. In multigravidae, the improvement was less perceptible as anaemia was mainly due to iron deficiency. There is a need to reinforce malaria prevention strategies and micronutrient supplementation in both groups.

**P645:** Western versus Eastern African experimental huts for the evaluation of products: a SWOT analysis from comparative test of repellents and insecticidal products in Benin.

Welbeck A. Oumbouke,1 Augustin Fongnikin,1 Sarah Moore,1 Raphael Nguessan,1,3

1Centre de Recherche Entomologique de Cotonou, Cotonou; 2Environmental Health and Ecological Sciences Thematic Group, Ifakara Health Institute, Ifakara, Tanzania; 3Department of Disease Control, London School of Hygiene and Tropical Medicine, London, UK

**BACKGROUND:** The Western and Eastern African experimental huts are used to assess the efficacy of products targeting mosquitoes. The range is vast and includes toxicants and spatial repellents that keep mosquitoes away from human. Owing to differing design of the west versus east type, the suitability of either hut to evaluate a given product is questionable. The present study compared the efficacy of Spatial Repellents (coils) versus Long Lasting Insecticidal Net in both design and highlighted their Strength and Weaknesses.

**METHODS:** Olyset Net and metofluthrin 0.00625% 0.0097% were evaluated in Southern Benin. The Western huts have mosquito entry slits on the sides and large screened verandah to prevent egress of mosquitoes. The Eastern design has entry baffles and eave gaps surrounding the roof through which mosquitoes escape or access huts. Only Culex quinquefasciatus collected in abundance was analyzed further and reported.

**RESULTS:** Both type of huts reduced entry of Culex into huts in presence of the coils or LLIN but the rate of entry was reduced by 78-79% in the western design compared to only 28-49% in the eastern format (P<0.05), with Olyset deterring the least. Without treatments, the proportions of mosquitoes exiting the Western huts by dawn (64%) were greater than those caught in veranda of the western huts (34%) and this was still evident with the treatments (P<0.05). The overall personal protection levels were similar in the eastern and western huts for the Olyset Net (94-95%) but significantly higher in the eastern hut than the western hut for the spatial repellents (49-62% vs 39-52%) (P<0.05). Induced mortality was lower in the western hut for all treatments compared to the eastern design.

**CONCLUSIONS:** The study showed the suitability of both type of huts to evaluate key properties (blood feeding inhibition and toxicity) induced by non-spatial repellent or lowly deterrent intervention like olyset Net though a slight but significant improvement was observed with the Ifakara experimental hut. Spatial repellents are better suited to the eastern experimental huts to deliver protection through higher exophily than the confined western design, which current structure is not suited to assess accurately deterrence and exophily induced by chemicals.

**P646:** Malaria in Pregnancy, How to prevent it.

Mensah Owusu

Each year, 25–30 million women become pregnant in malaria-endemic areas of Africa, and similar numbers are exposed to malaria in Ghana, Asia Ocean and South America. Malaria is an important cause of severe anaemia in pregnant African women, and by this mechanism malaria causes an estimated 10,000 maternal deaths each year. Unlike other infections, malaria infections result in 75,000–200,000 low birth weight (LBW) babies each year, due to combinations of preterm delivery (PTD) and fetal growth restriction (FGR). Effects on miscarriage and still birth are unknown, but adequate malaria control alone could prevent 3–8% of infant deaths.

To tackle this enormous burden, we have two proven tools. First, insecticide treated nets (ITNs) decrease parasite prevalence in all gravidities, decrease LBW and still birth in first to fourth pregnancy, and show trends toward benefits against anaemia and clinical malaria. Secondly, intermittent preventive treatment in pregnancy (IPTp), using regular treatment doses of the antimalarial sulphadoxine pyrimethamine (SP) has been shown to decrease peripheral and placental parasitemia, and to increase maternal hemoglobin and infant birth weight, especially in primi- and secundigravidae. Unfortunately, high-level coverage with SP IPTp and ITNs has not yet been achieved. The development and evaluation of programs to prevent malaria in pregnancy can be facilitated by a better understanding of the pathogenesis of malaria. This article will review aspects of malaria parasite biology and the pathogenesis and immunity of malaria in pregnancy. We will highlight areas where these aspects can inform future study of how best
P648: HbC and HbS modify distinct Plasmodium falciparum binding interactions.

Oumar Attah1, Almahamoudou Mahamari1, Moussa Kanoute1, Kadidia Cisse2, Bakary Diarra1, Patrick Duffy2, Alassane Dicko2, Michal Fried2.

1Malaria Research & Training Center, Faculty of Medicine, Pharmacy and Dentistry, University of Sciences Techniques and Technologies of Bamako; P.O Box 1805, Bamako, Mali.
2Laboratory of Malaria Immunology and Vaccinology; National Institute of Allergy and Infectious Diseases, National Institutes of Health, Twinbrook 1, 5640 Fishers Lane, Rockville, Maryland 20852 USA.

Background: Plasmodium falciparum is the deadliest of the human malaria parasites, and kills up to a million African children each year due to severe syndromes. Hemoglobinopathies reduce severe malaria risk, and existing data suggest that their protective effect may be related to an effect on parasite adhesion. Because HbS protects from all severe syndromes while HbC may preferentially protect against cerebral malaria, we hypothesized that host factors like HbS and HbC may differentially modify falciparum parasite binding to specific receptors.

Methods: In assays using clinical isolates collected from children participating in longitudinal cohorts in Ouelessebougou, Mali, we identified novel endothelial molecules that support infected erythrocyte binding including extracellular matrix molecules and members of the integrin family.

Results: Infected erythrocytes collected from children with sickle cell trait were less likely to bind to the receptor CD36 but not to other receptors, while infected erythrocyte collected from children with hemoglobin AC were less likely to bind to other endothelial receptors(E-selectin, P-selectin, ICAM1, Integrin α5b1 and ICAM2) but not to CD36.

Conclusions: In summary, our results support our hypothesis that different host factors differentially modify infected erythrocyte binding to endothelial receptors.

P647: Lack of evidence for the re-emergence of chloroquine-sensitive falciparum malaria in Lagos, South-Western Nigeria

Yetunde A Olukosi1,*, Muyiwa K OyeboI1,2, Oluwaseun S Ajibaye2, Bassey B Orok2, Olugbenga O Aina1, Chimere O Agomo1, Bamidele A Iwalokun1, Samuel K Akindele1, Vera N Enya1, Hilary I Okoh1.

1Malaria Research Unit, Biochemistry Division, Nigerian Institute of Medical Research, 6, Edmond Crescent, PMB 1393, Yaba, Lagos, Nigeria; 2Parasitology and Bioinformatics Unit, Department of Zoology, University of Lagos, Nigeria.

Background: A recovery in chloroquine efficacy following a period of cessation has raised the possibility of its re-introduction for malaria chemotherapy. Eight years after the withdrawal of chloroquine and the subsequent introduction of artemisinin-based combination therapies in Nigeria, there has not been sufficient information on the susceptibility of the existing Plasmodium falciparum populations to the drug. This study aimed to assess the current distribution of the major markers of chloroquine resistance.

Methods: Finger prick blood samples were collected from participants presenting with symptoms of malaria in two selected health centres each representing Lekki and Ijede communities of Lagos, Nigeria. Thick and thin blood smears were prepared for microscopy and dry blood spots made from malaria-positive participants for parasite DNA extraction. The detection of mutations in the Plasmodium falciparum chloroquine resistance transporter (pfcrt) and P. falciparum multidrug resistance (pfmdr1) genes was performed by nested polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP).

Results: Of the 527 blood samples that were confirmed by PCR to be P. falciparum positive, 412 and 344 were typed for the molecular detection of pfcrt and pfmdr1 gene mutations respectively. The mutant alleles of pfcrt were present among 290 (77%) parasite individuals while the pfmdr1 mutant allele was found in 117 (33%) of the total parasite populations. There were higher distributions of the mutant alleles for the two loci in Ijede than in Lekki. The observed frequencies of pfcrt mutant alleles in the two parasite populations were in keeping with the expected frequencies predicted by Hardy-Weinberg. Comparing data with studies conducted from year 2000 to 2002 in Ijede, we observed an increase in the prevalence of mutant type pfcrt against the marginal decline in the pfmdr1 mutant type.

Conclusions: The high level distributions of CO-resistant parasites are suggestive of a persistent drug pressure and an enduring inefficacy.
2.0 (95% CI=1.8-2.4) and 1.3 (95% CI=1.2-1.6) in the asymptomatic, uncomplicated and severe malaria groups respectively. Polyclonality was significantly higher in asymptomatic (61%) and uncomplicated malaria (60%) groups compared to the severe malaria (34%) group (P<0.001). A total of 32, 35 and 28 distinct MSP-2 alleles were found in the asymptomatic, uncomplicated and severe malaria groups respectively. Sequence analysis showed that the FC27-type sequence was characterized by two distinct subtypes and a hybrid sharing amino acid sequences from the two subtypes while the 3D7-type sequence was characterized by three subtypes of repetitive domains.

CONCLUSIONS: Isolates in this region are genetically diverse but severe malaria isolates have low MOI and most of the infections are monoclonal. Data on the sequence haplotypes found in this study will serve as baseline for future studies in this region and lends support for allelic inclusion in the design of a multi-component malaria vaccine.

P650: Diagnostic Reliability of Rapid Diagnostic Test Compared to Routing Microscopy in Guiding Malaria Treatment Decisions for Febrile Patients

Isaac Oyewole, Timothy Salawu and Akinwale Ogunlade.
Department of Biosciences and Biotechnology, Babcock University, Ilisan Remo, Nigeria.

Effective management of malaria requires the use of rapid diagnostic tests (RDTs) for prompt and accurate parasitological confirmation of malaria parasites especially in areas where microscopy diagnoses are not available. Here, we evaluate the reliability profile of a FirstResponse® device for PHRP2 compared to routing Microscopy which may guide the decision in treating febrile patients. A descriptive and cross-sectional study was carried out on 250 febrile patients for parasitological examination during medical consultation. Blood samples were collected and tested using two methods: Light microscopy of Giemsa-stained blood slides and RDT (FirstResponse® device for PHRP2). The overall prevalence of malaria by blood slide microscopy was 66.8% (N=167) with a mean parasitemia of 272 ± 290 parasites/µL of blood, while by FirstResponse RDT it was 36.8% (N=92). In comparison with microscopy as the gold standard, RDT exhibited high specificity (87.2%) and moderate sensitivity (43.2%) with positive predictive and negative values of 86.4% and 44.9% respectively. The sensitivity of RDT however increased significantly with increase in P. falciparum parasitaemia (Ps 0.05). For effective management of malaria where prompt and rapid diagnoses are required especially in the endemic areas where microscopy diagnoses are not available, RDT could be a preferred choice.

P651: A pilot analysis of the genetic diversity of Plasmodium falciparum merozoite antigens as markers of immunity and invasion

Lynette Isabella Oyier1, John Okombo1, Kevin K. A. Tetteh1, Kevin Marsh1
1 KEMRI Centre for Geographic Medicine Research, Coast, Kilifi, Kenya; 2 Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, United Kingdom

Several Plasmodium falciparum merozoite invasion genes are highly polymorphic, allowing the parasite to evade the host’s immune mechanisms and use alternative invasion pathways. The Reticulocyte binding homologue (Rh) (1, 2a, 2b, 4, 5), erythrocyte binding antigen (EBA) (175, 181, 140, 140), erythrocyte binding ligand-1), merozoite surface protein (MSP) 3-like (3, 6, 3.8, MSPDBL) families, apical membrane antigen 1 and MSP1 have been shown to be involved in invasion and antibodies to these proteins can also inhibit invasion in vitro. It is possible that through pilot screen of the genetic diversity of these merozoite antigens, markers of immunity and invasion can be identified.

The 15 genes were sequenced from an asymptomatic malaria population (2008-2009) (mean age 6.7) and an uncomplicated malaria drug trial population under 5 years of age (2007-2008), which consisted of pre- and post-treatment samples. Also a subset of the uncomplicated malaria population, were adapted to in vitro culture.

AMA1 showed the greatest diversity within an infection, a number of SNPs in Rh2b, Rh5, EBA175, AMA1, MSPDBL and MSP3.8 showed significant temporal heterogeneity in the uncomplicated malaria population and not the asymptomatic population. In the uncomplicated malaria population, a single SNP in Rh2b and AMA1 were significantly associated with age and a single SNP each for MSP3, MSP3.8 and 23 SNPs of AMA1 were associated with parasitemia. In the asymptomatic malaria population an indel in MSP1 and 2 SNPs in Rh5 were significantly associated with age and a single SNP in AMA1 was associated with parasitemia.

Highly diverse genes within an infection like AMA1 would provide a challenge as a vaccine candidate compared to a less diverse gene like Rh5. Temporal genetic heterogeneity is a potential sign of frequency dependent immune selection in the uncomplicated infections, while in asymptomatics there was a stable genotype maintained, suggesting a control of infections by semi-immune individuals or prolonged infection with one haplotype. There are a potential number of SNPs which can serve as markers of immunity or invasion (due to high parasite densities), which require verification in a larger sample set and using functional assays.

P652: Potential antimalarial activity of methyl jasmonate and its effect on lipid profiles in Plasmodium berghei infected mice

Oladapo E Oyinlouye,1 Akoulehin M Kosoko,2 Obukwo B Emikpe,3 Catherine O Falade,1,4 George O Ademowo,1,4
1 Department of Pharmacology and Therapeutics, college of Medicine, University of Ibadan, Ibadan, Nigeria; 2 Department of Biochemistry, college of Medicine, University of Ibadan, Ibadan, Nigeria; 3 Department of Veterinary Pathology, University of Ibadan, Ibadan, Nigeria; 4 Institute for Advanced Medical Research and Training, College of Medicine, University of Ibadan, Ibadan, Nigeria

BACKGROUND: Malaria is a major threat to public health and economic development in Africa. Efforts at controlling malaria has been hampered by parasite resistance to commonly used and affordable antimalarial drugs. There is an urgent need for search and discovery of new antimalarial agents. This study aimed to evaluate the in vivo antimalarial activity of methyl jasmonate (MJ) which is a plant hormone, known since as a fragrant component in the essential oil from flowers of Jasminum grandiflorum.

METHODS: The antimalarial activity of MJ against Plasmodium berghei (P. berghei) was evaluated using the Rane test procedure. Albino mice weighing between 18g and 30g were distributed into 10 groups of seven animals each. Four groups were uninfectected, a group received ethanol, while others received 10mg, 25mg and 50mg MJ per kg body weight respectively. Six groups were infected with P. berghei, a group was not treated while the remaining five groups were treated with 3mg arteether (AE), 10mg chloroquine (CQ), 10mg, 25mg and 50mg MJ respectively. Parasitaemia was monitored daily and screened under X 100 magnification using a light microscope. The PCV and lipid profiles were also investigated. Data were analyzed computed and processed using SPSS version 15.0 at P<0.05.

RESULTS: The suppressive effect of 50mg MJ, CQ and AE on parasite growth were demonstrable from day 1 (post treatment) and with suppression reaching 81.9%, 96.9% and 97.3% respectively by day 3. There was recrudescence from day 5 in AE treated group, 70.3% and 70.4% suppression of parasitaemia were observed in 10mg and 25mg MJ treated groups respectively on day 10 post infection as against AE and CQ (94.8% and 86.6%) respectively.

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P653: Benchmarking 2 multisite Intermittent Preventive Treatment of Malaria in Pregnancy (IPTp) clinical trials in 5 sub-Saharan countries

Golbahar Pahlavan on behalf of the MiPPAD Consortium
Barcelona Centre for International Health Research (CRESIB, Hospital Clinic-Universitat de Barcelona), Barcelona, Spain

The “Malaria in Pregnancy Preventive Alternative Drugs _ MiPPAD” trials, funded by the Malaria in Pregnancy Consortium and the EDCTP, compare the efficacy and safety of Mefloquine as malaria Intermittent Preventive Treatment in pregnancy (IPTp) in HIV-negative and positive women in 5 sub-Saharan countries. Respectively 4750 and 1070 women were recruited during 2009 - 2013 with the subsequent follow-up of their children during 1-year and 2-month periods.

In addition to the logistic and standardisation challenges presented by the simultaneous implementation of these trials in varied settings, other critical factors were the existing local health systems, the associated regulatory structures and how the trials were embedded therein.

Interventions in developing countries, particularly those directed at vulnerable populations including pregnant women and children need to consider both short-term trial objectives as well as longer-term ones such as integral health service delivery, empowerment of local health service providers and acceptance by the community at large.

Within this framework an on-site evaluation by the Trial Management Team was carried out between 2010 and 2011. The primary objectives of this evaluation were to overview study procedures, benchmark study implementation, and to evaluate the impact of MiPPAD trials on the study site health system. To this end, spot observations and open-ended interviews were carried out across the participating sites with all the staff involved including those from the Ministries of Health.

The results of this benchmarking shall be presented.

P654: Developing new vector control tools for metabolic resistance; responding to GPIRM

Paine, M.J.I., Poupardin, R., Lycett, G.J. and Ranson, H
Vector Biology, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool L3 5QA, UK

BACKGROUND: Control of malaria and other insect borne diseases is seriously threatened by rising insecticide resistance in the vector populations, precipitating the WHO Global Plan for Insecticide Resistance Management for Malaria Vectors (GPIRM). The development of new tools to aid vector control, in particular for monitoring and overcoming resistance, is a central pillar of GPIRM. Over the past few years there have been great strides in our understanding of the biology of resistance. The challenge now is to exploit this for the production of new tools for detecting insecticide resistance

METHODS: Studies of field mosquito populations have identified a core set of insecticide metabolising enzymes (P450s) from the major malaria transmitting Anopheles species in Africa. These have been used as a platform for the development of new products for vector control.

RESULTS: We have produced commercial quantities of recombinant enzymes from this core set. These have been used to screen all four classes of WHO recommended insecticides for their ability to metabolise insecticides and their susceptibility to PBO inhibition. These results provide base-line information on the insecticide-P450 interactions associated with insecticide resistance in African malaria vectors.

CONCLUSIONS: As well as predicting likely cross-resistance issues with current compounds in malaria control programs, this new resource has great value for the development of resistance diagnostics and early phase development of new insecticides to flag potential problems associated with P450 mediated resistance.

P655: Local Illness Concepts and their Relevance for the Prevention and Control of Malaria During Pregnancy in Ghana, Kenya and Malawi: Findings from a Comparative Qualitative Study

Arantzaz Menaca1,2, Christopher Pell1,4,5,6,7, Lucinda Manda1, Samuel Chatio1, Nana A. Afrahi1, Florence Were1, Abraham Hodgson7, Peter Ouma1, Linda Kalilani1, Harry Tagbor1, Robert Pool1,2
1Centre de Recerca en Salut Internacional de Barcelona (CRESIB), Hospital Clinic-Universitat de Barcelona, Barcelona, Spain; 2Departamento de Antropología Social, Universidad Complutense de Madrid, Madrid; 3Centre for Social Science and Global Health, University of Amsterdam, Amsterdam; 4College of Medicine, University of Malawi, Blantyre, Malawi; 5Navrongo Health Research Centre, Navrongo, Ghana; 6Department of Community Health, School of Medical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana; 7The Kenya Medical Research Institute (KEMRI) and Centers for Disease Control and Prevention (CDC) Research and Public Health Collaboration, Kisumu, Kenya

BACKGROUND: In sub-Saharan Africa, the burden of morbidity and mortality linked to malaria during pregnancy (MiP) is significant and compounded by its unclear symptoms and links with other health problems during pregnancy. Mindful of the biomedical and social complexity of MiP, this article explores and compares local understandings of MiP and their links with other pregnancy-related health problems.

METHODS: A comparative qualitative study undertaken at four sites in three countries: Ghana, Malawi and Kenya. Individual and group interviews were conducted with pregnant women, their relatives, opinion leaders, other community members and health providers. MiP-related behaviours were also observed at health facilities and in local communities.

RESULTS: Across the four sites, local malaria concepts overlapped with biomedically defined malaria. In terms of symptoms, at-risk groups, outcomes and aetiology of malaria during pregnancy, this overlap was however both site-specific and partial. Moreover, the local malaria concepts were not monolithic and their descriptions varied amongst respondents. The symptoms of pregnancy and malaria also overlapped but, for respondents, symptom severity was the distinguishing factor. Malaria was generally, though not universally, perceived as serious for pregnant women. Miscarriage was the most widely known outcome, and links with anaemia, low birth weight and congenital malaria were mentioned. Nonetheless, amongst many potential causes of miscarriage, malaria was not recognized as the most important, but rather interacted with other pregnancy-related problems.

CONCLUSIONS: Given the overlap of common pregnancy problems with the symptoms of malaria, and the limited association of malaria with its main outcomes, a comprehensive ANC programme is the most appropriate strategy for the provision of health education, prevention and treatment for MiP. Variations in locally shared understandings of MiP must however be taken into account when designing and promoting MiP intervention strategies.
P656: Prevention and management of malaria during pregnancy: findings from qualitative studies in Ghana and Malawi

Christopher Pell1,2, Arantza Menaca1,2, Nana A. Afrah3, Lucinda Manda-Taylor3, Samuel Chatto4, Abraham Hodgson2, Linda Kalliani3, Harry Tagbor4, Robert Pool1,2
1Centre for Social Science and Global Health, University of Amsterdam, Amsterdam Centrum De; 2Recerca en Salut Internacional de Barcelona (CRESSIB), Hospital Clinic-Universitat de Barcelona, Barcelona, Spain; 3Department de Antropología Social, Universidad Complutense de Madrid, Madrid, Spain; 4Department of Community Health, School of Medical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana; 5College of Medicine, University of Malawi, Blantyre, Malawi; 6Navrongo Health Research Centre, Navrongo, Ghana

BACKGROUND: In endemic regions of sub-Saharan Africa, malaria during pregnancy (MiP) is a major preventable cause of maternal and infant morbidity and mortality. Current recommended MiP prevention and control interventions include intermittent preventive treatment (IPTp), distribution of insecticide treated bednets (ITNs) and appropriate case management. This article explores the social and cultural context to the uptake of these interventions at three sites across Africa.

METHODS: A comparative qualitative study was conducted at three sites in two countries: Ghana and Malawi. Individual and group interviews were conducted with pregnant women, their relatives, opinion leaders, other community members and health providers. MiP-related behaviours were also observed at health facilities and in local communities.

RESULTS: ITNs were generally recognized as important for malaria prevention. However, their availability and use differed across the sites. In Malawi, ITNs were sought-after items and reported usage was high but there were complaints about availability. In central Ghana, ITNs were less commonly available and women saved them until the birth of the child. Although, recent distribution programmes increased ITN availability, in northern Ghana, they were generally used seasonally. In central Ghana, pregnant women did not associate IPTp with malaria, whereas, in Malawi and northern Ghana, IPTp was linked to malaria, but not always with prevention. Although IPTp adherence was common at all sites, whether delivered with directly observed treatment or not, a few women did not comply with IPTp often citing previous side effects. With regard to case management, although opinions of diagnostic testing were generally positive, women's experiences of malaria testing varied in and Ghana and Malawi, and treatment was sometimes administered in spite of a negative diagnosis. Despite generally following the advice of health staff, personal experience, the availability and accessibility of antimalarials influenced the MiP treatment that women received.

CONCLUSION: Although social and cultural context is relevant, personal experience also plays an important role in non-adherence to preventive or curative MiP interventions, particularly IPTp.

P658: Reduced efficacy of Long-lasting insecticidal nets in pyrethroid resistance area in Ivory Coast

Cédric Pennetier1,2,6, Ludovic P. Ahoua Alou3,4,6, Olivier Pigeon5, Alphonsine A. Koñû3,6
1Institut de Recherche pour le Développement (IRD)/UMR 224 MIVEGEC, Cotonou, Benin; 2Centre de Recherche Entomologique de Cotonou (CREC), Cotonou, Benin; 3Institut Pierre Richet (IPR), Abidjan, Côte d’Ivoire; 4Laboratoire de Zoologie et Biologie Animale, Université Félix Houphouët Boigny, Abidjan, Côte d’Ivoire; 5Walloon Agricultural Research Centre (CRA-W), Agriculture and Natural Environment Department, Plant Protection Products and Biocides Physico-chemistry and Residues Unit, Gembloux, Belgium.; 6Anopheles Biology & Control (ABC) Network

Evidences of pyrethroid-treated nets reduced efficacies in areas of pyrethroid resistance are becoming more frequent in the literature. After the characterization of the resistance status of Anopheles gambiae population from M’Bé in central Ivory Coast, a standard experimental hut study was run with 3 long-lasting net (LLINs) brands distributed by the National Malaria Control Programme. These LLINs reached the WHOPES criteria (in the laboratory testing using WHO cone tests) and have been checked for the active ingredient contents. Nevertheless the unwashed and 3-fold washed LLINs did not induced more than 9% and 13% mortality respectively whereas the blood feeding inhibition ranged from 52-74% and 28-62% respectively. Pyrethroid resistance in An. gambiae appears to threaten the future of ITN and IRS in Ivory Coast as the mechanisms described in M’Bé populations have been reported in several areas across the country. These striking results stress the urgent need of alternative tools.

P657: Local understandings of malaria during pregnancy: results from a qualitative study in Madang, Papua New Guinea

Erin V.W. Andrew1,2, Christopher Pell1,2, Angeline Angwin1, Alma Auwum1, Su Phauanuonnon1, Robert Pool1,2
1Barcelona Centre for International Health Research (CRESSIB, Hospital Clinic - Universitat de Barcelona), C/ Rosselló 132 Sobre ático, 08036 Barcelona, Spain; 2Global Health, University of California, San Francisco, 50 Beale Street, Suite 1200, San Francisco, California, 94105, USA; 3Centre for Social Science and Global Health, University of Amsterdam, OZ Achterburgwal 185, 1012 DK, Amsterdam, The Netherlands; 4PNG Institute of Medical Research, PO Box 378, Madang, MP 511, Papua New Guinea

BACKGROUND: Malaria is the leading cause of illness and death in Papua New Guinea (PNG). Infection during pregnancy with *falciparum* or *vivax* malaria, as occurs in PNG, has health implications for mother and child, causing complications such as maternal anaemia, low birthweight and miscarriage. This article explores local understandings of malaria and risk perception during pregnancy and attitudes towards malaria in pregnancy (MiP) interventions in PNG.

METHODS: As part of a qualitative study in Madang, MiP, participatory techniques (free-listing and sorting) were conducted along with focus group discussions, in-depth interviews (with pregnant women, health staff and other community members) and observations in the local community and health facilities.

RESULTS: Attitudes towards and knowledge of MiP, its risks, and prevention varied amongst respondents. Although there was a general awareness of the term “malaria”, it was often conflated with general sickness or with pregnancy-related symptoms. Moreover, many preventive methods for MiP were related to practices of general healthy living. Indeed, varied messages from health staff about the risks of MiP were observed. In addition to ideas about the seriousness and risk of MiP, other factors influenced the uptake of interventions: availability and perceived comfort of sleeping under insecticide-treated mosquito nets were important determinants of usage, and women’s heavy workload influenced Chloroquine adherence.

CONCLUSION: The non-specific symptoms of MiP and its resultant conflation with symptoms of pregnancy that are perceived as normal has implications for MiP prevention and control. However, in Madang, PNG, this was compounded by the inadequacy of health staff’s message about MiP.

P659: Effect of Antiretroviral Therapy on Malaria parasitaemia and Clinical Episodes among HIV-infected Adults in Rural Uganda, 2010-A prospective Population-based Cohort Study

Kimbowa Peter

BACKGROUND: Untreated HIV-1 induced immune-suppression is associated with an increased incidence of clinical malaria ad parasitaemia. We assessed the effect of ART on clinical malaria (parasitaemia with fever) and malaria parasitaemia in HIV infected individuals.

METHODS: HIV-infected and uninfected participants in a cohort were
followed from 2005-09. Blood smears examined microscopically for malaria at quarterly visits and whenever participants had fever. CD4 cell counts were measured quarterly among individuals receiving ART, 6 monthly for those not yet on ART. From 2001, HIV-infected individuals received cotrimoxazole prophylaxis. The incidence of clinical malaria and parasitaemia was compared between HIV-uninfected and HIV-infected (not yet on ART and on ART) using rate ratios (RR). Random effects Poisson regression models were used to account for repeated events and adjustments were made for covariates (HIV/ART status current age and visit type).

RESULTS: Participants were: HIV-uninfected (206), HIV-infected not yet on ART (255) and HIV-infected on ART (280). Compared to HIV-uninfected, clinical malaria incidence was higher among HIV-infected individuals not yet on ART (adjusted RR [aRR] 1.80 [95%CI 1.30, 2.50]) and individuals on ART for less than 2 years (aRR 1.83 ([95%CI 1.35, 2.48]), but lower among individuals on ART for 2 or more years (aRR 0.63 [95%CI 0.45, 0.89]). The incidence of clinical malaria decreased with increasing age (p for trend=0.002) and increasing CD4 cell counts (p for trend<0.001). Similar observations occurred for the incidence of parasitaemia, according to HIV and ART status. *Plasmodium falciparum* (93.6%) was the commonest species identified. Before cotrimoxazole prophylaxis introduction (1995-2000), malaria incidence (per 100 pyr) among HIV-infected individuals was 89.1 (95%CI 79.2, 100.1) while during the period of cotrimoxazole prophylaxis alone (2001-2003) it was 94.3 (95%CI 82.7, 107.5).

CONCLUSION: HIV infection increases risk of both clinical malaria and parasitaemia. Over time, ART reduces both of these risks.

P660: Trans kunene malaria initiative (tkmi): partnering cross border toward malaria elimination

**Constance Njovu** Jackie Park1, Matondo Alexandre1, Christopher Lourence2, Susan Lassen1, S. Eliza Petrov1
1IC Flowers Foundation, Isdell: Flowers Cross Border Malaria Initiative; 2Anglican Aids Program, Namibia; 3Clinton Health Access Initiative (CHAI)

**BACKGROUND:** A malaria parasite knows no borders: highly mobile populations who freely cross between porous borders contribute to the malaria burden and make elimination more complex. The Trans Kunene Malaria Initiative (TKMI) recognizes that elimination cannot be achieved with bed nets alone and requires a continuum of services that extend cross border. To achieve elimination this program targets border communities in Angola/Namibia with comprehensive services and invests in synchronizing programs and policies between ministries. The majority of work is carried out in partnership with local ministries, the Anglican Church and local non-governmental organizations (NGOs).

**STATEMENT OF THE RESEARCH PROBLEM:** The majority of malaria cases in northern Namibia are imported from southern Angola. In order to eliminate, Namibia requires close cooperation from Angola. This research looks at whether Namibia can achieve elimination by targeting communities on both sides of border with parallel malaria programs and by securing cooperation between both governments.

**RESEARCH METHODOLOGY:** From 2011, 20 square kilometers on both sides of the border were targeted with community based malaria interventions including LLIN distribution; community education; trainings for Church leaders, malaria control agents and research assistants; rapid diagnostic testing and case management.

Both Angolan and Namibian governments agreed to the following: removal of custom duties for malaria commodities i.e. LLINs, IRS chemicals; information sharing; trial car stickers for malaria workers to easily cross the TKMI borders; identity cards for the malaria workers; distribution of LLINs, IRS chemicals; information sharing; trial car stickers for malaria workers to extend cross border. To achieve elimination this program targets border communities in Angola/Namibia with comprehensive services and invests in synchronizing programs and policies between ministries. The majority of work is carried out in partnership with local ministries, the Anglican Church and local non-governmental organizations (NGOs).

**SUMMARY OF RESULTS:** There has been a reported drop in case burden in the TKMI areas. Angola reported a reduction in morbidity of 35% and a reduction of mortality of 20% in the Kunene province in the 2011 season. In the mirroring TKMI region in Namibia, Ohangwena, there was a reduction of morbidity of 47% in 2011-2012.

**DISCUSSION:** Programs for elimination must target communities on both sides of the border, ensuring harmonization of interventions, government cooperation, surveillance and monitoring and evaluation. These results show that cross border work is both critical to elimination of malaria and possible despite working between different national governments with language and cultural differences.

P661: Intermittent Preventive Treatment of Malaria and Place of Ante-Natal Care: a Study of a South West Nigerian peri-Urban Community

**Abimbola S. Phillips**2, Babawale O. Obuboro1, Adesegun O. Fatusi2
1Department of Community Health, Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, Nigeria; 2Department of Obstetrics & Gynecology, Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, Nigeria

**BACKGROUND:** Intermittent preventive therapy (IPT) is a key strategy in reducing the prevalence and sequelae of malaria in pregnancy. Involvement of community resources (CRs) such as Traditional Birth Attendants, Faith-Based Organizations, etc, is a known approach to treatment delivery. Many studies have shown that a considerable proportion of pregnant women are more likely to access care in these places than in health facilities (HF) with skilled attendants. This study aimed to determine the level of access, and associated factors, to IPT with focus on CRs in a South-West Nigerian peri-urban community.

**METHODS:** Data were from the Nigeria-Ife 2010 baseline round of the Gates’ Partners Family Health and Wealth Study (FHWS) - a multi-country longitudinal study which follows up a cohort of at least 500 peri-urban families in nine sites in Asia and Africa, including Nigeria. The FHWS Nigeria-Ife cohort consists of 784 couples, residing in Ipetumodu, South-West of Nigeria, selected by multistage random sampling. All 518 women who had a birth in the last 5 years preceding the survey, and had ANC in their last pregnancy, were analyzed. The outcome measure – use (or non-use) of IPT during pregnancy – was regressed on place of ANC, adjusting for socio-economic variables (such as wealth quintile), parity, and education, using binary logistic regression.

**RESULTS:** Over one-tenth (11.2%) of the women reported receiving ANC from CRs compared to 88.8% from HFs. Among women who had ANC from CRs, 62% of them had IPT compared to 75% from HFs (p=0.03). Women who had ANC from CRs were 61% less likely to use IPT than those from HFs (AOR, 0.39; 95%CI: 0.19-0.78). The predicted probability of IPT use was on the average 26.4% higher for the poorest women having ANC from CRs than from the poorest having ANC from HFs.

**CONCLUSION:** Pregnant women who patronize CRs are less likely to use IPT. The effect of place of ANC on use of IPT varies on the levels of wealth. It is important to strengthen the capacity of the CRs and make IPT available at all avenues in order to improve malaria prevention.

P662: IPTp policy in Zanzibar towards pre-elimination of malaria: results from a study of placental parasitemia

**Mwinyi Isa Msellemi**1, Marya Plotkin1, Khadija Said2, Natalie Hendler1, Asma Ramadhan Khamis1, Maryjane Lacoste1, Elaine Roman3, Chonge Kitojo4, Julie Gutman4
1Zanzibar Malaria Control Programme, Zanzibar Ministry of Health; 2Jhpiego Tanzania; 3Jhpiego Baltimore; 4Centers for Disease Control and Prevention and President's Malaria Initiative; 5United States Agency for International Development

**BACKGROUND:** Rapid scale-up up of malaria prevention and treatment has brought Zanzibar to the malaria pre-elimination phase. *Plasmodium falciparum* prevalence in the general population has been < 1% since
2008, and screening of antenatal care (ANC) attendees using malaria rapid diagnostic tests (mRDTs) indicated 0.2% peripheral parasitemia prevalence in 2011-12. Intermittent preventive treatment for pregnant women (IPTp) with sulfadoxine-pyrimethamine (SP) was introduced in 2004, but coverage with two SP doses has not exceeded 50%. Zanzibar is considering a transition from IPTp to more intensive efforts to screen and treat pregnant women. To help inform Zanzibar’s policy revisions for control of malaria in pregnancy, this study assessed placental malaria positivity at time of delivery among Zanzibari women who had not received IPTp.

METHODS: A prospective observational study was conducted to estimate placental parasitemia positivity among women who had not received SP for IPTp during pregnancy. From September 2011 to September 2012 we enrolled a convenience sample of pregnant women on day-of-delivery at six hospitals on Zanzibar’s two main islands, Pemba and Unguja. Dried blood spots on filter paper were prepared from placental blood specimens subsequently analyzed via polymerase chain reaction (PCR) to detect Plasmodium infection.

FINDINGS: 1,423 deliveries were enrolled from Pemba (52%) and Unguja (48%), representing 6% of the total deliveries at the six facilities. Of the 1,349 placental specimens processed, nine (0.8%, 95% confidence interval 0.2–3.3%) were PCR positive for *P. falciparum*, with no other plasmodium species detected. Six (66%) of the nine placental infections were from Unguja deliveries. Eight placental infections were accompanied by a normal birth weight delivery (≥ 2500 g). Placental infections were not more common during the seasonal transmission increases of 2011-12.

CONCLUSIONS: Low placental parasitemia positivity among women who had not received IPTp triangulates with population-based prevalence and ANC screening data in Zanzibar and indicates low risk of malaria in pregnancy. Zanzibar has introduced screening with mRDT in ANC services to increase likelihood of early detection and prompt treatment of malaria. Decisions regarding the future of IPTp in Zanzibar must also consider insecticide treated net coverage and risk of malaria resurgence.

P664: Evaluation of the immunomodulatory function of *Plasmodium falciparum* Hsp70

**Offense J. Poore**, Addmore Shonhai

1Department of Biochemistry & Microbiology, University of Zululand, P. Bag X1001, KwaDlangezwa, 3886, South Africa

BACKGROUND: Heat shock proteins (Hsps) are conserved molecules that constitute a major part of the cell’s protein folding machinery. *Plasmodium falciparum* Hsps play an important cytoprotective role ensuring that the malaria parasite survives under the harsh conditions that prevail in the host environment. In addition, the parasite exports some of its Hsps to the erythrocyte of infected red blood cells. The exported proteins augment malaria pathogenicity by promoting the cytoadherence properties of the parasite infected erythrocytes. Recent studies have suggested their chaperone (protein folding) role, some Hsps exhibit ‘chaperokin’ (signal transduction) function. Because of this some Hsps, especially the Hsp70 family have been implicated in the host immune suppression. *P. falciparum* Hsp70 (PfHsp70) is a ubiquitous, cytosol localised Hsp70 that is essential for parasite survival. Patients living in malaria endemic areas have been shown to possess antibodies that recognise PfHsp70. In the current study, we investigated the immunomodulatory function of PfHsp70.

METHODS: Recombinant forms of the full length PfHsp70 and its N-terminal ATPase domain were expressed in *E. coli* XL1 Blue cells. The proteins were expressed with N-terminal polyhistidine tags to facilitate their purification by nickel affinity chromatography. To remove lipopolysaccharides (LPS) contaminants, the proteins were run through an Endotrap Red coloum (Hyglos). The successful removal of LPS was confirmed by analysing the LPS content of the purified proteins, compared to the original batch purified by nickel affinity chromatography. Freshly isolated human neutrophils (PMN) were labelled with annexin-V–FITC (Roche Molecular Biologicals, Mannheim, Germany) as recommended by the manufacturer. Labeled cells were resolved by flow cytometry. The FACs data was analyzed using a FACS Calibur flow cytometer and the CellQuest Pro software (BD Biosciences, San Diego, USA). We further subjected TLR-2 and TLR-4 knock-out macrophage to both full length PfHsp70 and its ATPase domain respectively, and monitored IL8 production.

RESULTS: Based on L-selectin shedding, general morphological appearance and Annexin-V binding, both full length PfHsp70 and its ATPase subdomain appeared to be capable of stimulating PMN cells. Although the recombinant proteins contained traces of LPS, the level of stimulation that the recombinant proteins exhibited was significantly high compared to LPS only controls. Full length PfHsp70 appeared less capable of stimulating TLR-4 knock-out macrophages compared to its ATPase subdomain. However, none of the two proteins stimulated TLR-2 macrophages.

CONCLUSIONS: Our findings provide data supporting a role for PfHsp70 in host immunomodulation. We intend to express the recombinant proteins in LPS-free *E. coli* cells in order to obtain LPS-free protein for further validation of the findings.

P663: Impact of 2010 Universal Coverage Bednet Distribution Campaign on Malaria Transmission in Sofala Province, Mozambique

**Mateusz M. Plucinski**, Silvia Chiceuciu, Eusebio Macete, S. Patrick Kachur, Graça Matsinhe, Caterina Guinovart, Juliette Morgan

1Centers for Disease Control and Prevention, Atlanta, GA, United States, 2Manhiça Health Research Centre, Manhiça, Mozambique, 3National Malaria Control Program, Ministry of Health, Maputo, Mozambique, 4Barcelona Center for International Health Research, Hospital Clinic-Universitat de Barcelona, Spain

INTRODUCTION: Malaria is the leading cause of death in Mozambique in children under five years old. In 2009, Mozambique developed a novel bednet distribution model to optimize universal coverage. During the pre-distribution enumeration, in addition to household (HH) size, data are collected on sex, age, and relationship to head of HH. These data, together with assumptions about sociocultural sleeping patterns, determine the number of bednets to distribute. We evaluated the impact of a pilot-test of this distribution model on malaria transmission in four districts in Sofala Province, Mozambique.

METHODS: We conducted a HH cross-sectional survey one month after the 2010 distribution of 140,000 bednets and a second survey 13 months later in 2011. During HH visits, we performed blood smears on HH members and collected data on bednet ownership, access, and usage; these indicators were analyzed at the individual, HH, and community levels. We performed logistic regression to evaluate the impact of the bednet distribution campaign on prevalence of malaria infection, and to evaluate predictors of malaria blood smear positivity.

RESULTS: Malaria parasitemia prevalence in children under five years old declined from 46% in 2010 to 37% in 2011. Adjusting for possible confounders, this represents a 32% reduction in the odds of a child having malaria (aOR: 0.68, CI: 0.48–0.96). The reduced risk of malaria was significantly associated with community-level frequency of use of bednets, showing a stronger association than community access, HH access, HH frequency of use of bednets or individual use of bednets.

DISCUSSION: The significant decrease in malaria parasite prevalence, together with the strong protective effect of community use of bednets, suggests that this alternative distribution model introduced in Mozambique is effective in limiting community transmission of malaria.
BACKGROUND: Insecticides used in malaria vector control program might affect the perception of host cues and change behaviour of *Anopheles* spp females. There are evidences that the presence of insecticide-based control tools alter mosquito behaviour. Nevertheless the effects of insecticide on host-seeking behaviour sequences are poorly understood. Objectives of this study were to assess 1) how pyrethroid insecticide on a net modulates the activation and the choice between two human hosts and 2) if the Kdr resistance mutation alter these behaviours.

METHODS: Two strains of *Anopheles gambiae*, sharing the same genetic background, Kis (susceptible) and KdrKis (resistant), were used. Female batches were released in a dual-choice olfactometer. Attractive odor plume came from the 2 olfactometer arms through air flows coming from tents in which two men were standing under a net. We used a permethrin-treated net and an untreated. Two parameters were recorded: the activation rate (the proportion of females which left the release zone) and the choice (proportion of active female who chose one or other arm).

RESULTS: Total of 224 *A. gambiae* batches were released (175 of Kis and 184 of KdrKis). The activation rate was higher among susceptible strain than the resistant strain. The presence of permethrin on one net did not induce a preferential choice between the two human hosts regardless the strain.

CONCLUSION: In this study we showed that the presence of permethrin on a net did not modulate the long range choice between 2 protected human hosts. Further investigation are undergoing on short range behaviour and the ability of malaria vectors to find a hole in a torn bed net. It is also crucial to better understand how mosquitoes perceive and integrate human host olfactory cues in presence of insecticide treated material to understand why some mosquitoes avoid treated houses.

**P665: Long range behavioural response of Anopheles gambiae to pyrethroid-treated net**

Angélküe Porciani1,2, Malal Diop1,2, Oulydou Boussari3, Amal Dahount3,4, Laurent Dormont5, Cédric Pennetier1,2

1Institut de recherche pour le développement (IRD), Benin, 2 Centre de recherche entomologique de Cotonou (CREC), Benin, 3 Centre d’Ecologie Fonctionnelle et Evolutive (CEFE), UMR 3, France

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**P666: Diversity, host switching and evolution of Plasmodium vivax infecting African great apes**

Franck Prugnolle

*Plasmodium vivax* is considered to be absent from Central and West Africa because of the protective effect of Duffy negativity. However, there are reports of persons returning from these areas infected with this parasite and observations suggesting the existence of transmission. Among the possible explanations for this apparent paradox, the existence of a zoonotic reservoir has been proposed. May great apes be this reservoir? We analyze the mitochondrial and nuclear genetic diversity of *P. vivax* parasites isolated from great apes in Africa and compare it to parasites isolated from travelers returning from these regions of Africa, as well as to human isolates distributed all over the world. We show that the *P. vivax* sequences from parasites of great apes form a clade genetically distinct from the parasites circulating in humans. We show that this clade’s parasites can be infectious to humans by describing the case of a traveler returning from the Central African Republic infected with one of them. The relationship between this *P. vivax* clade in great apes and the human isolates is discussed.

**P667: Quality of malaria case management among children under five years at lower level health facilities in Tororo district, Uganda**

Puleh Steven Sean1,2, Yeka Adoke1,2, Waiswa Peter2

1School of Public Health, Makerere University College of Health Sciences, Kampala, Uganda; 2Uganda Malaria Surveillance Project, Kampala, Uganda;

**BACKGROUND:** Prompt and effective treatment of malaria cases is a key control strategy. Effective case management entails quality in diagnosis, and treatment of cases. Quality of care ensures safe, effective, patient centered, timely, efficient and equitable treatment of cases.

**OBJECTIVE:** To assess the quality of malaria case management among children under the age of five years at lower level health facilities in Tororo district, Uganda.

**METHODS:** A cross-sectional survey was conducted in 3 level IV and six level III public health facilities in Tororo district. A total of 384 exit interviews were conducted with caretakers of children under five years treated for malaria to assess their understanding of information on and satisfaction with malaria case management. Healthcare workers were observed and interviewed while treating malaria cases. A total of 8 Key informant interviews were conducted with heads of health facilities. A composite index was utilized to assess overall quality. Uni-variate, bivariate and multivariate analysis were conducted to determine predictors of quality malaria case management.

**RESULTS:** About 52.2% of the children had optimal quality of care. Predictors of optimal quality of malaria case management were a younger (36-60 months) age group (OR= 2.51; 95% CI 1.32 - 4.80), supervision of health workers in the last 6 months prior to the survey (OR=4.71; 95% CI 2.03 - 10.96), adequate knowledge of malaria prevention (AOR=5.57; 95% CI 2.070 - 14.968) and getting treatment from level IV health facility (AOR=6.26; 95% CI 3.83 - 10.24).

**Conclusion:** Quality of malaria case management among children under five years was sub-optimal because of non-adherence to national guidelines. Ministry of Health leadership and district leaders should supervise and mentor health workers to improve quality of malaria case management.

**KEYWORDS:** Quality, malaria, case management, Uganda

**FUNDING:** Quality, malaria, case management, Uganda

**P668: Malaria prevalence in a district with three distinct ecological zones in Southern Ghana**

Alberta Amu Quartey1, David Schellenberg2 and Tsiri Agenyega3

1School of Medical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana; 2Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, UK

**BACKGROUND:** An understanding of the epidemiology of malaria in an area is critical for the design and evaluation of control efforts and research studies. The latest malaria data from Dangme West District, southern Ghana, comes from 1993 when the parasite prevalence was 42% in Dodowa and 20% in Pampram in the dry season. We set out to describe the current epidemiology of malaria in the district.

**METHODS:** We measured the prevalence of malaria in the then Dangme west district, which has three distinct ecological zones - forest (Dodowa), lakeside (Osudoku) and coastal savannah (Pampram). Multistage cluster sampling cross sectional surveys included 3,456
people of all ages at the end of the dry season in March 2012. Following written informed consent, participants were interviewed and a blood sample collected for blood films and preparation of filter paper blots for ELISA evaluation of MSP1α antibodies.

RESULTS: A similar proportion of participants were recruited from each zone (32% (n=1,099) from Forest, 33% (n=1,142) from Lakeside and 35% (n=1,215) from the Coastal Savannah). ITN use was 24% in the Forest, 40% in the Lakeside and 28% in the Coastal Savannah. The overall parasite prevalence was 6.5%, ranging from 2.7% at the Lakeside to 8.5% in the Coastal Savannah areas. The dominant parasite species was Plasmodium falciparum (96%). The age-specific parasite prevalence was 9% in 0-9 year olds, 8% in 10-19 year olds, 5% in 20-29 year olds, 3% in 30-39 years and 4% in those aged 40 years and above. The corresponding age-specific MSP1α prevalence was 37%, 58%, 60%, 66% and 67% respectively.

CONCLUSION: This data shows a reduction in malaria in the area in the last 20 years, with marked reduction in the Forest compared to the Coastal Savannah. The Lakeside zone with the highest ITN use had the lowest parasite prevalence rate. The age specific parasite prevalence rate was almost the same for the 0-9 and 10-19 year old groups. Further analysis of this and related entomological and intervention coverage data will facilitate ongoing efforts to control malaria in the district.

P669: Interferon gamma (IFN)-g promoter haplotypes influence erythropoietic responses in Kenyan children residing in a holoendemic Plasmodium falciparum transmission area

Evans Raballah1, Gregory C. Davenport2, Collins Ouma3, Samuel B. Anyona4, Tom Were5, Prakash Kempalai6, John M. Vukule7, James B. Hittner8, Douglas J. Perkins9, and John M. Ong’o10

1University of New Mexico Laboratories of Parasitic and Viral Diseases, Centre for Global Health Research, Kenya Medical Research Institute, Kismu, Kenya; 2Department of Medical Laboratory Sciences, Masinde Muliro University of Science and Technology, Kakamega, Kenya; 3Center for Global Health, Department of Internal Medicine, University of New Mexico, Albuquerque, NM, USA; 4Centre for Global Health Research, Kenya Medical Research Institute, Kismu, Kenya; 5Department of Psychology, College of Charleston, Charleston, SC, USA

BACKGROUND: Plasmodium falciparum malaria is among the leading causes of morbidity and mortality among African children. In P. falciparum holoendemic transmission areas of western Kenya, severe malaria commonly manifests as severe malarial anaemia (SMA); haemoglobin (Hb) <5.0 g/dL) in children. Interferon-gamma (IFN)-g is a pleotropic cytokine associated with both protection and pathogenesis in severe malaria. Since the functional role of IFN-g variants in conditioning susceptibility to SMA is inconclusive, the relationships between IFN-g polymorphisms (-183 G/T, rs2069709 and -1616 A/G, rs2069705), their haplotypic structures, SMA and erythropoietic responses were investigated.

METHODS: Parasitaemic children (aged 3-36 mos.; n=744) presenting at a rural hospital in western Kenya were recruited. DNA was extracted from buccal swabs (epicentre). 3.0 mL of whole blood was obtained and full haemogram performed (Beckman Coulter A cT diff2). Parasitaemia was determined on Giemsa-stained blood smears. IFN-g -183 G/T was genotyped by RFLP, while IFN-g -1616 A/G was genotyped using a Taqman® 5′ allelic discrimination assay. IFN-g concentrations were determined as part of a Cytokine 25-plex Antibody Bead Array.

RESULTS: Bivariate logistic regression analysis, controlling for age, gender, sickle-cell trait, parasitaemia, and HIV-1 status, demonstrated that relative to homozygous -1616 A (wild) individuals, carriage of GG genotype was associated with a reduced risk of inefficient erythropoiesis (OR=2) (OR, 0.564; 95% CI, 0.323-0.983; P=0.043), while carriage of AG genotype showed a trend towards reduced risk of inefficient erythropoiesis (OR, 0.556; 95% CI, 0.280-1.104; P=0.094). Additionally, carriage of GA haplotype was associated with an increased risk of inefficient erythropoiesis (OR, 2.005; 95% CI, 1.573-2.555; P<0.001), while GG (OR, 0.525; 95% CI, 0.412-0.668; P<0.001) and TG (OR, 0.147; 95% CI, 0.031-0.691; P=0.015) haplotypes were associated with reduced risk of inefficient erythropoiesis. However, none of the genotypes or haplotypes were significantly associated with the development of SMA.

CONCLUSIONS: Although the IFN-g promoter genotypes and haplotypes investigated are significant predictors of the erythropoietic response in children with malaria, they appear only indirectly associated with the development of SMA. An explanation for these findings may be related to differences in the temporal scale of events in which inefficient erythropoiesis precedes the clinical outcome of SMA.

P670: Assessment of anaemia and iron status in pregnant women with co-infections of malaria, intestinal helminthes and HIV in Southwest Nigeria

Olawunmi Rabiu1,2, Hannah Dada-Adegbola1, Ayokulehin M Kosoko1, Catherine O Falade1,4, Ganiyu O Arinola3,4, Alexander B Odaibo1, Olusegun G Ademowo1,6

1Department of Zoology, 2Department of Medical Microbiology and Parasitology, 3Department of Biochemistry, 4Department of Pharmacology and Therapeutics, 5Immunology Unit, Department of Chemical Pathology, 6Institute for Advanced Medical Research and Training, University of Ibadan, Nigeria.

BACKGROUND: Malaria, helminthiasis and HIV infections are diseases of public health menace in Africa particularly in the sub-Saharan region. Pregnant women remain a symbolic risk group to these infections. Furthermore, a staggering statistics of iron deficiency has been reported among these women. Hence, this study was aimed at evaluating the prevalence and association of anaemia and iron status with malaria, helminth and HIV infections.

METHOD: Three hundred and twenty-three pregnant women were recruited into the study from the ante-natal and HIV clinics of a secondary healthcare facility. Blood samples were obtained for haematocrit determination, preparation of thick smears for malaria microscopy while serum samples were used for ferritin and iron level estimation using ELISA technique and Atomic Absorption Spectrophotometry respectively. Direct and Kato-Katz methods were used for identification and quantification of helminth ova in stool samples collected. Data were computed for analysis using SPSS version 16.0.

RESULTS: Twenty-three (7.1%) of the women were positive for malaria only, 11 (3.4%) and 65 (20.1%) for HIV only while 175 (54.2%) had no infection. There were 2 (0.6%), 46 (14.2%) and 1 (0.3%) cases of malaria/helminthiasis, malaria/HIV and helminthiasis/HIV co-infections respectively. Preliminary results showed that 190 (60.9%) were not anaemic while 64 (20.5%), 57 (18.3%) and 1 (0.3%) had mild, moderate and severe anaemia respectively. A significantly lower haematocrit value was observed among those positive for HIV (p=0.000), malaria (p=0.000) and malaria/HIV (p=0.000) infections relative to those negative for the three infections. Coinfection of malaria and HIV caused a significantly lower haematocrit value compared to helminth only (p=0.022). Also, women positive for malaria only or coinfected with helminth had higher ferritin levels compared to those with no infections. Furthermore, no observable difference was detected in serum iron levels among the groups.

CONCLUSION: Despite a lot of interventions in Nigeria, the burden of malaria either singly or in coinfection with helminth or HIV is still relatively high thus aggravating the anaemic tendencies in pregnancy. Pregnant women infected with malaria and/or HIV are more prone to anaemia relative to those infected with helminthes only. However, differences in iron status based on infection are still further investigated.
INTRODUCTION: Historically, the majority of Anopheles gambiae complex mosquitoes collected from the Vlakbult region of Mpumalanga have been the minor malaria vector Anopheles merus. However, recently, entomological surveillance has revealed that the majority of malaria vectors collected in Vlakbult was Anopheles arabiensis. The aim of our study was to obtain baseline data on the vector prevalence and susceptibility status of the malaria vector mosquitoes in the Vlakbult region, Mpumalanga Province of South Africa. MATERIALS AND METHODS: This is an experimental study based on World Health Organization (WHO) bioassay testing procedure. A total of 130 mosquitoes were collected from November 2012 to January 2013. Larval collection was conducted and adult mosquitoes were collected using three different methods: Collection from Pit traps, CO$_2$ traps and Human landing catches. Mosquitoes belonging to the Anopheles gambiae Complex were assayed for insecticide susceptibility. In this procedure, mosquitoes were exposed to 4% DDT and to 0.05% deltamethrin. A 24-hour post exposure to insecticides was recorded to ascertain susceptibility to insecticides. Species identification was conducted through PCR and Morphological keys. Eliza test was performed on wild caught adult mosquitoes to detect parasites in the mosquitoes. RESULTS: Mosquito identification by morphological keys together with PCR revealed that 27% (35/130) of samples collected were An.arabiensis, followed by 20% (26/130) of samples identified as An.funestus group. The funestus groups were identified as An. leesoni and An. vaneenedi. Mortality recording after 24 hours revealed 100% mortality from 4% DDT exposure (22/22) and also 100% mortality from 4% deltamethrin (Pyrethroid) exposure (8/8). The ELIZA test for parasites detection also revealed parasite prevalence of zero (0/32), indicating 0% parasites infectivity rate. The non-vector species identified included anophelines from the An.marshalli group. CONCLUSION AND WAY FORWARD: This study suggests that there has been a change in the dominant malaria vector species prevalent in Vlakbult, these results will be key to informing malaria control and elimination efforts of Mpumalanga Province. It is therefore recommended that the malaria control in Mpumalanga start including the Vlakbult area for seasonal spraying and entomological surveillance should be intensified to keep check on the mosquitoes and their insecticides susceptibility status.

P672: Consequences of an interrupted integrated malaria control initiative: a case study from Gaza Province, southern Mozambique
Jaishree Raman1, Dayalan Govender1, Natasha Morris1, Ishen Seocharan1, Reshma Gayaram2, Pedro Mulanga1, Rajendra Maharaj1
1Malaria Research Unit, Medical Research Council, 491 Ridge Road, Durban, KwaZulu-Natal, 4001, South Africa; 2Gaza Province Directorate of Health, Xai, Gaza Province.

Building on the successes observed in Maputo Province where malaria prevalence declined from by 45% following seven years of aggressive malaria control, the initiative was extended into Gaza Province in 2006. The integrated intervention aimed to reduce the malaria burden in a sustainable manner.

Indoor residual spray (IRS) operations commenced at sentinel sites across Gaza Province during 2006. Impact of IRS together with definitive diagnosis and effective treatment had on the malaria burden was assessed by annual malaria prevalence surveys, monthly entomological surveys and monthly MIS malaria notification reports. Efficacy of the IRS insecticides and antimalarials were determined using molecular surveillance for resistance markers.

A year after the implementation, malaria cases and deaths had declined by 26% and 44%, respectively. This steady decline continued until 2011 when sub-optimal spray coverage enabled malaria vector populations to resurge enhancing transmission intensity by 23% compared to 2010. The An. gambiae s.l vectors (An. arabiensis and An. merus) prevalence had rebounded to baseline levels (OR: 0.84, 95 CI: 0.65-1.26, p=0.323) while Anopheles funestus s.s prevalence was almost double that of 2006 (OR: 1.61, 95% CI: 1.43-1.81, p<0.0001). This dramatic increase was due to inadequate spray coverage rather than emerging resistance as the mutant kdr gene was absent. In contrast the quintuple mutation associated with sulphadoxine-pyrimethamine (SP) treatment failure increased over the study period (OR: 0.12, 95 CI: 1.14-1.42, p<0.0001) approaching saturation (84.4%) by 2011, raising concerns over the continued use of SP for intermittent preventative treatment. Even more alarming was the dramatic increase in the mdr1 186N allele, from 25.3% at baseline to 83.6% in 2011, associated with lumefantrine tolerance, given the high regional drug pressure and emergence of artemisinin resistance in South East Asia.

The breakdown of control measures in Gaza Province facilitated marked increases in malaria cases, deaths and vector numbers which negatively impacted regional control efforts. If elimination is to become a reality in southern African, then sustainable regional malaria control efforts must become the order of the day. Funding organisations and governments embarking on unsustainable interventions must be held accountable as malaria rebounds with severe consequences once interventions are interrupted.

P673: Côte d’Ivoire – home to highly resistant Anopheles gambiae: consequences for sustained effective malaria control in the region.
Jaishree Raman, Reshma Gayaram, Ashokoornar Saikoolal, Rajendra Maharaj, Franco Maartens
1Malaria Research Unit, South African Medical Research Council, Durban, KwaZulu-Natal, South Africa; 2Integrated Malaria Control Consulting, Durban, KwaZulu-Natal, South Africa.

BACKGROUND: Malaria control in Africa is highly dependent on insecticide-based interventions. However insecticide resistance is threatening to severely compromise the effectiveness of these interventions. Efficacy of insecticide-based methods together with the prevalence of molecular markers of insecticide resistance should be regularly determined to ensure sustained malaria control. This paper reports on the species composition, infectivity and resistance status of malaria vectors from Côte d’Ivoire in 2012.

METHODS: Species vector larvae were collected from two sites and reared to adulthood in an insectary. Two day old Anopheles gambiae senu lato adult females (n = 250) were subjected to insecticide susceptibility assessment using WHO tubes and insecticide impregnated filter papers. Sibling species identity, molecular form, infectivity and presence of knockdown resistance (kdr) and insensitive acetylcholinesterase (ace-1) mutations in mosquitoes used in susceptibility testing (n = 119) were determined using conventional and qPCR methods.

RESULTS: Members of the An. gambiae complex constituted over 90% over the samples collected, with An. gambiae senu stricto (s.s), the most...
prevalent species. Susceptibility testing revealed insecticide resistance was firmly entrenched with mortality never exceeding 20% during testing. Both the M and S molecular forms were common among the indoor resting mosquitoes while the M form was the dominant form (97%) among larval collections. The kdr mutation was approaching saturation (90%) while the homozygous ace-1" mutation was absent. 

**DISCUSSION:** The high level of multi-insecticide resistance detected in this study does not bode well for sustained insecticide-based malaria control in Côte d’Ivoire and threatens the success the country has made rolling back. Resistance levels and efficacy of insecticide-based interventions must be closely monitored, particularly as carbamate resistance appears to have a metabolic rather than genetic basis. A strategy must be put in place to deal with possible large scale failure of insecticide-based interventions, while alternative insecticides are being sought.

**RESULTS:** In the 18 surveys, the majority of mosquitoes caught belonged to *Anopheles gambiae* s.l. (91.10%), and 8.9% *Anopheles funestus* was observed. Man bit rate (M.B.R) of *Anopheles gambiae* s.l in January was 9.03 bit / man / night (MBR) and February (MBR = 5.99). The parity rate of *Anopheles gambiae* s.l varies between 1.38 in mid-January and 43.4 at the end of August.

**CONCLUSION:** Malaria transmission was ensured by *Anopheles gambiae* s.l in March, June and August and we have found the lower *Anopheles funestus* number.

**P676: Analyse of antibody responses against three blood stage antigens (AMA-1, MSP-142, GLURP-R2) of *P. falciparum* in naturally exposed children aged from 1 to 5 years old in Bandiagara, Mali**

**Amassou Raymond**

**INTRODUCTION:** Development of effective antimalarial vaccine is still a challenge for researcher. Several malaria candidate vaccines have been tested with limited success. One of the most important issues is lack of correlate of protection. Antimalarial immunity is age dependent and require many mosquitoes bites during several years. The results of the vaccine trials show that a single antigen could not be sufficient to induce protection.

**OBJECTIVES:** Measure the association between antibody response against several vaccine candidate antigens and protection against malaria

**MÉTHODOLOGIE:** We have conducted cohort study in Bandiagara (Mali) in 211 children. We measured IgM, IgG and IgG subclasses specific to AMA-1, MSP-1 et GLURP-R2, 3 blood stage antigens by ELISA. A six month follow up of the cohort were performed to evaluate the malaria incidence, by thick smears.

**RESULTS:** Malaria incidence was 0.69 per children per year. Anti-malaria IgG seropositivity was high in children having parasites at the start of transmission season.

Levels of d’IgG, d’IgG1 and d’IgM to AMA-1 were higher in children aged from 3 to 5 years compared to younger children. IgG levels to MSP-1 and and GLURP-R2 in children from 3 to 5 years were comparable to those less than 3 years. There was no correlation between antibody levels and protection against malaria episodes.

**DISCUSSION:** As usually described the natural acquisition of antibodies against these 3 antigens was age dependent. No association between the presence of any isotype of malaria antigen specific antibody and protection against the occurrence of clinical episode has been observed as described in studies of Dodoo and Kusi in Ghana. This result needs more investigation.

**CONCLUSION:** The antibodies against three antigens is not associated with protection against malaria in this population of children 1-5 years old in Bandiagara a setting of seasonal transmission area.

**P677: Anophele fauna and their role in the malaria transmission in the Soudano-sahelian zones of Northern Cameroon**

**Raymond Tabue**1,2, Jude Bigoga1,2, Parfait Awono-Ambe2, Josiane Etang1, Salomon Patchoke1, Jean Atangana1, Jean-Claude Toto2, Etienne Fondjo3,5, Jean Atangana5, Jean-Claude Toto4

1National Malaria Control Programme, 2 Faculty of Medicine and Biomedical Sciences, 3,5 jean Atangana5, jean-claude toto4

**BACKGROUND:** Malaria is major health problem in Cameroon. Knowledge of the vectors involved in malaria transmission in any given
area is fundamental to the design and implementation of policies that would be easily amenable for intervention. Therefore, we have studied the Anopheles fauna and their roles in malaria transmission in 38 villages belonging to three health districts in the northern savannah region of Cameroon.

METHODS: Mosquitoes were sampled at night on human volunteers between 6:00pm and 6:00am during two consecutive nights. Following morphological identification, the ovaries of a proportion of the female anophelines were dissected to check for parity. Members of the Anopheles gambiae complex were further identified using PCR. Infectivity was determined through circumsporozoite protein ELISA and the entomological inoculation rates determined. Blood meal ELISA studies were used to determine the anthropophilic index.

RESULTS: A total of 8964 mosquitoes were collected. Major Anopheles species were An. gambiae s.l. (70.64%), An. pharoensis (11.30%), An. rufipes (8.78%), An. funestus (5.63%). The anthropophilic rate of vector species varied from 12.43% to 100 % and the Entomological Inoculation Rate (EIR) of the malaria vectors ranged from 13.76 ib/m/n and 0.03 ib/m/n.

CONCLUSIONS: The intensity of malaria transmission greatly varied between the villages and health districts. It was lowest in the in highland areas but peaking in areas that are highly implicated in rice cultivation. Interestingly An. rufipes generally known to be a zoophilic species was identified as an important local vector of P. falciparum. The observed differences in transmission pattern in relation to varying ecology should be taken into account in the design and implementation of intervention policies.

P678: Role of an.Ziemanni in malaria transmission in Ndop health district (North West region Cameroon)

Raymond Tabue1,2,3, Thomas Nem4, Jean Atangana3,4, Salomon Patchoke4,5, Frederic Thcouine4, Etienne Fondjo4,5, Jude Bigoga1,2, Barrière Flojo1,2, Rose Leke2

1 Molecular Parasitology and Disease Vector Research Laboratory, National Reference Unit for Vector Control, The Biotechnology Center, University of Yaoundé I, Cameroon. 2 Faculty of Science, Department of Biochemistry, University of Yaoundé I, Cameroon. 3 Ministry of Public Health. 4 Ministry of Public Health. 5 Faculty of Medicine and Biomedical Sciences, University of Yaoundé I, Cameroon

BACKGROUND: Suitability of ecological conditions determines malaria vector species distribution in space and time. Thus, knowledge of the baseline malaria transmission in a given environment is important to guide malaria control interventions. So a 3-years entomological study of the baseline malaria transmission in a given environment is important to guide malaria control interventions. Therefore, we have studied a further study on the behavior of An. ziemanni and the characterization of its breeding sites is essential.

P679: Evaluation of the performance of SD BIOLINE malaria Ag Pf/Pv and CareStart™ malaria Pf/Pv Combo tests for the diagnosis of malaria in two malarious areas in central Ethiopia,

Abeba Gebretsadik 1, Ato Abebe Animitu 1, Dr. Girmay Medhin2, Prof. Berhanu Erko 1

BACKGROUND: Early and accurate diagnosis of malaria followed by prompt treatment reduces morbidity and mortality in endemic regions. Presumptive treatment of malaria is widely practiced where microscopy or rapid diagnostic tests are not readily available. Introduction of rapid diagnostic tests (RDTs) for the treatment of malaria in many low-resource settings need evaluation of their performance. This study evaluated the performance of two RDTs in two health centers from November-December, 2011 in Adama and Amaya.

OBJECTIVE: To evaluate the diagnostic performance of SD BIOLINE malaria Ag Pf/Pv and CareStart™ malaria Pf/Pv Combo test relative to microscopy for the diagnosis of P. falciparum and P. vivax malaria in Ethiopia.

METHODS: In this cross-sectional study, patients who had malaria symptoms and visited two health facilities in Oromia Region were recruited. Thin and thick blood smears were prepared from finger prick and stained by 10% Giemsa. Microscopic examination was done under 100x magnifications for Plasmodium species identification and determination of parasitaemia. The two RDTs were performed as per the manufacturers instructions.

RESULTS: A total of 547 febrile patients were diagnosed, of which 127 were microscopy positive for Pf (n=38) and Pv (n=85). The sensitivity, specificity, positive and negative predictive value of SD BIOLINE malaria Ag Pf/Pv test were 92.1%, 99.1%, 95.9% and 98.2%, respectively; and for CareStart™ malaria Pf/Pv Combo tests were 94.5%, 99.6%, 98.4% and 99.6%, respectively.

CONCLUSION: The diagnostic performance of SD BIOLINE malaria Ag Pf/Pv test and CareStart™ malaria Pf/Pv Combo test were very good with respect to malaria microscopy.

P680: EBA and PfRh invasion ligands are targets of antibodies that inhibit invasion and facilitate immune evasion by phenotypic variation

Linda Reiling1, Kristina E. M. Persson2, Freya J. J. Fowkes3, Fiona J. McCallum1, Nimmo Gicheru4, Jack S. Richards5, Christine Langer6, Faith Osier1, Alan F. Cowman1, Peter M. Siba2, Chetan Chitnis3, Takafumi Tsuboi2, Ivo Mueller1, Kevin Marsh1, James G. Beeson1

1 Burnet Institute for Medical Research and Public Health, Melbourne, Victoria, Australia; 2 Karolinska Institutet, Microbiology, Tumor and Cell Biology, Stockholm, Sweden; 3 The Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia; 4 Centre for Geographic Medicine Research, Coast, Kenya; 5 Papua New Guinea Institute of Medical Research, Goroka, PNG; 6 International Centre for Genetic Engineering and Biotechnology, New Delhi, India; 7 Ehime University Graduate School of Medicine, Toon, Japan

BACKGROUND: Antibodies are important in acquired immunity against Plasmodium falciparum malaria, and may be involved in a range of effector functions. However, specific targets and effector mechanisms of functionally active antibodies are largely undefined. Direct inhibition of invasion is one mechanism by which antibodies against P. falciparum invasion ligands may contribute to protective immunity. The erythrocyte binding antigens (EBAs, including EBA140, EBA175, EBA181) and reticuloocyte binding homologues (PFRhRs, including PFRh1, PFRh2, PFRh4, PFRh5) are two families of invasion ligands that are important in the invasion of erythrocytes. The redundancy in invasion ligands facilitates the switching of invasion pathways and immune evasion, thereby
contributing to the capacity of *P. falciparum* to cause chronic and repeated infections.

**METHODS AND RESULTS:** We have used parasite lines with targeted disruption of different *eba* and *Pfrh* genes and sera from exposed children and adults in functional assays to demonstrate that EBAs and PFRhs are targets of invasion inhibitory antibodies, and that phenotypic variation contributes to immune evasion. Furthermore, we affinity-purified human antibodies against EBAs and PFRhs and used these in invasion assays to demonstrate directly their role in invasion inhibition. We also examined the association between antibodies to different PFRhs and EBAs ligands and protection from malaria in a longitudinal cohort study of children.

**CONCLUSIONS:** Considering all data together, our findings provide important evidence that PFRhs and EBAs are major targets of invasion-inhibitory and protective human antibodies, and that variation in the expression and function of the PFRhs and EBAs mediates evasion of acquired antibodies. This knowledge will be useful to advance malaria vaccine development and to understand how the immune response targets multiple invasion ligands to overcome the capacity of *P. falciparum* for immune evasion.

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**P681:** Holistic approach to malaria elimination: Novel multi-stage malaria vaccine candidates, GMP-process development, automated plant-based production and automated microscopy-based malaria diagnostics.

Alexander Boes¹, Holger Spiegel¹, Véronique Beiss¹, Güven Edgü³, Helga Schinkel¹, Marcel Houdelat¹, Stefan Schillberg¹, Markus Sack³, Thomas Rademacher¹, Pia Dahmi¹, Robin Kastlan¹, Johannes Buyel¹, Tanja Holland², Daniel Blessing³, Nadja Vöpel³, Stephan Hellwig³, Jürgen Drossard³, Rolf Fendel³, Stephanie Kapelski³, Dominika Maskus¹, Otthère Addal-Mensah⁴, Melanie Seidel³, Stefan Barth¹, Markus Geese³, Kristian Reis³, Christian Wenzel³, Christian Hügel³, Oliver Scholz³, Günther Kostka³, Michaela Benz³, Erik Haßlmeyer³, Christian Müzenmayer³, Christian Weigand³, Matthias Scheuermeyer³, Gabriele Pradel³, Edmond Remarque³, Bart Faber³, Rolf Horstmann³, Egbert Tannich³, Rainer Fischer³, Andreas Reimann³

¹ Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Aachen, Germany; ² Fraunhofer Institute for Production Technology (IPT), Aachen, Germany; ³ Fraunhofer Institute for Integrated Circuits, Erlangen (IIS), Germany; ⁴ Institute of Biology VII (Molecular Biotechnology), RWTH Aachen University, Aachen, Germany; ⁵ Bernhard Nocht Institute For Tropical Medicine, Hamburg, Germany; ⁶ Institute for Molecular Infection Biology, University of Würzburg, Würzburg, Germany; ⁷ Biomedical Primate Research Centre, Rijswijk, the Netherlands

**BACKGROUND:** Malaria is a devastating infectious disease caused by parasites of the genus *Plasmodium*. It affects more than 200 million people worldwide and causes an estimated 700,000 deaths every year, primarily children in developing countries. Effective vaccines against malaria are not yet available and anti-malarial drugs are becoming less effective as the parasites develop resistance. In addition to the disease burden malaria has a severe impact on public health and economic welfare, hindering progress in countries where the disease is endemic. Urgent research is therefore required to holistically address the global burden of malaria.

**APPROACH/METHODS:** Based on the funding of the German Fraunhofer Future Foundation a large multidisciplinary project consortium was formed to combine expertise from the infection biology, biotechnology, engineering and medical technology fields for the development of innovative and complementary approaches towards malaria elimination.

**RESULTS:** We generated novel protein-based multi-stage malaria vaccine candidates against *Plasmodium falciparum* (*Pf*) covering antigens from the pre-erythrocytic, erythrocytic and sexual stages. The vaccine candidates were expressed in yeasts (*Pichia pastoris*) and plants (*Nicotiana benthamiana*) and elicited strong, balanced immune responses in mice and rabbits. Binding studies and immunofluorescence assays demonstrated the native conformation of the vaccine candidates. Affinity-purified pAbs showed strong inhibitory effects in functional assays for each stage.

In preparation for clinical testing, GMP-compliant production processes are being established for the yeast- and plant-based malaria vaccine candidates, the latter benefiting from a groundbreaking production facility with integrated vertical farming and 2D/3D-plant scanners. The facility is under construction at the IME in Aachen and will permit the automated large-scale manufacturing of the malaria vaccine candidates in plants according to GMP standards as well as the production of certified transgenic seeds.

In addition to the active vaccination, a potential passive vaccination approach against *Pf* is being explored via a novel technology platform for the generation of human monoclonal antibodies from peripheral blood mononuclear cells of semi-immune donors. First inhibitory antibodies have been isolated and are being evaluated.

To complement the therapeutic approach and to strengthen malaria control a novel diagnostic platform for the automated microscopic acquisition and analysis of Giemsa-stained thin and thick blood smears is being developed. A prototype system has been set up, first segmentation and classification algorithms were generated and a training database was built including images of 1,087 annotated *Pf* parasites and 11,932 artifacts from thick blood smears. Preliminary evaluation of a non-overlapping test set including 1.098 *Pf* images and 2,277 artifacts provided a detection accuracy rate of 93% with combined sensitivity and specificity values >90%.

**CONCLUSION:** The Fraunhofer Future Foundation Malaria Project has introduced a holistic concept to support the elimination of malaria by focusing not only on the generation of innovative malaria vaccine candidates but also on GMP-compliant process development, novel enabling technologies for manufacturing and accurate, automated malaria diagnostics.

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**P682:** The impact of financing the obtention of treated mosquito bed nets by inhabitants of developing countries

Nde Jean Romain.

International Organization for Health and development, Bafoussam-Cameroon

**BACKGROUND:** The financing of the purchase of treated mosquito nets remains a major problem in the fight against malaria in Cameroon. Up to 2010, less than 10% of Cameroonians were in possession of the nets. In 2011, the Global fund sponsored the purchase and distribution of these nets in Cameroon. Our study is aimed to show how making available nets to the local population, has encouraged the use of the nets and how it has triggered investment in this means of malaria prevention.

**METHODS:** Roughly, 15 000 000 mosquito nets bought under the framework of Global Fund sponsorship were distributed in about 70% of households in Cameroon in November 2011. The nets were distributed such that there were a net for two persons per bed. CSOs supported distribution and follow up of the nets, and ORISADE took charge of the Mifi Division, West Region. In course of our study, information with regard to the behavior of beneficiary population vis-à-vis the mosquito nets was obtained monthly through the health areas, and that concerning malaria was obtained from the health services in the Mifi health district.

**RESULTS:** From 4% before the operation, the rate has risen to 60%, just after 2 months of distribution within the general population. In the same vein, the rate of malaria related consultation has moved from 55% to 20% in the Mifi division, and the rate of hospitalization has dropped from 45% to 18%. Within the 30% of households in the division that have not yet gotten mosquito nets, 10% have expressed interest in buying the nets. This is due to pieces of advice and the positive remarks giving to them by beneficiaries.

**CONCLUSION:** From this study, it is concluded that the non utilization of treated mosquito beds nets in developing countries is due to their absence. Financing the purchase of these nets will push the population to obtain them and to start investing in it purchase.
P683: Harmonizing Malaria in Pregnancy Guidance: A Review from Five African Countries

Elaine Roman1, Patricia Gomez2, Aimee Dickerson1
1Jhpiego, An Affiliate of Johns Hopkins University

BACKGROUND: Thirty-nine countries in sub-Saharan Africa have malaria in pregnancy (MIP) policies in place, including intermittent preventive treatment (IPTp), insecticide treated bed-nets (ITNs) and effective case management. Nonetheless, IPTp and ITN coverage among pregnant women remains well below international goals. MIP policies are typically produced by National Malaria Control Programs (NMCP), but are implemented by National Reproductive Health Programs (RHP).

METHODS: We reviewed MIP policy documents from the NMCP and RHP in Kenya, Mali, Mozambique, Tanzania and Uganda to understand 1) how closely national MIP documents reflect WHO MIP guidance and 2) how consistent documents produced by the NMCP and RHP are with each other. We developed a framework to compare MIP documents from RHP and NMCP according to WHO guidance for MIP, including IPTp timing and dosing, directly observed therapy, linkages to HIV prevention programs, promotion and distribution of ITNs, and diagnosis and treatment.

RESULTS: All countries have national documents promoting IPTp, ITN use, and case management of MIP. WHO guidance was not always reflected in these documents: four countries restrict dosing of the first and second IPTp doses to specific gestational weeks, provide inconsistent guidance on MIP prevention in HIV+ women, and fail to provide clear guidance on the different antimalarial treatment that should be administered in the first vs. later pregnancy trimesters. All countries had discordant guidance between RH and NMCP in at least one official MIP guidance document. For example, all countries had conflicting guidance on the timing or dosing of SP and the mechanism pregnant women should use to obtain ITNs. Considerable discrepancies exist between MIP guidance documents from NMCP and RHP.

CONCLUSIONS: These discrepancies contribute to confusion by health workers implementing MIP programs, contributing to the low coverage of IPTp and ITNs. Harmonization of national MIP documents is urgently needed, with effective re-orientation and supervision of health workers to updated materials to help accelerate implementation. While this review is targeted primarily at country level stakeholders, the information is important for regional and global level stakeholders as well. This exercise should be repeated in other malarious countries.

P684: Plasmodium falciparum gene expression associated with variations in malaria transmission intensity and host immunity

Martin Rono1, Mary Nyonda1, Joyce Mwongeli1, Joan Simam1, Kevin Marsh2, Zbynek Bozdech3, Margaret Mackinnon1
1KEMRI Wellcome Trust Research Programme Kilifi, Kenya; 2School of Biological Sciences, Nanyang Technological University, Singapore

Plasmodium parasites have shown remarkable success to adapt despite strong and highly diverse selection pressures in their environment, both natural and man-made. To understand the underlying features of the parasite biology we used whole-genome transcriptome analyses to test for adaptive differences between natural populations of Plasmodium falciparum parasites evolved under different transmission intensities in East Africa. Comparisons from three pairs of parasite populations from high vs. low transmission intensity environments revealed differences in functions relating to red cell invasion, energy metabolism, protein turnover, transport and export to the red cell cytosol. This suggests that parasites evolve different levels of investment in replication and immune evasion to suit their prevailing environments. The molecular systems and genes involved in this adaptation have been identified.

P685: “They just told me to go buy drugs from the shop.” How public sector stock outs of ALu affected malaria case management and care-seeking behaviors in the Mtwara, Mwanza, and Mbeya Regions of Tanzania.

Denise Roth Allen1, Clarence Mkoba2, Admirabilis Kalolella3, Emmy Metta4, Salim Abdulla5, Catherine Goodman4, S. Patrick Kachur2
1Centers for Disease Control and Prevention, Atlanta, GA, United States; 2Ifakara Health Institute, Dar es Salaam, United Republic of Tanzania, 3London School of Hygiene and Tropical Medicine, London, England

BACKGROUND: In 2010, Tanzania began experiencing severe disruptions in the procurement of artesmether-lumefantrine (ALu) for the public sector. These disruptions coincided with a national rollout of interventions to improve access and targeting of ALu to confirmed cases. A multidisciplinary evaluation to assess the effectiveness of these interventions in the Mtwara, Mwanza, and Mbeya Regions began in 2009 and was completed in 2012.

METHODS: Baseline qualitative data were collected twice in Mtwara Region (2009 and 2010) and once in Mwanza and Mbeya Regions (2012). Endline data were collected in 2011 (Mbeja) and 2012 (Mtwara and Mwanza). 117 interviews were conducted at baseline and 106 at endline, including 28 community focus groups (FGD), 113 illness narrative interviews (INI), and 84 in-depth interviews (IDI) with health workers and drug shop vendors. Discussions were held with regional and district authorities to document malaria-related strategies and challenges. Interview transcripts were entered into NVivo8 for content analysis. Being told to “go and buy” ALu in a drug shop emerged as a key theme in this study, being mentioned in a substantial proportion of interviews in 2010 and 2012.

RESULTS: Although some health workers said they provided their patients with prescriptions for drug shop referrals, several INI participants reported being left on their own to figure out where to go and what drug to buy. Others noted they avoided government facilities altogether because of prior experiences of being told there were no drugs. Economic consequences of ALu stock outs also emerged. FGD and INI participants at all sites complained about having to pay facility registration fees only to be told there were no drugs; some resorted to buying half doses due to limited funds. District authorities adopted several measures to address the stock out including rationing of ALu to children under-five years, moving ALu stocks between facilities, and treatment with available antimalarials, although they expressed concern about the overuse of SP and quinine.

CONCLUSIONS: Health facility stock outs of ALu had negative effects on timely access to effective antimalarial treatments. Improving timely acquisition and distribution of first line antimalarials is of utmost importance.

P686: Endogenous antioxidant defenses in malaria comorbidly occurring with typhoid

Oladipo Adeyemiwa1, David A. Ojo1, Regina N. Ugbaja1, Solomon O. Rotimi1
1Department of Biochemistry, Federal University of Agriculture, Abeokuta, Nigeria; 2Department of Microbiology, Federal University of Agriculture, Abeokuta, Nigeria; 3Department of Biological Sciences, Covenant University, Canaan Land, Ota, Nigeria

BACKGROUND: Malaria and typhoid are two diseases of public health importance that have similar symptomatology and epidemiology and are associated with significant morbidity, mortality and economic loss. Individuals in areas endemic for both diseases are often at risk of contracting both of these diseases, either concurrently or an acute infection superimposed on a chronic one. Although studies have indicated that malaria parasites induce an oxidant stress on the host erythrocytes...
during malarial infection, there is a dearth of information on the host oxidative response during typhoid infection and co-morbidity of the two diseases. Based on the mounting evidence that oxidative stress might be an important pathobioc medicinal factor as well as an effective therapeutic principle in disease conditions the host antioxidant responses during malaria, typhoid and typhoid + malaria infections was assessed.

METHODS: Spectrophotometric techniques were used to assay for the activities of glutathione transferase (GST), catalase (CAT) and the concentrations of glutathione (GST) in plasma and erythrocytes of patients in Abeokuta, Nigeria (n= 115).

RESULT: The presence of either or both parasitic infections resulted in significant alterations in the antioxidant response of the subjects (p<0.05). Depletion of erythrocyte GSH, increase in plasma GSH and increased expression of CAT in both plasma and erythrocyte, characterized the antioxidant response of the subjects. While the highest erythrocyte CAT activity was observed in typhoid-infected males, the highest plasma CAT activity was observed in females infected with typhoid (p<0.05). GST activity in malaria infection was not significantly different from control (p>0.05), whereas the activity of the enzyme reduced in typhoid infection and increased when typhoid and malaria infections were present (p<0.05).

CONCLUSION: Our findings indicate that sex differences might play a significant role in the antioxidant response of subjects in typhoid infection alone and in co-morbidity with malaria.

P687: Epistatic interactions between Apolipoprotein E and Hemoglobin S genes in regulation of malaria Parasitemia2.


Apolipoprotein E is a monomeric protein secreted by the liver and responsible for the transport of plasma cholesterol and triglycerides. The APOE gene encodes 3 major isoforms E4, E3 and E2 with APOE Ɛ 4 responsible for the transport of plasma cholesterol and triglycerides. Apolipoprotein E is a monomorphic protein secreted by the liver and responsible for the transport of plasma cholesterol and triglycerides. The APOE gene encodes 3 major isoforms E4, E3 and E2 with APOE Ɛ 4 responsible for the transport of plasma cholesterol and triglycerides. A cross sectional survey was performed in 508 children aged 1 to 12 years in Southeastern Gabon during the wet season. Children were screened for Plasmodium spp. infection, APOE and hemoglobin (Hb) Polymorphisms. Overall median parasite densities were significantly higher in APOE Ɛ4 children for Plasmodium spp. densities compared to non-APOE Ɛ4 children. When stratified for HbS polymorphisms median Plasmodium spp. densities were significantly higher in HbAA children if they had an APOE Ɛ4 allele compared to those without the APOE Ɛ4 allele. When considering non-APOE Ɛ4 children, there was no quantitatively reduction of Plasmodium spp. parasite densities for Hb AS compared to Hb AA. Similar results were found in P. falciparum specific analyses. No influence of APOE Ɛ4 on successful Plasmodium liver cell invasion was detected by multiplicity of infection. These results show that the APOE Ɛ4 allele is associated with higher median malaria parasite densities in West African children likely due to the importance of cholesterol availability to parasite growth and replication. Moreover, we observe an epistatic interaction between APOE and Hb S genes such that sickle cell trait only had an effect on malarial parasite density in APOE Ɛ4 children. This suggests a linked pathway of regulation of parasite densities involving expression of these genes. These findings have significance for understanding host determinants of regulation of malaria parasite density, the design of clinical trials as well as studies of co-infection with Plasmodium and other pathogens. The pyrethroid resistance threat – is it undermining malaria control and what can be done? The past decade has seen unprecedented progress in malaria control, resulting in major declines in malaria morbidity rates in many countries. This progress is attributed to a significant scale-up of vector control interventions, as well as better diagnostic testing and wider availability of effective anti-malarias. The next few years will be critical in the fight against malaria. Vector control, primarily through the use of long-lasting insecticidal nets and indoor residual spraying, will remain at the centre of our efforts. While the tools for controlling malaria vectors remain highly effective in almost all settings, mosquitoes are rapidly developing resistance to insecticides and countries in sub-Saharan Africa, characterized by high levels of malaria transmission and widespread reports of resistance, present the graviest concern. The main factor driving resistance has been the heavy reliance by vector control programmes on a single class of insecticides, the pyrethroids. While it is uncertain that action will prevent the further selection of resistance, what is the evidence that present levels of resistance are undermining control interventions? The evidence will be reviewed together with research and development priorities to maintain malaria control.

P688: Sustained Declining Burden of Malaria at Community level in Northeastern Tanzania

Acleus SM Rutta, Filbert Francis, Bruno P Mmbando, Deus S Ishengoma, Samwel Sembuche, Ezekiel K Malecela, Johari Y Sadi, Mathia K Kamugisha, Martha M Lemenge

National Institute for Medical Research, Tanga Medical Research Centre, Tanzania

BACKGROUND: The reported decline of malaria in most parts of Tanzania has some implication on accuracy of malaria diagnosis and management, especially following the introduction of expensive artemisinin combination therapy (ACT) with artemether/lumefantrine (ALu). Traditionally, fever has been the back-bone of malaria case management; but with declining malaria and introduction of expensive ACTs, this approach poses a major challenge. In our previous and ongoing passive case detection (PCD) of febrile illnesses in 4 villages of Korogwe, northeastern Tanzania, we demonstrated that provision of early diagnosis and treatment of malaria by community owned resource persons (CORPs) using rapid diagnostic tests (RDTs) and ALu is an effective strategy for malaria control. This paper gives updates of sustained impact of deployed interventions within an on-going study which aims at assessing the use of CORPs in provision of early diagnosis and prompt treatment and to estimate the burden of malaria at community-level.

METHODOLOGY: In 2006, individuals reporting to CORPs with history of fever within 24 hours or fever (≥37.5°C) at presentation were presumptively treated with sulphadoxine/pyrimethamine. Between 2007 and 2012, individuals aged 5 years and above with positive RDT were treated with ALu while under-fives were treated irrespective of RDT results. The CORPs were supervised weekly by a clinician and a technician to ensure adherence to SOPs.

RESULTS: A total of 18,981 cases were attended and 17.2% were positive for malaria parasites by microscopy. Malaria prevalence and incidence decreased across the years, from 34.6% to 1% and 235/1000 to <8/1000 person years at risk for 2007 and 2012, respectively. The highest incidence of malaria shifted from children aged 5-9 years to individuals aged 10-19 years from 2009. Despite these changes, fever prevalence remained high at >40.0% in under-fives and >20.0% among individuals aged 5 years and above.

CONCLUSION: The significant reduction in malaria prevalence and incidence observed might be attributed to different interventions including early diagnosis and prompt treatment by CORPs. Studies to investigate causes of fevers other than malaria are recommended for better case management. The current remarkable and sustained decline in malaria suggests that these areas might be moving from control to pre-elimination levels.
BACKGROUND: Brazil has 300,000 malaria cases/year about 60% of cases in Americas. In 2012, Acre State showed a 37% increase in malaria cases compared to 2011. Long-lasting insecticidal mosquito nets (LLIN) were installed in each household in 2007, 2010 and 2012. This study describes knowledge, attitudes and practices of the population in relation to LLIN in three municipalities of Acre State, located in Brazilian Amazon region.

METHODS: A cluster survey (30×7) was performed, considering prevalence of 50%, design effect 2.0 and accuracy 10%. A semi-structured questionnaire was used to interview 15 years old and older. Prevalence ratios were analyzed.

RESULTS: Among 196 respondents, 131(69%) were women, mean educational level was 7 years (0-17) and annual salary was $4,000. 170(87%) of households had incomplete walls and 180(92%) of the respondents have had malaria at least once in life. The average ownership of LLIN was 2.7(SD=0.17) by household which had 3.0(SD=0.07) sleeping places, 52% individuals have slept under a LLIN the night before, and the mean use of LLIN was 2.0(SD=0.11) years. 161(82%) of interviewers know that malaria is transmitted through mosquito bites and 132(67%) know that LLIN can be used to prevent it. Statistically association was found between: better condition of life (RP=1.28) and complete basic education (RP=1.26) with malaria transmission knowledge; being under social aid (RP=1.11) and complete basic education (RP=1.14) with prevention knowledge; electricity absence (RP=1.14), sleeping outside the house (RP=1.25), had received explanation on LLIN use by the health agent (RP=1.40), enjoying to use LLIN (RP=1.60) and perceiving its positive effect (RP=1.30) with preventive attitude with LLIN; performing malaria risk activities (RP=1.14), electricity absence (RP=1.14), enjoying to use LLIN (RP=1.60) and sufficient ownership of LLIN per sleeping places (RP=1.19) with actual use of LLIN.

CONCLUSIONS: Interviewees were well-informed about knowledge of transmission. Despite numerous campaigns undertaken in Acre, there is still need to improve knowledge on LLIN importance through increasing basic education of this population and better explanation on LLIN use during distribution. Although LLIN use is better were malaria risk and LLIN protection effect are perceived there is a need to increase ownership through further bednets distribution. Further knowledge on durability of bednets and its relation to malaria increase in this area is needed.

P690: IgG responses to Anopheles gambiae salivary gSG6-P1 peptide as sensitive biomarker for human exposure to Anopheles bites and to malaria risk: applications to low/seasonal malaria area and to dry season

A. B. Sagna1,2, L. Gaayeb1, J. B. Sarr1,3, P. M. Drame1, M. O. Ndiath1, S. Senghor1, C. S. Sow1, A. Poinssignon3, M. Seck1, E. Hermann1, A. M. Schacht1, L. Fayé1, F. Remoué14 and G. Riveau14
1Centre de Recherche Biomédicale Espoir Pour La Santé (CRB-EPLS), Saint-Louis, Sénégal; 2Laboratoire de parasitologie, Département de Biologie Animale, Université Cheikh Anta Diop, Dakar, Sénégal; 3Institut de Recherche pour le Développement (IRD), UMR 224 MIVEGEC, Montpellier, France; 4Institut de Recherche pour le Développement (IRD), UMR 198 URMIITE, Dakar, Sénégal; 5Centre d’Infection et d’Immunité de Lille (CIIL), Inserm U1019, Université Lille Nord, Lille, France; 6Centre de Recherche Entomologique de Cotonou (CREC), Cotonou, Bénin.

BACKGROUND: Current methods for monitoring malaria, such as entomological and parasitological measures, present limitations in sensitivity in contexts of low transmission intensity. In the framework of elimination/eradication objective, it needs new sensitive and robust surveillance tools. In this study, we assess the IgG antibody responses to Anopheles gambiae salivary peptide (gSG6-P1) as a sensitive biomarker for discriminating micro-geographical heterogeneity of exposure to Anopheles bites in low and seasonal transmission area. In addition, such application was evaluated in the specific context of the dry season to assess its potential as biomarker of Plasmodium falciparum exposure.

METHODS: A longitudinal survey was performed in 410 children aged 1 to 9 years from five villages of northern Senegal, and five visits corresponding to different season were carried out during the 16 months study period. Vector data was obtained using both Human Landing Catches (HLC) and Pyrethrum Spray Catches (PSC). Human IgG Antibody response to gSG6-P1 salivary peptide was assessed by ELISA and compared according to the season and villages. Parasitological and clinical data (asymptomatic or symptomatic) also were correlated with anti-gSG6-P1 IgG levels during the dry season.

RESULTS: Specific IgG Ab levels to gSG6-P1 varied considerably according to the villages (p<0.001), suggesting it is sensitive enough to detect micro-geographical heterogeneity of Anopheles exposure. Moreover, the specific IgG Ab levels to gSG6-P1 increased during the peak of Anopheles exposure/transmission season, and decreased immediately after the end of the exposure/transmission season (all p<0.05). Interestingly, children with P. falciparum infection showed higher IgG response levels to gSG6-P1 than uninfected children during the dry season (p<0.01), suggesting that positive IgG response to gSG6-P1 could be a useful indicator of P. falciparum infection risk in children. IgG response to gSG6-P1 also seemed to discriminate non-infected children to asymptomatic carriers of the parasite (p<0.01).

CONCLUSIONS: The gSG6-P1 salivary peptide seems to be a sensitive tool to discriminate the micro-geographical heterogeneity of exposure to Anopheles bites and to detect P. falciparum infection risk in areas of very low and seasonal malaria transmission. This immunoenepidemiological marker could be a very useful surveillance tool for monitoring malaria in the scope of malaria elimination.

P691: Relationship between alpha+–thalassaemia and glutathione-S-transferases polymorphisms in children with severe malaria in Tanzania

Fredy Saguti1, Sakurani Balthazary1, Alphaxard Manjurano2,3, Robert Max2, Filemon Tenu4, Filibert Francis1, Seif Shekalah3 and Reginald Kavishe1
1National Institute for Medical Research, Tanga Medical Research Centre, Tanga, Tanzania; 2Kilimanjaro Clinical Research Institute, Kilimanjaro Christian Medical College of Tumaini University, Tanzania, Moshi, Tanzania; 3Sokoine University of Agriculture, Morogoro, Tanzania; 4National Institute for Medical Research, Amani Research Centre, Muheza, Tanzania; 5Jakara Health Institute, Bagamoyo, Tanzania; 6National Institute for Medical Research, Mwanza Research Centre, Mwanza, Tanzania; 7Joint Malaria Programme, Moshi, Tanzania.

BACKGROUND: Alpha+-thalassaemia is well known for conferring partial protection against severe malaria. In addition, Glutathione –S-transferase (GST) polymorphism has recently been associated with severe malaria in children. Aretrospective cross sectional study was carried out to determine the relationship between genotypic polymorphisms of alpha+-thalassaemia and glutathione-S-transferase in children with severe malaria.

METHODS: A total of 148 DNA samples from children aged between 1 and 15 years with mild and severe malaria were retrieved and determined by polymerase chain reaction.

RESULTS: Children with Glutathione-S-transferase-p1 (GSTP1)-polymorphism were observed to have three fold risk (OR = 2.9; 95% CI
were found to have 3% decreased protective effect of alpha+-thalassaemia although this was not statistically significant [OR = 0.81 (95% CI = 0.5-1.5; P = 0.5) to OR = 0.7895% CI = 0.4-1.5; P = 0.44)].

CONCLUSIONS: We conclude that Glutathione-S-transferase-p1 polymorphism increases risk of developing severe malaria due to Plasmodium falciparum in children. The observed inverse relationship between GSTP1 polymorphisms and alpha+-thalassaemia in children with severe malaria need further investigation.

P692: Knowledge and use of malaria preventive measures by caregivers of under-fives in rural South Western Nigeria

Dr. Mobolaji M. Salawu*, Dr. Oluwaseun O. Akinyemi**, Dr. Akindele O. Adebiyi*
*Dept. of Community Medicine, UCH, Ibadan. ** Dept. of Health Policy and Management, College of Medicine, University of Ibadan.

BACKGROUND: Malaria is highly endemic in sub-Saharan Africa. Nigeria is one of the worst hit countries causing about 30% of child mortality. Several common and effective measures had been used to control malaria; and some African countries have reported up to 50% reduction in malarial cases. However, data from Nigeria have not shown significant reduction. This study therefore assessed the use of malaria preventive measures by caregivers of under-fives in rural South Western Nigeria.

METHODS: This was a cross sectional survey conducted in 2010 among 274 caregivers of under-fives who were selected using the cluster sampling technique. Logistic regression analysis was used to model for predictors of use of malaria preventive measures at 5% level of significance.

RESULTS: About 86.1% of the caregivers were females and their mean age was 28.95±8.1 years. Almost all respondents (92.7%) were married. They were largely unskilled workers (63.5%). About 95.6% of the care givers knew that malaria can be prevented and 78.1% had good knowledge of known malaria preventive measures. However, the use of malaria preventive measures was varied as only 19.7% used indoor residual spraying, 15.0% used anti malaria drug, 8.4% used personal and environmental hygiene and 8.0% used insecticide treated nets. Respondents who were professionals were more likely to have a good knowledge of malaria preventive measures p<0.05. The predictor of use of malaria preventive measures was respondents who had a good knowledge of causes of malaria (OR 9.3, 95% CI: 1.35-64.3).

CONCLUSIONS/RECOMMENDATION: The use of malaria preventive measures among the caregivers of under-fives in this region is poor. There is therefore a need to educate and encourage them on proper use of malaria preventive measures in order to prevent their children from developing malaria infection.

P693: Impact of the intermittent Preventive treatment with amodiaquine plus sulfadoxine/pyrimethamine on the prevalence of dhfr 59codon mutation

Salif Sombié1, Issiaka Soulamà1, Yaro Jean Baptiste, Edith C. Bougouma, Sodionmon B. Sirimà1, Amadou T. Konate1
1Centre National de Recherche et de Formation sur le Paludisme, Ouagadougou, Burkina Faso,

Intermittent preventive treatment of malaria in children (IPTc) is a promising new approach to the control of malaria in areas of seasonal malaria transmission. The Sulfadoxine-Pyrimethamine/Amodiaquine combination is known in selecting mutant parasite. The objective of the present study was to assess the impact of the Intermittent preventive treatment of malaria in children based using the Sulfadoxine-Pyriméthamine/Amodiaquine combination on the prevalence of the dhfr 59 condon in children aged 3 to 59 months living in seasonal malaria transmission setting in Burkina Faso. The study was conducted in three different villages located in the province of Kourweogo at 40km northwest of Ouagadougou the capital city. Children were then randomised to receive three courses of IPTp with SP plus AQ or placebos given at monthly intervals during the peak malaria transmission season.

RESULTS: The prevalence of children with mutants parasites for codon 59 of the dhfr gene were 17.70%, 43.60% and 48.10% , respectively before the administration of intermittent preventive treatment, one month after the preventive treatment intermittent and one year after the administration of intermittent preventive treatment. These results show that the prevalence of the mutation of codon 59 of the dhfr gene increases after treatment with the Amodiquine + sulfadoxine / pyrimethamine combination and confirm the positive selection of mutant parasite by the Amodiquine + sulfadoxine / pyrimethamine combination.

P694: Improving better pregnancy outcomes: Use of Community Health Workers in identification and referral of pregnant women not accessing intermittent preventive treatment (IPTp) in Kenya

Sombie Salif, Augustine Ngindu

INTRODUCTION: Malaria in pregnancy is associated with anemia, low birth weight, miscarriages and death. Despite availability of effective MIP interventions intermittent preventive treatment using Sulfadoxine Pyrimethamine (IPTp-SP) and insecticide treated nets (ITNs), coverage rates in Kenya have remained low; IPTp2 uptake- 25% and ITN coverage- 41%. To increase coverage rates Kenya has adapted a community strategy approach sensitizing pregnant women to start antenatal care (ANC) early to receive comprehensive care throughout pregnancy. This includes access to an ITN at first ANC visit and IPTp uptake beginning in the second trimester to increase coverage rates.

METHODS: Trained community health workers (CHWs) registered pregnant women in their Community units for follow up monthly. CHWs conducted monthly follow up of all registered pregnant women to identify those not attending ANC and referred them for ANC services as well as counseled those not using ITNs. CHWs received supportive supervision from district managers and MCHIP staff to assess performance skills on quality of data and mentorship on MIP interventions. Data collected was analyzed for pregnant women registered, accessing IPTp and referred for ANC services.

RESULTS: 3,212 pregnant women were registered and 1,541 (48%) of the registered pregnant women were referred for ANC services because they were either late in starting ANC attendance or IPTp after 1st trimester or defaulters of scheduled visits. Among the registered pregnant women 81% had taken one or more IPTp doses.

DISCUSSION AND CONCLUSION: Use of CHWs in identification of pregnant women not accessing IPTp and referral of 48% of them has shown an effective methodology of identifying defaulters in IPTp uptake among pregnant women. Scaling up of this community-based approach would ensure early ANC attendance and access to the available effective MIP interventions including IPTp.
P695: Reverse Transcriptase qPCR as a Tool to Investigate in vitro Immunomodulation caused by Visceral Leishmaniasis and Malaria co-infection.

Aurélien Saliki1*, Erika van den Bogaart1, Laura de Bes1 & Henk Schallig1
1Department of Biomedical Research, Royal Tropical Institute (KIT), Amsterdam, the Netherlands

BACKGROUND: Visceral leishmaniasis and malaria are two major public health threats in developing countries. Epidemiologic and single case studies suggest a high risk and possible clinical severity of these concomitant infections in areas where the two diseases overlap. Particularly at immunological level, where this condition is largely unassessed, little substantial information concerning the effects of this co-infection is available. In this study, we describe the use of reverse transcriptase qPCR to monitor the immunomodulatory effects caused by in vitro exposure of human dendritic cells to P. falciparum and L. donovani.

METHODS: The mRNA expression levels of toll like receptors (TLR2, TLR4, TLR8 and DC-SIGN (CD209), were obtained from healthy donors and stimulated with different amounts of parasites and/or lipopolysaccharide (LPS). Thereafter, nucleic acids were isolated, subjected to DNase treatment to remove genomic DNA and transformed into a cDNA library for evaluation. Amplification of the human housekeeping gene B-2-microglobulin was included in all PCR reactions to control for accuracy, efficiency and sensitivity. The relative mRNA expression level of each pattern recognition receptor (PRR) was calculated against the unstimulated control using the ΔΔCt-method.

PRELIMINARY RESULTS AND CONCLUSIONS: Optimal amplification of all targeted genes was achieved with a two-step qPCR (average efficiency 98%, n=5) using standard amounts of MgCl2 (1.5 mM) for TLR2, TLR4 and TLR8 and additional MgCl2 (2 mM) for DC-SIGN. Samples should be processed on the same day, as cDNA degradation may occur over time. As the qPCR sensitivity correlates with the available amount of targeted nucleic acids, a high concentration of dendritic cells (> 10^6 DCs/ml) is required for cDNA synthesis. The RT-qPCR optimized in this study appears suitable to accurately measure the transcriptional level of targeted PRRs and will be a valuable tool for visceral leishmaniasis and malaria co-infection studies.


Badara SAMB1,2, Charles S. WONDJI1, Ibrahima DIA1, Lassana KONATE1, Ousmane FAYE1
1Laboratoire d’Ecologie Vectorielle et Parasitaire, Département de Biologie Animale, Université Cheikh Anta Diop de Dakar, Dakar, Senegal
2Liverpool School of Tropical Medicine, Liverpool, United Kingdom
3Unité d’Entomologie Médicale, Institut Pasteur de Dakar, Dakar, Senegal

BACKGROUND: The insecticide resistance status of the major malaria vector Anopheles funestus and the underlying resistance mechanisms remain uncharactised in Senegal. To fill this gap in our knowledge, we assessed the susceptibility of a population of this species in Gankette Balla, Northern Senegal and investigated the potential resistance mechanisms.

METHODS: Insecticide susceptibility assays were carried out using 2–5 day old F1 adults generated from indoor-collected blood-fed female of An. funestus from Gankette Balla. Microarray and quantitative real-time PCR analysis were used to investigate the metabolic resistance mechanism.

RESULTS: WHO bioassays indicated that An. funestus is resistant to lambda-cyhalothrin 0.05% (74.64% mortality / n = 222). Suspected resistance was observed to DDT 4% (83.36% mortality / n = 158), deltamethrin 0.05% (88.53% mortality / n = 114), permethrin 0.75% (91.19% mortality / n = 139), bendiocarb 0.1% (94.13% mortality / n = 157) and dieldrin 4% (96.41% mortality / n = 306). However this population is fully susceptible to malathion 5 % (100% mortality / n = 50) and fenitrothion 1% (100% mortality / n = 55). The microarray and q-RT-PCR analysis indicated that the lambda-cyhalothrin resistance in Gankette Balla is conferred by metabolic resistance mechanism under the control of the P450 CYP6M7 gene. The absence of over-expression of the duplicated P450 genes, CYP6P9a and CYP6P9b, indicates that the resistance mechanism in Senegal is different to that observed in Southern Africa.

CONCLUSIONS: This study represents the first report of pyrethroid resistance in An. funestus from Senegal and shows that resistance to insecticides is not only confined to An. gambiae as previously thought. These findings should be taken into account by future malaria control programs.

P697: The effect of amodiaquine plus artesunate combination therapy on malaria and outcome on anaemia in children in a rural area of Cameroon.

Moses Samiel1*, Irene Sumbele2, Elsy Ngwa1, Theresa Nkou Akenji3
1Dep’t of Medicine, University of Bamenda, Cameroon; 2Department of Zoology and Animal Physiology; and 3Department of Microbiology and Parasitology University of Buea, Cameroon

BACKGROUND AND AIM: Malaria infection in humans by Plasmodium species, especially Plasmodium falciparum, remains an important health problem and frequently leads to anaemia. To gain more insight into the contribution of the infection on the severity of anaemia in children (<15 years), the prevalence and density of malaria parasites as well as haemacotric was assayed in children residing in Muea, a malaria endemic area in Cameroon.

METHODS: A total of 201 children harbouring malaria parasites were recruited in a longitudinal study during which the children were followed up weekly for six weeks. Malaria parasitaemia and packed cell volume (PCV) were determined weekly respectively by microscopy and haematocrit was assayed in children residing in Muea, a malaria endemic area in Cameroon.

RESULTS: Of the 201 children that were followed up, anaemia prevalence (PCV<31%) was 43.4%. Following appropriate treatment for malaria, there was a progressive increased in mean PCV, mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and WBCs while mean corpuscular volume (MCV), platelets and neutrophils levels dropped by D42. Generally, the severity of anaemia correlated positively with parasite intensity. The difference between pre-and post-treatment mean PCV was significant (P<0.05).

DISCUSSIONS: Our results show that malaria exerts a negative effect on PCV (malaria anaemia) and leads to haematological changes. The scenario is circumvented by proper treatment with artesinim combination therapy. Assistant to the local community in the form of education on the advantages of proper/prompt treatment and frequent control of local drug stores to ascertain the sales of proper antimalarial drug is imperative in controlling the disease.

P698:Health Manpower Development: The Need for Postgraduate Training in Sierra Leone

Babalola Ayodele Samuel

OBJECTIVE: To justify the need for postgraduate specialist training of medical doctors in Sierra Leone.

METHODS: A descriptive study and questionnaires were administered to government registered doctors inclusive of interns.

RESULTS: 66.3 % (126) doctors registered under the Ministry of Health and Sanitation, participated in this study. 77.8% were male and 22.2%
P699: Optimized Pan-species and species duplication real-time PCR assays for Plasmodium parasites detection in malaria vectors.

Maurice Marcel Sandeu1,2, Azizath Moussiliou1, Nicolas Moiroux1, Achille Massougbodi1,2, Vincent Corbel1, Nicaise Tulkue Ndame1,2. 
1Ecolgie, Genetique, Evolution et Controle (MIVEGEC), UMI-CNRS 5290-IRD 224, Institut de recherche pour le developpement (IRD), Maladies Infectieuses et Vecteurs, O1 BP, 4414 RP Cotonou. 
2Centre d’Etudes et de Recherche sur le Paludisme Associe a la Grossesse et à l’Enfant (CERPAGE), Faculte des Sciences de la Santé (FSS), Cotonou, Benin.

BACKGROUND: An accurate method for detecting malaria parasites in the mosquito’s vector remains an essential component in the vector control. The Enzyme linked immunosorbent assay specific for circumsporozoite protein (ELISA-CSP) is the gold standard method for the detection of malaria parasites in the vector even if it presents some limitations. Here, we optimized multiplex real-time PCR assays to accurately detect minor populations in mixed infection with multiple Plasmodium species in the African malaria vectors Anopheles gambiae and Anopheles funestus mosquitoes collected in two localities in southern Benin.

METHODS: Complementary TaqMan-based real-time PCR assays that detect Plasmodium species using specific primers and probes were first evaluated on artificial mixtures of different targets inserted in plasmid constructs. The assays were further validated in comparison with the ELISA-CSP on 200 field caught Anopheles gambiae and Anopheles funestus mosquitoes collected in two localities in southern Benin.

RESULTS: The validation of the duplex real-time PCR assays on the plasmid mixtures demonstrated robust specificity and sensitivity for detecting distinct targets. Using a panel of mosquito species, the real-time PCR showed a relatively high sensitivity (88.6%) and specificity (98%), compared to ELISA-CSP as the referent standard. The agreement between both methods was “excellent” (κ=0.8, P<0.05). All infected mosquito samples contained Plasmodium falciparum DNA and mixed infections with P. malariae and/or P. ovale were observed in 18.6% and 13.6% of An. gambiae and An. funestus respectively. Plasmodium vivax was found in none of the mosquito samples analyzed.

P700: School performance after six years of intermittent preventive treatment using artemisinin-based combination therapy in Mali

Hamma Maiga,1 Breanna Barger-Kamate,2 Issaka Sagara,2 Oumar Bila Traore,2 Mamadou Tekete,1 Intimbeye Termine,1 Antoine Dara,2 Zoumana Issac Traore,2 Modibo Diarra,3 Samba Coumame,3 Aly Kodio,1 Bouran Sidibe,1 Aboubacrine Haidara,1 Nouhoum Diallo,2 Ogobara K. Doumbo,1 Abdoulaye A. Dijoum,1 
1Malaria Research Training Center, Department of Epidemiology of Parasitic Diseases, University of Science, Technique and Technologies of Bamako (USTTB), Mali; 2School of Medicine, Johns Hopkins University, Baltimore, MD

BACKGROUND: Previous studies showed that in areas of seasonal malaria transmission, intermittent preventive treatment of school children (IPTsc) targeting the transmission season, reduced the rates of clinical malaria. The efficacy of ACTs in the context of longitudinal IPTsc is poorly investigated and school performance has not been thoroughly evaluated.

METHODS: This was an open randomized controlled trial of seasonal IPT among school children aged 6–13 years in Kolle, Mali. The study began in September 2007 and completed follow-up in June 2013. Students were randomized to one of three study arms: Sulphadoxine–pyrimethamine plus artesunate (SP/AS), Amodiaquine plus artesunate (AQ/AS) or Control (C). All students received two full treatment doses, given 2 months apart during the season of high transmission from September to December. Groups were compared with respect to school performance, incidence of clinical malaria, asymptomatic parasitemia and anaemia.

RESULTS: A total of 296 students were randomized, and retention in the study was 99.3%. Yearly grade average and success rate in the SP/AS and AQ/AS arms were (5.37; 79.1%) and (4.87; 70.5%) respectively vs. control (4.81; 68.7%) (P < 0.05). Clinical malaria incidence in the SP/AS and AQ/AS arms was reduced by 50.9% and 20.6%, respectively, vs. control (P < 0.001). There were fewer all-cause clinic visits among the children receiving SP/AS or AQ/AS (P < 0.001). The prevalence of asymptomatic parasitemia was higher in the control group than in the SP/AS or AQ/AS (P < 0.001) groups. At the end of the transmission period, children treated with IPT showed a trend towards lower rates of anaemia (SP/AS, 4.2%; AQ/AS, 7.8%; Control, 12.7%; P = 0.012).

CONCLUSION: IPTsc with SP/AS reduced the rates of clinical malaria, all-cause acute clinic visits and asymptomatic parasitemia and trended towards a reduction in anaemia among school-aged children while improving markers of school performance.

P701: Surveillance in Easy Access Groups as a Tool for Evaluating District-level Malaria Control Progress: is it time to revisit convenience sampling?

Sanlé S. S. Sesay1,2, Emanuele Giorgi1, Peter J. Diggle1, David G. Lalloo3, Arantxa Rocas-Feltrer1, Feiko O. ter Kuile1, Miguel Sanjoaquin1, Dianne J. Terlouw4. 
1Mali-Liverpool-Wellcome Trust Clinical Research Programme, College of Medicine, Blantyre, Malawi; 2Lancaster University, Lancaster, United Kingdom; 3Liverpool School of Tropical Medicine, Liverpool, United Kingdom.

BACKGROUND: Malaria transmission intensity and burden can vary widely within countries and even between sub-districts. Timely, valid, low-cost district and local level estimates of short- and medium-term progress in control of malaria are needed urgently to support the move towards more targeted control and transmission reduction. Surveillance in aggregations of sub-groups of the population, so-called Easy Access Groups (EAGs), offers a less resource intensive method of deriving estimates of control progress, but concerns about biased estimates have held back their use. This study aims to evaluate the accuracy of estimates from several EAGs by comparison with population-based household survey in Chikhwawa District, a high malaria transmission region in Malawi.

METHODS: We field tested several EAGs to determine both population estimates and heterogeneity of malaria burden and uptake of control interventions in the catchment area of a clinical trial in a rural area of Malawi. EAG estimates were compared to that of a ‘gold standard’ continuous household level Malaria Indicator Survey (eMIS) in the same population, combining classical epidemiological and geospatial statistical methods.

RESULTS: Findings from the first two years (May 2011 – April 2013) of the study will be presented. Results will focus on the comparison of estimates derived from children attending Expanded Programme on Immunization Clinics, pregnant women attending Antenatal Clinics and community market EAGs with the eMIS.

CONCLUSIONS: The rapid developments in geospatial statistics are opening up exciting opportunities for use of EAG monitoring to guide more targeted malaria control efforts.
P702: Assessing the role of patent medicine vendors in malaria case management for sustainable malaria control in Nigeria

Abubakar Sanusi, Muhammad Lawan Umar, Zubairu Iliyasu, Jamila Abdulkadir
Faculty of Medicine, Bayero University Kano. Nigeria

BACKGROUND: Nassarawa local government is located in Kano state, Nigeria with a population of 596,669. Patent medicine vendors (PMVs) are ubiquitous street level drug sellers found all over Nigeria. Malaria is endemic in Nigeria and a leading cause of morbidity and mortality especially amongst children and pregnant women. There is a need to assess the ability of PMVs to effectively manage and refer community members with malaria using the malaria case management approach as stipulated in the national treatment guidelines.

METHODS: This was a cross-sectional study which used simple random sampling to select 120 PMVs from a list of 240 registered PMVs in Kano. A structured interviewer administered questionnaire was used for data collection after obtaining ethical approval. The data was analyzed using MINITAB® 12.21 software. Percentages were used to describe categorical variables while quantitative variables were described using means. The chi square test was used to assess the significance of associations.

RESULTS: Respondents were mainly married men with secondary school education and a mean age of 32.3 years. A third of the respondents had good knowledge of uncomplicated malaria but (63.8%) had poor knowledge of severe malaria, (94.8%) of the respondents had poor knowledge of rapid diagnostic tests to confirm malaria and their knowledge of ACTs was poor. Most of the respondents had good knowledge about long lasting insecticidal nets and their protective value in pregnancy. Educational status of respondents and good practice of malaria case management had a statistically significant association.

CONCLUSION: There still exist significant knowledge and practice gaps regarding key components of malaria case management amongst PMVs in Kano despite series of trainings conducted by NMCP and NGOs. There is need to review strategies used for training of PMVs on national malaria treatment guidelines so that trainings by different stakeholders are delivered in a coordinated manner using harmonized training tools and capacity building approach for sustainable malaria control in Nigeria.

P703: “The Ownership Gap”: Socioeconomic Differential and Scaling up the use of ITN through Ownership in Africa

Sarpong Doris¹, Mary Atta-Pomaah¹, Elizabeth Awini¹, Margaret Gyapong¹
¹Dowoda Health Research Centre

BACKGROUND: Many curative and preventive strategies have been employed at different times in many malaria-endemic countries across Africa in the quest to reduce the burden of malaria on their populations. Yet, the utilization of insecticide treated net (ITN) has been far from targeted due to ownership gap.

OBJECTIVE: To investigate the differential impact of household socioeconomic status on ITN ownership.

METHOD: Study made use of descriptive and cross tabulations data from recent demographic and health surveys (DHS) from 2006 to 2011 in 13 countries across Africa involving 146,963 households.

RESULTS: Consistently the proportion of households with an ITN was lower than the proportion of households with any type of bed net in all countries including Ghana. ITN ownership gap among households ranged from 0.7% in Rwanda and to 14.2% in Ghana with 4% in Ghana. Households’ wealth index positively influenced ITN ownership in four countries excluding Ghana. Households in lowest wealth quintiles were less likely to have ITN relative to highest wealth quintile households in eight countries. Malawi had the highest proportional difference (33.3%) and the lowest in Kenya (6.5%). The reverse was true in countries like Ghana where the former households were 9% higher to own ITN than the latter households.

DISCUSSIONS AND CONCLUSION: Despite the various policies and programmes as national strategies in combating malaria in the quest of reducing annual financial lost to malaria in the forms of treatment cost, prevention and loss to productivity due to work time loss, there still exists a gap in ITN ownership in African households. More pragmatic measures are therefore needed in not only meeting the Abuja Declaration of ensuring that 60% of communities have ITN but achieving the universal access for the at-risk population of under-five children and pregnant women which have direct impact on MDGs 4, 5 and 6.

P704: Malaria epidemiology in Accra, Gana

Josephine Sasaraku

BACKGROUND: Plasmodium vivax and Plasmodium falciparum malaria remain highly endemic in some deprived area in the capital of Ghana. The Ghana Gold field Ghana Limited is conducting mining activities and funded an integrated vector control intervention within the villages surrounding the mine. The aim of this study was to assess the impact of such programme by comparing the epidemiological trends of malaria in different parts of the island.

METHOD: Two cross-sectional surveys were conducted before and after the intervention (2006–2010) to determine malaria prevalence in mine-impact (MI) and non-MI areas. Incidence of malaria was estimated for the maamobi Medical Centre catchment area using island population denominators and a health-centre passive case detection ongoing from 2006–2011.

RESULTS: A total of 2,264 and 1,653 children <15 were surveyed in the cross-sectional studies. The prevalence of any malaria parasitaemia initially was 31.5% in MI areas and, 34.9% in non-MI (POR 1.17; 95CI 0.97 – 1.39). After four years there was a significant reduction in prevalence in the MI areas (5.8%; POR 0.13, 95CI 0.09–0.20), but reduction was less marked in non-MI areas (26.9%; POR 0.69, 95CI 0.58-0.81). 28,747 patients were included in the evaluation of incidence trends and overall malaria in local and deprived villages, while it remained at similar high levels among migrants. The age-incidence analysis showed that for each higher age range the malaria incidence declines compared to that of the previous stratum.

CONCLUSIONS: There was a substantial reduction in prevalence and incidence rates of both P. vivax and P. falciparum in the mining area following implementation of a malaria control intervention, which was not seen in the area outside the mining activities.

P705: Public health impact of RTS,S malaria vaccine candidate in children estimated using a Markov cohort model and Phase 3 trial results

Christophe Sauboin1, Ilse Van Vlaenderen2, Laure-Anne Van Bellinghen2, Baudouin Standaert1
1GlaxoSmithKline Vaccines; 2CHESS In Health for GlaxoSmithKline Vaccines

BACKGROUND: Efficacy data from a Phase 3 trial of the RTS,S candidate malaria vaccine candidate have been published1, 2. We estimated the potential public health impact of the vaccine based on these results.

METHODS: Our static Markov cohort model used three categories of transmission intensity (Malaria Atlas Project definitions: Low, parasite prevalence [PfPp] ≤5%; Moderate, PfPp 5–40%; High, PfPp>40%). Malaria incidence without vaccination in each category was estimated using data from the Phase 3 trial and a concurrent malaria transmission intensity study. Natural immunity was assumed to increase with successive infections. Vaccination was assumed to influence only infection risk. Two vaccination strategies were considered: doses at 6, 10 and 14 weeks; or at 6, 7.5 and 9 months. Data on vaccine efficacy (VE) against clinical malaria came from the Phase 3 trial. VE was assumed to decline over time. The 2017 birth cohort in each country was divided between no malaria risk and the three PfPp categories and followed to age 5 years. Vaccination was applied using diphtheria-tetanus-pertussis-3 vaccine coverage with 25% reduction for the second strategy.
RESULTS: The table shows the estimated number of malaria cases/deaths without vaccination and those averted by each vaccine strategy.

<table>
<thead>
<tr>
<th>Country</th>
<th>Birth cohort</th>
<th>Without vaccination</th>
<th>Vaccination at 6, 10 Vaccination at 6, and 14 weeks</th>
<th>7.5 and 9 months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases</td>
<td>Deaths</td>
<td>Cases</td>
<td>Deaths</td>
</tr>
<tr>
<td>Burkina Faso</td>
<td>839,221</td>
<td>8,882,027</td>
<td>32,781</td>
<td>1,007,646</td>
</tr>
<tr>
<td>Democratic</td>
<td>3,143,100</td>
<td>22,613,484</td>
<td>98,731</td>
<td>2,170,912</td>
</tr>
<tr>
<td>Republic Congo</td>
<td>810,886</td>
<td>5,276,364</td>
<td>26,773</td>
<td>722,225</td>
</tr>
<tr>
<td>Ghana</td>
<td>1,704,467</td>
<td>2,617,689</td>
<td>18,310</td>
<td>384,316</td>
</tr>
<tr>
<td>Nigeria</td>
<td>7,250,554</td>
<td>63,845,839</td>
<td>266,826</td>
<td>4,020,086</td>
</tr>
<tr>
<td>Tanzania</td>
<td>2,252,093</td>
<td>7,142,537</td>
<td>46,274</td>
<td>1,091,608</td>
</tr>
<tr>
<td>Global Alliance</td>
<td>33,381,206</td>
<td>192,457,809</td>
<td>886,514</td>
<td>18,038,516</td>
</tr>
</tbody>
</table>

CONCLUSION: Vaccination would be expected to reduce malaria cases/deaths. The model has limitations, e.g. it does not account for herd effects, seasonality or heterogeneity. It is a simplified tool to help improve understanding of the public health impact of the RTS,S candidate vaccine.


P706: Neonatal malaria: In vitro assessment of fetal haemoglobin and serum on growth rates of Plasmodium Falciparum

Ulrich Sauerzopf1, Ghyslain Momo-NGoma1, Jean-Rodolphe Mackanga1, Benjamin Mordmüller1,2, Michael Ramharter3,4
1Centre de Recherche Médicale Lamboréné, Albert Schweitzer Hospital Gabon; 2Dept. Of Medicine I, Div. of Infectious Diseases and Tropical Medicine, Medizinische Universität Wien; 3Institut für Tropenmedizin, Universität Tübingen

BACKGROUND: The epidemiological observation of low incidence of malaria in children below the age of 6 months is commonly attributed to fetal hemoglobin providing a certain level of protection against P. falciparum infection. This study aimed at investigating the effect of HbF on in vitro parasite growth as well as, a growth inhibitory effect of antibodies persisting in the neonatal circulation on P. falciparum.

METHODS: Blood specimens were taken from umbilical cords, as well as from the peripheral maternal blood. The anti-coagulated blood specimen were split into a erythrocyte and plasma fraction which were separately put into culture in a complete RPMI 1640 based parasite medium. In vitro growth was compared between maternal and child blood components and with non-malaria exposed donors. Growth assays were performed using standard techniques and HRP2 assay.

RESULTS: Use of maternal and fetal erythrocytes in in vitro culture led to lower HRP2 levels than for non-malaria exposed donor erythrocytes. Fetal erythrocyte cultures showed however a trend towards higher growth rates than maternal erythrocyte cultures. Standard in vitro culture supplemented with maternal and fetal serum showed significantly lower growth rates than non-malaria exposed serum.

CONCLUSIONS: These data challenge the current believe that young children are protected from malaria infection by the presence of fetal haemoglobin. Fetal haemoglobin is perfectly suitable for parasite growth. Maternal antibodies may play a role in prevention of disease, however further studies are needed to elucidate the pathophysiology of innate and acquired protection against neonatal malaria.

P707: Targeting male mosquito behaviour for malaria control

Sawadogo S. P.1, Niang A.1, Maiga H.1, Dabiré K. R.1, Triplet F.2, and Diabaté A.1
1IRSS/Centre Muraz, Bobo-Dioulasso, Burkina Faso; 2University of Keele, United Kingdom

BACKGROUND: Following the emergence of insecticides resistance used for malaria vectors control, a new tool to control malaria is to reduce the high reproductive rate of mosquitoes by mass killing of males during swarming. This strategy requires a proper understanding of mosquito swarming and mating behaviour in the field. The goals of this study are to characterize the distribution of swarms in time and space, to assess the feasibility of attracting and mass killing males within swarms and to measure the impact of swarm control in association with Insecticide Residual Spray approach on local density.

METHODS: The swarming and mating systems of natural populations of An. gambiae were investigated through longitudinal surveys conducted between July to November 2012 in Vallée du Kou (VKS), a rural areas of south-western Burkina Faso. In the study village, the presence or absence of swarms, their numbers, frequency of occurrence and size were recorded and correlated with the ecological variables such as human density, presence of refuges for males, availability and distribution of discrete swarm markers, sources of nectar, major breeding sites. All swarming place were geo-referenced in order to map the swarming location.

PRELIMINARY RESULTS: Swarms of Anopheles gambiae were observed within the villages regularly to the same site associated with visual markers. The distribution of swarms in the villages was not uniform but clustered and seemed to correlate with the distribution and density of visual markers. The peak of the swarms number and density was observed between August and September and is correlated with the density of mosquitoes in the village.

CONCLUSION: Our preliminary results showed a good correlation between the distribution of swarms, the density and distribution of visual markers in Vallée du Kou (VKS).

P708: Malaria Transmission after Artemether-Lumefantrine and Dihydroartemisinin-piperazine ? a randomized trial.

Patrick Sawa

BACKGROUND: Artemisinin-combination therapy (ACT) reduces malaria transmission potential compared to non-ACT treatment; it is unclear whether this effect differs between ACTs.

METHODS: 298 children (6 m-10y) with uncomplicated falciparum malaria were randomized to artemether-lumefantrine (AL, n=153) or dihydroartemisinin-piperazine (DP, n=145) in Mbita, western Kenya. Gametocyte carriage was determined by molecular methods at days 0, 1, 2, 3, 7, 14, 28 and 42. Infectiousness to mosquitoes was determined by mosquito feeding assays on day 7.

RESULTS: The cumulative risk of recurrent parasitemia on day 42 after initiation of treatment, unadjusted by PCR, was 20.7% (95% CI 14.4-28.2) for AL compared to 3.7% (95% CI 1.2 -8.5) for DP, P<.001. The mean duration of gametocyte carriage was 5.5 days (95% confidence interval 3.6-8.5) for AL and 15.3 days (95% CI 9.7-24.2) for DP (P<.001). The proportion of mosquitoes that became infected after feeding on blood from AL treated children was 1.88% (43/2293) compared to 3.50% (83/2371) for DP (P = .06); oocyst burden was lower after AL (P=.005)

CONCLUSIONS: While DP is associated with a longer prophylactic
time after treatment, gametocyte carriage and malaria transmission to mosquitos are lower after AL.

**P709: Community-based scheduled screening and treatment of malaria in pregnancy for improved maternal and infant health: a cluster-randomized trial**

Pètra F. Mens & Henk D. F. H. Schallig

On behalf of the COSMIC consortium at Royal Tropical Institute, Parasitology Unit, Meibergdreef 39, 1105 AZ Amsterdam, The Netherlands A EU funded interdisciplinary research consortium. COSMIC comprising partners from Medical Research Council (The Gambia), Centre de Recherches Entomologiques de Cotonou (Benin), Centre Muraz (Burkina Faso), Institute of Tropical Medicine (Belgium), Imperial College (UK), WHO - Special Programme for Research and Training in Tropical Diseases and Royal Tropical Institute (Netherlands) has recently started its activities. The consortium aims at evaluating scheduled intermittent screening of pregnant women with rapid diagnostic tests (RDTs) by Community Health workers (CHW) at community level and treat positive women with anti-malarial treatment (SST). CHWs will also encourage pregnant women to attend antenatal clinics (ANC) for other pregnancy-targeted interventions such as intermittent preventive treatment with sulphadoxine-pyrimethamine (IPTp/SP), thereby improving its coverage. This approach combines existing IPTp/SP with SST at village level as an extension of community based case management of malaria (CCMm) . This simple (diagnosis by RDTs) and low cost intervention capitalizes on an already existing interventions (CCMm) to improve maternal and newborn health. The main project objectives are: 1) to identify bottlenecks for implementation of SST by CHW involved in CCMm; 2) to determine the impact of introducing SST in pregnancy on the quality of CCMm; 3) to determine the impact of SST on ANC attendance and IPTp/SP coverage; 4) to determine the impact of SST on low birth weight, anaemia and placenta malaria; 5) to estimate cost-effectiveness of the intervention, and 6) to formulate recommendations for possible implementation.

**P710: Malaria – visceral leishmaniasis co-infections in East Africa**

Erika van den Bogaart, Marieke Berkhout, Pètra Mens, Emily Adams, François Chappuis, Koert Ritmeiter, Bakri Nour, Henk Schallig

**BACKGROUND:** Due to geographic overlap of malaria and visceral leishmaniasis (VL), co-infections may exist but have been poorly investigated.

**METHODS:** Two studies have been undertaken to further gather data on malaria-VL co-infections in East Africa. To describe prevalence, features and risk factors for VL-malaria co-infections, a case-control analysis was conducted on data collected at Amudat Hospital, Uganda by Médecins sans Frontières. A second retrospective study was performed using medical records of VL patients admitted to Tabarakallah and Gedafir Teaching Hospitals (Gedarif State) and Al`Azaza kala-azar Clinic (Sennar State), Sudan.

**RESULTS:** Two studies have been undertaken to further gather data on malaria-VL co-infections in East Africa. To describe prevalence, features and risk factors for VL-malaria co-infections, a case-control analysis was conducted on data collected at Amudat Hospital, Uganda by Médecins sans Frontières. A second retrospective study was performed using medical records of VL patients admitted to Tabarakallah and Gedafir Teaching Hospitals (Gedarif State) and Al`Azaza kala-azar Clinic (Sennar State), Sudan.

**CONCLUSION:** Co-infections do occur in endemic VL areas with malaria transmission, indicating that routine screening of VL patients living in malaria endemic-areas and close monitoring of co-infected patients should be implemented.

**P711: Quality of malaria case management among children under five years at lower level health facilities in Tororo district, Uganda.**

**Schallig**

**BACKGROUND:** Early diagnosis and prompt effective treatment of cases is a key malaria control strategy. Effective case management entails quality in diagnosis and treatment of cases. We assessed the quality of malaria case management among children under the age of five years at lower level health facilities in Tororo district, Uganda.

**METHODS:** The study was conducted at 3 level IV and six level III public health facilities. Health workers were assessed while managing 384 children with suspected malaria. Quality of malaria case management was assessed against the national guidelines. Caretakers’ attitudes about the quality of malaria case management and their understanding of instructions given by health workers were assessed at exit interviews. Key informant interviews were conducted with 8 health facility heads. A composite index was utilized to determine the optimal quality of malaria case management and its predictors.

**RESULTS:** Clinicians took adequate history of the sickness in 75.3% (289/384) of the cases. Temperature and weight were measured in 57.3% (220/384) and 13.0% (50/384) of the cases respectively. Parasitological diagnosis was performed in 48.4% (186/384) of the cases. Majority 82.6% (317/384) of the children were prescribed an ACT for malaria. Quality of care was optimal in 46.6 % of the cases. Optimal care was significantly associated with supervision of health workers in the last 6 months prior to the survey (AOR=4.7; 95% CI 2.0 - 10.9), adequate understanding of instructions by caretakers (AOR=5.6; 95% CI 2.1 - 14.9), getting care from a level IV health facility (AOR=6.3; 95% CI 3.8 - 10.2) and treating older children (AOR= 2.5; 95% CI 1.3 - 4.8).

**CONCLUSION:** Quality of malaria case management among children under five years was largely suboptimal. Quality of care was influenced by training and supervision of health workers, adequacy of infrastructure at the facilities and health system factors. The Ministry of Health and district leaders should ensure continuous training and supervision of health workers and provide the required infrastructure to improve quality of malaria case management.

**P712: Identifying the malaria hotspots in South Africa - an important precursor for elimination**

1M Hlatshwayo, 2M Blom, 1S Swanevi, 3E Misiani, 1A Mabuza, 1D Chetty, 2D Kruger, 1J Nawn, 1M Groep, 1E Rossiw, 1j Kleinschmidt, 1D Moonsaz

1National Department of Health, Pretoria, South Africa; 2Malaria Unit, National Department of Health, Pretoria, South Africa; 3Malaria Unit, Medical Research Council, Durban, South Africa; 4Provincial Department of Health, Mopumalanga, South Africa; 5Malaria Unit, Provincial Department of Health, Limpopo, South Africa; 6Provincial Department of Health, Kwa-Zulu Natal, South Africa; 7World Health Organization Pretoria, South Africa; 8London School of Hygiene and Tropical Medicine, London, United Kingdom

**BACKGROUND:** Local malaria transmission occurs in three provinces of South Africa, namely Limpopo, Mopumalanga and Kwa-Zulu Natal. Through the robust implementation of multiple interventions and cross-border collaboration with neighbouring countries, South Africa has significantly reduced malaria cases and embarked on a goal of reducing local cases to
zero by the year 2018. While the country prepares for elimination, it is crucial that malaria hotspots and transmission foci are accurately located and classified.

OBJECTIVE: To evaluate the trends of local malaria cases in South Africa between 2010/2011 and 2012/2013 malaria seasons and determine the location of malaria hotspots in the country.

METHODS: Secondary data on local malaria cases was analyzed to identify and stratify malaria hotspots by municipality and localities. The hotspots in each locality were mapped using GIS.

RESULTS: There has been a reduction in the local malaria incidence (39% decrease from 0.7 to 0.27 per 1000 population at risk during the malaria seasons 2010/2011 to 2012/2013) since South Africa embarked on reorientation towards elimination of malaria in the year 2010. The reduction of local cases was more prominent in Limpopo province (64% decrease, 2740 in 2010/11 to 995 cases in 2012/2013) compared to KZN (56%, 100 cases to 44) and Mpumalanga (50% decrease). Eight (8) municipalities in Limpopo and Mpumalanga reported more than 100 local cases per year. Further analysis of the local cases in these high burden municipalities indicated that reduction of specific hotspots at locality level (five (5) cases or more per annum), was not significant in provinces like Mpmulangala where the number of such localities only reduced from 12 to 11 localities.

CONCLUSION: Despite the overall reduction of local malaria cases, there was no corresponding reduction in the number of hotspot areas due to the emergence of new hotspots. Malaria elimination in South Africa will depend on reduction of malaria hotspots through increased surveillance and multiple targeted interventions in the most affected districts, such as Vhembe, Mopani and Ehlanzeni. Vector data will be incorporated to these results for mapping of foci.

P713: Acquisition of antibodies to merozoite surface protein 3 among residents of Korogwe, north eastern Tanzania.

Segeja MD, Mmbando BP, Seth MD, Lusingu JP, Shahi M, Seth MD, Mmbando BP, Lusingu JP, Lemnge MM.
National Institute for Medical Research, Tanga Medical Research Centre, Tanga, Tanzania.

BACKGROUND: A polymorphic malaria parasite antigen, merozoite surface protein 3 (MSP3), is among the blood stage malaria vaccine candidates. It is believed to induce immunity through cytophilic antibodies that disrupt the process of erythrocytes invasion by merozoites. This study aimed at assessing natural acquisition of antibodies to MSP3 in individuals living in an area with different malaria transmission intensity in preparation for malaria vaccine trials.

METHODS: The study was conducted in individuals aged 0-19 years from villages located in lowland, intermediate and highland strata in Korogwe district, northeastern Tanzania. Blood samples from 492 study participants were collected for malaria diagnosis and immunological investigations. Reactivity of MSP3 to different types of antibodies (immunoglobulin M, G and IgG subclass 1 and 3) were analysed by Enzyme Linked ImmunoSorbent Assay (ELISA).

RESULTS: Malaria parasite prevalence was higher in the lowland (50%) compared to the intermediate (23.1%) and highland (9.8%) strata. Immunoglobulin G subclasses 1 and 3 (IgG1 & IgG3), total IgG and IgM were found to increase with increasing age. IgG3 levels were significantly higher than IgG1 (p < 0.001). Furthermore, Plasmodium falcioparum infection was associated with higher IgG3 levels (p = 0.008). Adjusting by strata and age in individuals who had positive blood smears, both IgG and IgM were associated with parasite density, whereby IgG levels decreased by 0.227 (95%CI: 0.064 - 0.391; p = 0.007) while IgM levels decreased by 0.165 (95%CI: 0.044 - 0.286; p = 0.008).

CONCLUSION: Individuals with higher levels of IgG3 might be partially protected from malaria infection. Higher levels of total IgG and IgM in highlands might be due to low exposure to malaria infection, recent infection or presence of cross-reactive antigens. Further studies of longitudinal nature are recommended. Data obtained from this study were used in selection of one village (Kwashemshi) for conducting MSP3 phase 1b malaria vaccine trial in Korogwe district, northeastern Tanzania.

P714: Ownership and access to mobile phones in rural Malawi: a new channel for communicating health messages?

Monica Shabu1, Kim A. Lindblade2, Dycon Mwandama2, Laura Steinhardt1, Adam Wolkon1, Andy Bauleni3, Exton Mtande2, and Don P. Mathanga2
1Centers for Disease Control and Prevention, Division of Parasitic Diseases and Malaria, Malaria Branch, Atlanta, Georgia, USA; 2University of Malawi College of Medicine, Malaria Alert Center, Blantyre, Malawi

BACKGROUND: Mobile phone access in sub-Saharan Africa has expanded rapidly in recent years, but dissemination of health information via mobile phones is limited.

METHODS: To explore the feasibility of using mobile phones to communicate health information, we conducted a household census, as part of a larger malaria study, in six rural villages in Machinga District, Malawi in 2012. Residents were asked about access to and household ownership of mobile phones, factors related to ownership and their extent of text messaging.

RESULTS: We censused 2657 households, of which 2430 (91.5%) had an eligible respondent who consented to participate. Respondents were mostly female (76%) and had a median age of 33 years. Of the households surveyed, 42% owned at least one mobile phone and 4% owned more than one phone. In households without a phone, a majority (59%) knew a neighbor with a phone and almost half (46%) received personal messages from the neighbor’s phone. Most respondents from households with phones had attended primary school or above (73%), could sign their own name (70%) and many could correctly read a short text message in Chichewa, Chiyao, or English (53%). Household mobile phones were used for sending (39%), receiving (69%) and reading (46%) text messages, which contained personal messages and advertisements. Only 7% of households received health information text messages in the week prior to the interview. Mobile phone ownership was significantly associated with ability to read a text message (OR 2.2; 95% CI 1.8-2.6), increased household size (OR 1.3; 95% CI 1.2-1.4), bednet ownership (OR 2.1; 95% CI 1.6-2.8) and was negatively associated with having children under the age of five years (OR 0.6; 95% CI 0.5-0.7).

CONCLUSIONS: Household mobile phone ownership is approaching the same level of coverage as radios (55%) in our study area and phones should be explored as a communication route for behavior change messages. Additional studies are needed to compare sociodemographic factors related to ownership of mobile phones and to determine whether text or voice messages would be the most effective method for disseminating information through mobile phones in rural populations.

P715: Unpacking untraceable malaria cases in South Africa in view of elimination.

Bridget M Shandukani1, Eunice A Misiani1, Mary A Groepe1, Ntsieni Ramalwa1, Eric Raswiswi2, Philip Kruger2, Immo Kleinschmidt3 and D Moonasar1
1National Department of Health, Pretoria, South Africa; 2Provincial Departments of Health, KwaZulu-Natal, Limpopo, South Africa; 3South Africa Country Office, World Health Organization, Pretoria, South Afric; 4London School of Hygiene and Tropical and Medicine, London, United Kingdom

INTRODUCTION: To monitor progress towards malaria elimination, it is crucial to be able to distinguish between locally acquired and imported malaria. WHO (World Health Organization) recommends that countries in the elimination phase should have sufficient resources to investigate each case to ascertain whether it is imported or locally acquired and undertake appropriate control measures. South Africa has been reporting cases as local and imported cases for the past three years; however despite efforts to classify cases into these categories, there remains a large group of cases that are untraceable and whose origin is therefore unknown. These unclassifiable cases constitute a large proportion of all cases. The reasons for being unable to trace cases are related to population migration and reluctance to divulge travel
history. In this study we investigated the factors that are associated with being unable to trace cases in Limpopo and KwaZulu-Natal, and discuss the impact this problem has on the progress towards malaria elimination.

**MATERIALS AND METHODS:** A retrospective descriptive analysis was conducted on malaria data collected in Limpopo and KwaZulu-Natal from the period 2010-2012. Classified and unclassified cases were stratified by age, gender, geographical distributions.

**RESULTS:** Within the study period, there were 1464 malaria cases in KwaZulu-Natal and 9683 cases in Limpopo province. Of these 407 (4.2%) were reported as untraceable in Limpopo, of which 143 (35.1%) were females. The health districts with the significant number of untraceable cases were Vhembe 244 (59.9%) and Mopani 154 (37.8%). KwaZulu-Natal reported 441 (30%) untraceable cases, with 125 (29.3%) female whilst Ethekwini health district reported 361 (81.9%) untraceable cases. Data categorised by age and state of pregnancy were sparse for the untraceable cases.

**CONCLUSIONS:** The number of untraceable malaria cases is a significant challenge to the malaria elimination program as this hinders the true representation of progress made. Improving surveillance and case investigation systems in KwaZulu-Natal and Limpopo province is vital for accurately monitoring of progress towards the elimination goal. Strategies such as administration of case investigation form at the point of care so as to obtain source of infection information and training of malaria programme personnel is essential in improving data quality and proper classification of malaria cases.

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**P716: Clinical outcome and antibiotic prescription rate using a new Algorithm running on mobile technology for the Management of Childhood Illnesses (ALMANACH) in Tanzania**

Amani Shagi1,2, Clotilde Rambaud-Althaus1,3, Seneca Perri1, Nendria Swali1, Judith Kahama-Maro1, Marc Mitchell1, Blaise Genton1,2, Valerie D’Acremont1,4

1Swiss Tropical and Public Health Institute, Basel, Switzerland; 2University of Basel, Basel, Switzerland; 3National Institute for Medical Research, Dar es Salaam, Tanzania; 4Harvard School of Public Health, Boston, USA.

**INTRODUCTION:** The decline of malaria and use of malaria rapid diagnostic tests (mRDT) worsen overprescription of antibiotics. This, together with new evidence from etiology of fever studies, calls for revision of the IMCI strategy. A new algorithm (ALMANACH) running on mobile technology was developed based on i) results from a study on etiologies of fever in children, ii) systematic review of published studies on clinical and laboratory predictors of febrile diseases, iii) international IMCI expert opinions. The aim of this study was to assess health outcome and rate of antibiotic prescription using ALMANACH in urban and rural areas of Tanzania.

**METHODS:** In this controlled non-inferiority study, enrolled children aged 2-59 months with an acute illness were managed by study clinicians using ALMANACH [2 intervention health facilities (HF)], or clinicians using standard practice, including mRDT (2 control HF). At day 7 (D7) all patients were reassessed. Patients ill in-between or not cured at D7 were followed until recovery or death. Primary outcomes were proportion of children cured on D7 and of children who received antibiotics on D0. The secondary outcome was proportion of children admitted secondarily or who died.

**RESULTS:** 1465 children were included. 131/842 (15.6%) in ALMANACH and 241/623 (38.7%) in control arm were diagnosed as having an infection to be treated with antibiotics. Malaria prevalence was 4% and 9% respectively. 815/838 (97.3%; 96.1-98.0%) were cured at D7 with ALMANACH versus 573/623 (92.0%; 89.8-94.1%) with standard practice (p<0.001). Of 23 children not cured at D7 with ALMANACH, 44% had been diagnosed with skin infection at D0, 30% pneumonia, 26% upper respiratory tract infection and 13% likely viral infection. Only one of them had developed pneumonia at D7. Secondary hospitalization occurred for one child in ALMANACH and two children (one died) in controls. At D0, antibiotics were prescribed for 15.7% (13.2-18.1%) of the children in ALMANACH versus 84.3% (81.4-87.1%) in controls (p<0.001).

**CONCLUSION:** Management of children with ALMANACH led to a better clinical outcome than standard practice and to 80% reduction in antibiotic prescription. This was due to more accurate diagnoses and thus better identification of whether children need antibiotic treatment.

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**P717: Drug design through molecular modeling studies for exploring novel drug targets in Plasmodium falciparum to combat drug resistance**

Deepika Bhaskar1, Ravikant Sharma2

1Dept. of Biochemistry, Shivaji College, University of Delhi, India; 2NCD Division, Indian Council of Medical Research, New Delhi, India

**BACKGROUND:** Malaria is a major threat for public health, especially in the tropical and sub-tropical countries. The biology of this parasite is well understood with the help of genomics and proteomics projects and has aided identification of new molecular targets that could be used to aid rational design of drugs or vaccines. Drug resistance acquired by the malarial parasites is a major problem in the treatment and control of malaria.

**METHODS:** Dihydropterin synthetase- pyrophosphokinase (DHPS-pppk) which catalyse the synthesis of dihydropterin from condensation reaction of para aminobenzoic acid and 6-hydroxymethyl-7,8-dihydropteridine and Dihydrofolate reductase (DHFR) in Pf (Pf DHFR-TS; TS refers to thymidylate synthase, bound to DHFR in Pf), which catalyzes the reduction of dihydrofolate to tetrahydrofolate, is one of the most widely studied enzymes in antimalarial drug design due to its potential role in DNA synthesis. DHPS and DHFR is also considered to be a good target for other protozoal diseases. Some of the resistance-causing mutations include single A437G, double S436A+ A437G or A437G+S581G, triple mutant S436A+ A437G+K540E or S436A+ A437G+S581G in DHPS and single S108N, double S59R+S108N, triple N51I+S59R+S108N, S59R+S108N+N164L and quadruple N51I+S59R+S108N+N164L in DHFR.

**RESULTS:** Currently there are four crystal structures of protozoal DHFR available in the Protein Data Bank, including the wild type PfDHFR-TS complexed with triazine inhibitor WR99210, a double mutant PfDHFR-TS complexed with pyrimethamine, a quadruple mutant PfDHFR-TS complexed with WR99210 and wild type Plasmodium vivax DHFR-TS complexed with pyrimethamine. Similarly computational molecular binding studies of sulfadoxine with PDDHPS has also been reported.

**CONCLUSION:** To overcome the resistance against presently used drugs, computer aided programmes could be employed for developing and designing of new drugs. The binding studies of new compounds with other protozoal DHFR proteins, in particular with PfDHFR, could be of importance in the future drug design efforts.

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**P718: DDT and its Epidemiological impact of Malaria and Visceral leishmanisis in India**

R.S.Sharma, A.P.Dash* and L.S.Chuahan

National Centre for Disease Control

India’s population suffers from a significant disease burden from vector borne diseases, in the form of morbidity and mortality from malaria, kala-azar (VL), filariasis, Japanese encephalitis, and dengue. To address this burden, the National Health Policy (2002) set a goal of reducing malaria, dengue and Japanese encephalitis mortality by 50 percent by 2010; eliminating kala-azar by 2010 which is now revised to 2015; and eliminating lymphatic filariasis by 2015. Malaria in India is now particularly entrenched in low-income rural areas of eastern and north-
eastern states, but important foci are also present in the more arid western parts of the country, for example near agricultural development projects and in many urban areas. Experience from recent years shows that well-targeted and effective preventive and curative interventions can lead to significant reductions in the burden. Besides treatment of malaria cases by appropriate drugs, Indoor residual spray by DDT, Malathion and Synthetic Pyrethroids are used for vector control in Malaria and Kala-azar in rural areas. In urban areas larvicides are introduced in water bodies for control of larvae population and space spray with Pyrethrum is done in urban areas during outbreak. In some areas, where settlements are scattered and it is not feasible to undertake IRS due to difficult terrain, Insecticide treated mosquito nets (ITNs)/ Long Lasting Insecticide Nets (LLINs) are used. It is a well known fact that chemical insecticides are toxic to human beings. If not used as per recommendations, they cause adverse health impact to the persons involved, community and contamination of environment. Kala-azar (leishmaniasis) is found in 88 countries & about 350 million people are at risk. About 0.5 million clinical cases of kala-azar are reported every year with 60,000 deaths annually and 90 percent of the cases are found in India, Bangladesh, Nepal, Sudan and Brazil (WHO) . In India kala-azar is endemic in eastern States of India. It is estimated 165.4 million population at risk in 4 states. Mostly poor socio-economic group of population primarily living in rural areas are affected. As per WHO guidelines, by using DDT as IRS, India has reduced the disease incidence by 60 percent since 1992. One of the major programme strategies of India Government for elimination of kala-azar is vector control through IRS with DDT up to 6 ft height from the ground twice annually.

### P719: Population genetics of *Plasmodium falciparum* in the Arabian Peninsula and prospect of malaria elimination

Salama Al-Hamidi1, Mohammed A.K. Mahdy2, Saad M. Bin Dajem4, Adel Ali H. Al-Sheikh2, Zainab Al-Hashami3, Hissa Al-Farsi1, Abdulsalam M. Almekhlafi4, Mohamed Ahmed Idris6, Albano Beja-Pareira6 and Hamza A. Babiker4

1 Department of Biochemistry, Faculty of Medicine and Health Sciences, Sultan Qaboos University, Oman; 2 Department of Microbiology and Immunology, Faculty of Medicine and Health Sciences, Sultan Qaboos University, Oman; 3 Department of Parasitology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia; 4 Biology Department, College of Science, King Khalid University, Abha, Saudi Arabia; 5 National Centre for Training and Research, MOH, Jazan, Saudi Arabia; 6 Department of Parasitology, Faculty of Medicine, Sana’a University, Sana’a, Yemen; 7 Research Centre in Biodiversity and Genetic Resources (CIBIO), University of Porto, Rua Padre Armando Quintas 7, Vairaõ 4485-661, Portugal

**BACKGROUND:** Malaria control efforts in the Arabian Peninsula have been boosted by political commitment and increased funding of governments of the Gulf States. Consequently, the burden of the disease is being reduced dramatically and transmission has been interrupted in a number of countries throughout the region. However, malaria remains endemic in limited sites, in Yemen and southwest of Saudi Arabia. In addition to local transmission, imported malaria sustains an extra source of parasites. The present study examined genetic diversity of *Plasmodium falciparum* parasites in Yemen, and Saudi Arabia to elucidate parasite structure, and how the current control efforts is reflected on parasite diversity and its structure.

**METHODS:** Ten microsatellites were genotyped in 108 *P. falciparum* isolates collected in three sites in Yemen (Taiz, Dhamar and Hodeidah) and 203 samples from Saudi Arabia (Jazan). All samples were collected from confirmed *P. falciparum* cases in 2008. All isolates were types for 10 putative neutral microsatellites located on 6 different chromosomes.

**RESULTS:** All of the examined microsatellites were found to be highly polymorphic in all sites in Yemen and Jazan (Saudi Arabia). Allelic diversity at each locus, summarized as unbiased heterozygosity (He) from the distribution of allele frequencies, revealed higher levels of genetic diversity among parasites in Hodeida (He = 0.515 ) and Taiz from (He = 0.66 ) than from Dhamar (He = 0.481 ). In Jazan (Saudi Arabia), the level of genetic diversity (He = 0.76) was higher than among parasites in Yemen. Most microsatellites were distributed widely across different populations, and ‘private’ alleles (detected only in one population) were at very low frequencies. Pairwise comparisons of populations showed that Yemen populations, Taiz and Hodeidah display low population differentiation values Fst (0.074). The three population can probably be considered as one population. However, parasites in Taiz was among the Yemen parasites that are most closer to Jazan (Saudi Arabia), followed by Hodeidah. Dhamar is clearly a distinct population.

**CONCLUSION:** Although current control efforts have reduced risk of malaria in Saudi Arabia, the extent of parasite diversity and genetic structure was similar to that seen in Yemen where malaria transmission is high. The current control efforts should consider strategies to curb flow of imported malaria into the region.

### P720: Enhancing malaria and syphilis diagnosis and surveillance in pregnancy through portable devices in Geita district, Tanzania

Shekalaghe, S1; Mazigo, H2; Abdulla S3; Changalucha, J4; Ferro, S5

1 IFAKARA Health Institute, Bagamoyo, Tanzania; 2 Catholic University of Health and Allied Sciences, Mwanza, Tanzania; 3 National Institute of Medical Research, Mwanza, Tanzania; 4 Fio Corporation, Toronto, Ontario, Canada

**INTRODUCTION:** Malaria and syphilis surveillance in Antenatal care (ANC) is a public health priority. This activity is limited by geographical access, low diagnosis offer in the rural context, and delayed and unreliable data availability to public health officials. Fio Corporation has developed a technology designed and validated to address these issues with a portable device that integrates automated interpretation of RDTs with cell phone network and cloud information systems (the Fionet System), to improve diagnosis accuracy and to provide close to real-time high quality data availability. In association with NIMR, we have tested the feasibility of Fionet in ANC clinics in the Geita District, Tanzania.

**MATERIALS AND METHODS:** Eight dispensaries and two health centers in a densely populated area of the Lake Victoria region were selected to participate in this pilot. Health Care Workers (HCWs) at these locations received training in a 2-day workshop to operate Deki Reader™ and process RDTs. Data collection forms were adopted in the software of the device. RDT images were captured and transmitted to the portal for QC purposes. HCWs also filled out the paper based form and read the RDTs for case management. The follow up was performed using Spirii™ (a web based data management solution). Two evaluations were conducted with CHWs, information system staff and decision makers on the utility of the Fionet System.

**RESULTS:** All 10 dispensaries and health centers have reported malaria test results and syphilis test results in ANC population. Tendencies of prevalence of the diseases have been confirmed using RDTs and automated reported system. The data collection was complete and accurate and included geo-location and date and time. For the first time in the country negative diagnosis events were reported along with the positive cases, providing a denominator to the National Malaria Program, and STD program performance indicators. System users (community health workers, information system staff and decision makers) were satisfied with the benefits and results of the system.

**DISCUSSION AND CONCLUSION:** ANC programs can greatly benefit of the new technologies such as the one tested in the present study, to improve on surveillance of important disease, compliance with guidelines for treatment of patients and contacts, monitoring quality and overall performance of the program.
P721: Evidence for a direct interaction between *Plasmodium falciparum* Hsp70 (PfHsp70) and the Hsp70-Hsp90 organising protein (PfHop)

Grace W. Gitau1, James Njunge2, Earl Prinsloo3, Addmore Shonhai4
1Department of Biochemistry & Microbiology, University of Zululand, P. Bag X1001, KwaDlangezwa, 3886, South Africa; 2Department of Biochemistry, Microbiology & Biotechnology, Rhodes University, Grahamstown, South Africa, 6140

**BACKGROUND:** Heat shock proteins (Hsps) play an important role in the cytoprotection and pathogenicity of the main agent of malaria, *Plasmodium falciparum*. Hsp70 (PfHsp70) and PfHsp90 are stress-inducible molecular chaperones that are essential for parasite survival. The coordinated function of these two proteins is thought to be crucial for the folding of molecules such as steroid hormone receptors and kinases. In other eukaryotic cells, the coordinated function of Hsp70 and Hsp90 is facilitated by an adaptor protein known as Hsp70-Hsp90 organising protein (Hop). Hop brings Hsp70 and Hsp90 together in order to facilitate exchange of peptide substrates from Hsp70 to Hsp90. We previously demonstrated that PfHop associates with both PfHsp70 and PfHsp90 in the parasite cytosol. In the current study, we investigated the direct interaction between PfHop and PfHsp70.

**METHODS:** Recombinant PfHop and PfHsp70 were expressed in *E. coli* XL1 blue cells. The proteins were expressed with N-terminal polyhistidine tags to facilitate their purification by Nickel affinity chromatography. Surface Plasmon Resonance (SPR) and Far-Western analyses were employed to investigate the interaction between the two proteins. The stress-inducible expression of PfHop was examined in *P. falciparum* (3D7) cells at the trophozoite stage. After heat shock, infected erythrocytes were washed in PBS and the erythrocyte membranes lysed in PBS containing saponin 0.1% (w/v). Parasites were collected by centrifugation at 5000 rpm for 4 min followed by extensive washing step with PBS and then analysed by Western blotting. In addition, the expression of both PfHop and PfHsp70 was investigated at the various intraerythrocytic stages of the parasite.

**RESULTS:** Based on SPR and Far-Western analyses PfHop interacts with PfHsp70 through the C-terminal domain of the latter. This is the first evidence suggesting that PfHop may indeed coordinate the function of the PfHsp70-PfHsp90 protein folding pathway. In addition, PfHop was found to be heat-inducible and its expression at various intraerythrocytic stages of the *P. falciparum* mirrored that of PfHsp70.

**CONCLUSIONS:** Based on sequence alignment data, plasmodial Hop mirrored that of PfHsp70. This result suggests that PfHop may indeed coordinate the function of the PfHsp70-PfHsp90 protein folding pathway. PfHop is a potential candidate for the development of new antimalarial drugs.

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P723: Assessing malaria burden during pregnancy in Fana, Mali.

Malaria Research and Training Center, University of Bamako, BP 1805 Bamako, Mali

**INTRODUCTION:** Malaria in the tropics is a public health problem among children under five years and pregnant women. The aim of the study was to assess the level of infection among pregnant women in Fana, Mali.

**MATERIALS AND METHODS:** With the technique of “Rapid Assessment” we conducted a cross-sectional study from November 2005 to February 2006 at the Centre for Health Reference of Fana. The thick film and the determination of hemoglobin were diagnostic methods used. The study involved 200 women in antenatal clinics and 200 women in childbirth.

**RESULTS:** At the ANC, anemia (74.37%) was not associated with gravidity (p = 0.112). Peripheral infection (25.13%) was associated with age (p = 0.001) and residence of women (p = 0.005). The primigravida and secondisgestes were more susceptible to malaria (p = 0.002). At delivery, peripheral infection (16, 50%) was associated with gravidity (p = 0.011). Placental infection associated with gravidity (p = 0.025) was 13, 57%. Low birth weight (11.50%) was not related to any measured factor. Pre maturity (11, 11%) was associated only with LBW (p<0.001).

**CONCLUSION:** This work has to have data bases and build an effective strategy for malaria cont rol.

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P722: Development of an insecticide impregnated wall lining

Mthokozisi M Sibanda and Walter W Focke
Centre for Sustainable Malaria Control, Institute of Applied Materials, Department of Chemical Engineering, University of Pretoria, Private Bag X20, Hatfield 0028

**BACKGROUND:** Dichlorodiphenyltrichloroethane (DDT), pyrethroids, organophosphates and carbamates are insecticides recommended by the World Health Organisation (WHO) for malaria vector control. Long Life Insecticide Treated Nets (LLITNs) and Indoor Residual Spray (IRS) are the flagship interventions recommended by the WHO for malaria control. LLITNs usually employ pyrethroids as active ingredients. IRS is most effective when using DDT, a persistent organic pollutant whose use is contentious. Insecticide treated wall lining (ITWL) is a slow release technology that combines the advantages of LLITNs and IRS. Current commercial wall linings are produced using a labour intensive fabric weaving or knitting methods. This study explored the use of inexpensive lignings produced by extruding insecticide impregnated polyethylene directly into a net format.

**METHODS:** For safety reasons, initial trials involved blowing blayer polyethylene films with pyrethroids incorporated in the inner layer only. Monofilaments of organophosphates impregnated in polyethylene-co-vinyl acetate (EVA) (ca. 0.5 wt.%) were extruded and knitted into a mesh. Monofilament mesh samples containing deltamethrin and alphachlorpyrmethin (ca. 0.5 wt.%) were extruded using polyethylene as base. Carbamate insecticides were not considered as, on heating to typical polymer processing temperatures, they decompose releasing toxic gases. Efficacy testing was done using standard WHO bioassay tube tests.

**RESULTS:** The WHO effectiveness criterion is a mortality exceeding 80% after 24 hours following a 30 minute exposure of *An. arabiensis* mosquitoes to test samples. The pyrethroid impregnated mesh samples achieved 100% mortality in this test even after eighteen months of storage. Samples impregnated with organophosphates failed to achieve the WHO criterion. EVA is polar in nature and the poor performance of the organophosphate insecticides in this matrix was probably due to the high solubility of these insecticides in this polymer.

**CONCLUSIONS:** Monofilament polyethylene mesh, impregnated with pyrethroid insecticides, was successfully produced by a simple direct extrusion technique. It is an inexpensive method for the production of slow-release polymer-based wall linings. Laboratory efficacy tests suggest that this ITWL technology may be a potential substitute for IRS and it may also complement LLITNs.

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P724: Awareness of Artemisinin Combination Therapy in the treatment of malaria at some rural communities in Ekiti State, Southwest Nigeria.

Abayomi SUIADE and Joseph FADARE
Department of Pharmacology, Faculty of Basic Clinical Sciences, College of Medicine, Ekiti State University, Ado-Ekiti, Nigeria

**BACKGROUND:** Many of the West African countries have changed antimalarial treatment policy to Artemisinin-based Combination Therapy (ACT) as an effective approach to limit the wide spread of *Plasmodium falciparum* resistance to commonly used antimalarial drugs. It is however still common for many people to use CQ or other conventional antimalarial drug for treatment of malaria in Ekiti State. This study therefore sought to assess the use of chloroquine (CQ) after change of policy in Nigeria.
and awareness of people to the use of ACT in the treatment of malaria. METHOD: A community-based cross-sectional study was conducted in Ekiti State, Southwest Nigeria. Questionnaire was administered to randomized selected communities in Ekiti. A total of 110 questionnaires were sent out and had 103 respondents, the remaining 7 were not returned. The questionnaire sought to know demography details, socioeconomic, educational status, drug use habit of individuals and their awareness of the antimalaria treatment policy. RESULTS: Thirty-three percent of the respondents were traders, 14.56% civil servant, 48.5% unemployed (majority are house wives) and 2.9% self-employed. Their age ranged from 17- 83 years (mean 37.5±17.1years). Out of the 87 respondents who have been using CQ, 12.6% used CQ less than a year ago, while 29.9% used CQ to treat malaria over a year ago, and 57.5% of the respondent cannot recall when they last took CQ. 17.3% prefer to use CQ for the treatment of malaria. Similarly, 65.0% of the respondents are not aware of the change in policy to ACT. Most respondents that are not aware of ACT were 59.7% of females, 44.7% unemployed and 8.9% were uneducated individuals. Among those that were aware, 45.5% of them have never used ACT before based on the report that the drug was not readily available and even expensive for them to buy.

CONCLUSIONS: Our findings showed that people in the rural communities of Southwest Nigeria are not aware of the policy change in the treatment of malaria. Therefore, there is need for health education of the populace on the appropriate treatment of malaria. This will contribute to a reduction in the level of antimalaria drug resistance in our community.

P725: HIV/Malaria Co-Infection Among Pregnant women in Adama, and ‘Awash Sebat Kilo’, Ethiopia: a cross-sectional study
Heven Sime1,2, Beyene Petros1 and Afewerk H/Marian3

1Department of Microbial, Cellular and Molecular Biology, Addis Ababa University, P.O. Box 1176, Addis Ababa, Ethiopia; 2 Ethiopian Health and Nutrition Research Institute, Malaria, Other Parasitic and Vector Born Disease Research Team, Addis Ababa, Ethiopia; 3 World Health Organizations, Ouagadougou, Burkina Faso

BACKGROUND: Due to the high prevalence of HIV and malaria in Sub-Saharan Africa, co-infections are very common. This study was undertaken to determine the prevalence and severity of malaria in HIV positive pregnant and non-pregnant women who receive antiretroviral therapy (ART).

METHODS: Demographic information was collected through questionnaire. Blood samples were taken from the study participants and thick and thin blood smears prepared. Malaria parasite detection and parasite density was done microscopically. CD4+ T cell count was determined by BD FACS Count (Becton, Dickinson and Company (BD), USA machine and Hb value determined by the CELL DYN 1800 machine (Abbott Company, USA).

RESULT: 500 HIV positive women from Adama hospital and ‘Awash Sebat Kilo’ health center participated in the study. Out of these, 22.2% were malaria infected. Among the pregnant HIV positive women, 44.6% were malaria infected. Pregnant HIV/malaria co-infected women, on the average, had a significantly higher (P=0.001) malaria parasite density (26,595±15,309 versus 15,400±12,278), a significantly lower (P=0.05) Hb values (7.49±3.34 versus 8.37±3.13) and lower mean CD4+ T cell count (195±123 versus 220±140) compared to non-pregnant HIV positive women. Compared to pregnant women infected with only HIV, malaria/HIV co-infected pregnant women had significantly lower (P=0.005) CD4+ T cell count (195±123 versus 279±151) and significantly lower (P<0.001) mean Hb level (7.49±3.34 versus 10.53±2.96). Lower CD4+ T cell count and Hb level and higher parasite density were recorded in primigravid HIV/malaria co-infected pregnant women than in the multigravid ones. CD4+ T cell count was 195±123 versus 279±151. Our study revealed high malaria parasite density, reduced Hb level and CD4+ T cell count in HIV positive pregnant women, indicating that pregnancy has an adverse effect leading to severe malaria in HIV positive pregnant women.

P726: Rapid Diagnostic Tests (RDTs) and microscopic diagnosis in two health facilities
Brice Pemben Singana

BACKGROUND: Microscope still remains an important tool to detect and distinguish among malaria parasite species and determine the parasite density in patients. However, the poor quality of microscopes and reagents, poorly trained microscopic examiners, and unavailability of microscopes and basic laboratory reagents in many situations render rapid diagnostic tests (RDTs) for malaria useful, particularly in Africa. During a study of drug efficacy in children under 12 years old in Owando city located in the Congo Basin, a lactate dehydrogenase (LDH)-based malaria RDT and thick film were systematically used and compared for malaria parasite screening.

METHODS: Febrile children attending the no. 1 and no. 2 health centres in Owando were registered and examined by health workers to establish the clinical diagnosis. Finger-prick blood samples were collected by laboratory technicians and processed for thick films and malaria RDT. Samples were tested with Advantage MaL Card device (J. Mitra & Co Pvt. Ltd, India), according to the manufacturer’s instructions. The immunosay contains an anti-Plasmodium falciparum LDH monoclonal antibody and anti-Pan specific malaria parasite LDH monoclonal antibody. Blood films were Giemsa-stained and examined according to standard procedures.

RESULTS: The survey was conducted from November 2012 to February 2013. Of 873 febrile children under 12 years old screened for malaria, 207 (23.7%) had a positive RDT and 172 (19.7%) positive thick film (chi-square test =0.8, p=0.4). From November to February, the prevalence of positive RDT and positive thick films increased. There were 11.2%, 19.5%, 38.7% and 37.9% positive RDTs in November, December, January, and February, respectively. During the same period, 9.6%, 18.2%, 32.6%, and 27.4% of thick films were positive, respectively.

CONCLUSION: The malaria prevalence reported here represents partially the malaria burden in Owando city because of the limited consultation of the general population at the health facilities. Due to seasonal influences, the frequency of malaria episodes greatly changes from one month to another. Despite the distribution of impregnated bednets purchased with Global Fund and the availability of free-of-charge artesunate-amodiaquine and artemether-lumefantrine in the public health sector, malaria is still highly prevalent in Owando.

P727: Genotypic characterization of Plasmodium vivax: an interpretational problem
V Singh

It is conventionally assumed that in the absence of reinfection or drug-related, temporary parasite quiescence, the hypnozoite stage (I coined this term 3.5 decades ago) is the source of recurrent Plasmodium vivax malaria caused by parasites that are genetically similar to those which were responsible for the initial manifestations. How frequently (if ever) this is the case, though, is uncertain, partly because hypnozoites are never generated by the prior blood-stage infection. Hypnozoites are thought to be directly sporozoite-derived, but it has not been proven that they are not post-divisional pre-erythrocytic forms (MB Markus, 2012, Trends Parasitol. 28: 39–45). If the former, it has yet to be shown that genetically homologous sporozoites inoculated by the mosquito can behave in two different ways, i.e. involving some sporozoites initiating early hepatic schizigony but others becoming dormant as hypnozoites. This might indeed happen, of course. However, could it normally or this term 3.5 decades ago) is the source of recurrent Plasmodium vivax malaria caused by parasites that are genetically similar to those which were responsible for the initial manifestations. How frequently (if ever)
to occur in the life-cycles of some non-primate mammalian plasmodial species. For reasons that are not readily apparent, uncertainty concerning the origin of recurrences could complicate molecular identification of drug-resistant P. vivax parasites. P. vivax recurrences often follow clinical P. falciparum malaria. The cause is unknown but the phenomenon is not unique to malaria: one type of related coccidian infection can lead to reactivation of another (MB Markus, 1988, Med. J. Aust. 149: 344).

P728: Histopathological & Clinical Correlation of ARF in Malaria
V Singh

INTRODUCTION: Acute renal failure complicates approximately 5% of hospital admission and up to 30% intensive care units, oligurea, (urine output <400 ml/day) in frequent but not invariable clinical feature. Most of ARF are reversible including of severe malaria. The kidney being relatively unique among major organs with ability to recover from almost complete loss of function.

MATERIAL AND METHODS: Patient admitted in tertiary care hospital with malaria (positive P. falciparum / P. vivax) with renal failure as per WHO guidelines and subjected to PBF (thick and thin film), optimal test, PCR and renal biopsy. The exclusion criteria were setup to rule out other causes of renal disease before and during illness.

OBSERVATIONS: Out of 933 patient of malaria during year 2008-2009. Acute renal failure was observed in 219 patients of malaria. (PV – 45 & PF – 174). Oliguric renal failure was in 54(24.65%) (PV – 12, PF – 33, Mixed 9 patient) while non oliguric renal failure was in 165(75.34%) (PV – 24, PF – 123, Mixed – 18). Maximum patient of renal failure were in age group of 20 – 40 years. Total death of malaria with acute renal failure was 63. Death of patient of malaria of oliguric renal failure 42 (65.62%) (PV – 9, PF – 24, Mixed – 9) and of non oliguric renal failure 21 (33.33%) (PV – 21, PF – nil, Mixed - nil). Histopathological findings of 39 patients were suggestive of acute tubular necrosis (ATN) and mesangioproliferative glomerulonephritis (MPGN) (oliguric RF – 30 (76.92%), non oliguric – 9 (23.07%)) and out of that ATN was in 21 (53.84%) cases (3 oliguric and 18 non oliguric) and MPGN in 18 (46.15%). The incidence of renal failure was 2.07% in 1994, 6.16% in 2001 and 23.47% in 2003 in this region of Northwest India. Though renal failure was common with PF malaria and rare with PV malaria but we confirmed P.

CONCLUSION: Renal failure prevalence of renal failure in hospitalized patient is 16.43%, oliguric renal failure was having poor prognosis. Acute renal failure in P. vivax confirmed by PCR. Major Histopathological finding in malarial ARF is ATN and mesangioproliferative glomerulonephritis.

P729: Study of Plasma Lactate level in Malaria and its correlation with severity
V Singh

S.P. Medical College Bikaner Rajasthan India

BACKGROUND: Every year 300 to 500 million people suffer from Malaria and almost 1.7 to 2.5 million succumb worldwide. Most cases are in tropical regions where health care facilities are limited so triage of Malaria cases is necessary but this is hampered by lack of tests that can be done at grass root level. This study explores the role of Plasma lactate as an independent marker for severity of Malaria.

METHOD: Study subjects were selected from in-patient and out-patient presenting to our hospital. Detailed history was taken and physical examination was done. Biochemical and hematological investigation were sent on admission and repeated as required. Patients were classified as severe/ complicated malaria according to WHO criteria. Plasma lactate level were estimated using Biochemistry Analyzer TRACE 30 v4.1. Subjects were followed closely till final outcome of disease.

RESULTS: Total 100 study subject were selected. Mortality was 10%. All who succumbed had severe/ complicated Malaria. Among survivors 47.7% were having severe/ complicated Malaria. The mean plasma lactate level in survivors was 3.86 (95% CI: ± 1.41) mMol/L whereas in those who succumbed the mean plasma lactate level was 6.72 (95% CI: ± 0.39) mMol/L. Amongst the survivors, mean plasma lactate level in those without any complications was 3.16 (95% CI: ± 1.41) mMol/L, but in survivors the mean plasma lactate level was 4.63 (95% CI: ±1.3) mMol/L. All the patients who had hemoglobin <5gm/dl were having raised plasma lactate and hemoglobin levels <5gm/dl were significantly associated with mortality (p<0.003). Similarly all the patients who had serum creatinine level >3mg/dl were having raised plasma lactate and raised serum creatinine levels were significantly associated with mortality (p<0.0007).

Overall raised plasma lactate level were significantly associated with mortality (p<0.03).

CONCLUSION: Plasma Lactate level were higher in those with greater number of complications. Mortality was higher with higher plasma lactate level. So plasma lactate level may be used as indicator of severity and to guide treatment.

P730: Prevalence of mixed plasmodium species infection associated with age in children: population based survey observations of selected communities in Zambia
Lungowe Sitali1,2, Mulenga C. Mwenda3, Hawela Moonga3, John Miller4, James Chipeta2,3, Charles C. Micheko2,4

1University of Zambia School of Medicine, Department of Biomedical Science, Lusaka, Zambia; 2The School of Medicine and University Teaching Hospital Malaria Research Unit (SMUTH-MRU), C/O University teaching Hospital, Department of Paediatrics and Child Health, Lusaka, Zambia; University of Zambia School of Medicine, Department of Paediatrics and Child Health, Lusaka, Zambia; 3Ministry of Health, National Malaria Control Centre, MACEPA, Chainama grounds, Lusaka, Zambia; 4University of Zambia, School of Medicine, Department of Public Health, Lusaka, Zambia

INTRODUCTION: Malaria remains one of the preventable and treatable killers among infectious diseases, and yet it still claims more that 1 million lives every year. Mortality and morbidity is high among children under 5 and pregnant women. In Zambia, Rapid diagnostic Test (RDTs) are used in most health facilities for diagnosis of malaria, that only detect Plasmodium falciparum. This study sought to determine the presence of mixed plasmodium species in Eastern and Luapula provinces, and to examine their association with age.

METHODOLOGY: Data stem from the 2012 National Malaria Indicator Survey conducted between March and May country wide. Background, social and behavioural information were collected from households. In addition, blood slides, dried blood spots were collected from children below 5 years. Slides were stained using giemsa immediately after collection and examined by microscopy but Polymerase Chain Reaction (PCR) was used to analyse the filter papers for malaria species.

RESULTS: Overall (n=873), the mean age was 2.4 years, prevalence of malaria by PCR was 54.3%, and the prevalence of the individual plasmodium species were P. falciparum 53.38%, P. malariae 5.0%, P. ovale 2.1% and P. vivax 0.2%. The prevalence of mixed infection was 5.6%. Furthermore, increasing age was associated with higher likelihood of malaria infection (Pvalue < 0.00) Prevalence in children aged <1 year was 1.5%, whereas at 1 year and two, three, four and five years it was 2.8% and 4.0%, 7.9%, 7.7% and 9.8% respectively.

DISCUSSION: The prevalence of mixed infections was 5.6%, and a gradual increase in percentage of mixed age were having raised plasmodium are less likely to have malaria, as they grow they tend to have more malaria infections, as they reach the age of 5 their immunity has built up and infections reduce. There is need to pay attention to other species but it’s may not be necessary to change the HRPII based RDTs as the species mostly occur as mixed infections.
P731: Malaria in children under five years: Knowledge, attitudes and perceptions among mothers in a semi-urban area of Benue state, Nigeria

Robert Soumay

BACKGROUND: Malaria remains a major public health problem in Nigeria with children under five years and pregnant women being the most vulnerable. Due to the paucity of published data on childhood malaria, this study was conducted to determine the prevalence of malaria among children under five years and to assess the knowledge, attitudes and perceptions of the children’s mothers towards malaria and preventive measures in Gboko, a semi-urban area of Benue State, Nigeria.

METHODS: The study was cross-sectional in design and undertaken between May and September 2012. Thick blood films were prepared for standard parasitological examination. A questionnaire was also administered to each mother to collect information on socio-demographic data, knowledge, attitudes and perceptions towards malaria and preventive measures.

RESULTS: Of the 220 children examined, 14.5% (32/220) were found to be infected with malaria parasites. Males and females were similarly infected (14.5% v 14.6%) (χ², 0.000, p = 0.989). With regards to the socio-demographic factors of the children’s mothers, malaria was highest, 100% (4/4) (χ², 24.40, p = 0.000) among children whose mothers were within the age group [41-50] years and among children whose mothers are divorced, 45.5% (5/11) (χ², 12.50, p = 0.006). With regards to education and occupation of the children’s mothers, prevalence of malaria was highest among children whose mothers claimed to have attained a tertiary level of education, 17.8% (16/90) (χ², 1.37, p = 0.503) and among children whose mothers are traders, 25.0% (13/52) (χ², 8.27; p = 0.142).

Mothers of the children were observed having good knowledge of: malaria, 99.5% (219/220); its vectors, 75.5% (175/220) and aetiological agent, 74.5% (164/220). Likewise, 90.9% (200/220) of the mothers perceived that high temperature was a common malaria symptom and 70.3% (161/220) of them always referred their children to a hospital for treatment. With regards to the mothers’ attitude towards prevention, 85.5% (188/220) of the mothers used Treated Nets (Insecticide or Long Lasting Treated Nets) as preventive methods.

CONCLUSION: The study revealed a prevalence of 14.5% and shows that malaria is endemic among children under five years in Gboko, Benue State, Nigeria. It is recommended that control measures should be intensified in the area to reduce level of infection.

P732: Impact of the agricultural development on the dynamics of Anopheles gambiae population, its infectivity to Plasmodium falciparum and its resistance to pyrethroids.

Arthur Sov1, Martin AkoGBEto1
1Centre de Recherche Entomologique de Cotonou

BACKGROUND: A study of the dynamics of malaria transmission was carried out from July 2011 to June 2012 in four villages: one (Itassoumba) is characterized by the presence of track of farming and piscicultural basins, the three others (Itakpako, Djohounkollé and Ko-Koumolou) are characterized by traditional food-producing agriculture systems and practices. The 4 villages are located in the same geographical area within a radius of 15 kms. The study is to investigate if local agricultural practices have an impact on malaria transmission and the level of pyrethroids resistance to malaria vectors.

METHODS: In the 4 villages, the mosquitoes were sampled monthly by human landing catches in order to evaluate their biting rates. These mosquitoes were identified morphologically. The presence of P. falciparum Circumsporozoitic antigen in the females of Anopheles gambiae s.l. was evaluated by ELISA. The entomological inoculation rate (EIR) was also calculated. Further, larval collections were carried out in each village. Bioassays with deltamethrin and permethrin were carried out on the adult’s females mosquitoes aged from 2 to 5 days using WHO cylinders test kits. The presence of the kdr mutation in these mosquitoes was analysed by PCR.

RESULTS: The EIR was ranged from 9.3 to 22 infected bites of Anopheles gambiae per human per year in Djohounkollé, Itakpako and Ko-Koumolou against 1210.6 in Itassoumba. An. gambiae s.s M form (82.3%) was identified as the main malaria vector. Its susceptibility level to pyrethroids was the same (p > 0.05) in all villages. The frequencies of kdr mutation were also similar in all villages except Ko-Koumolou. This mutation was found in both M and S molecular forms of An.gambiae s.s.

CONCLUSION: The development of a market-gardening area in Itassoumaba did not increase the level of resistance to pyrethroids. The heterogeneous character of the malaria epidemiology was confirmed. The creation of piscicultural basins and the development of regional market-gardening perimeter increased drastically the malaria transmission in Itassoumaba.

P733: Artemisinin Based Combination Therapy drug use among caregivers of under five children in Ibadan Northwest Local Government.

Tolulope Soyannwo2, Ikeoulouwapo O Ajayi1, Dolapo Ijarotimi1
1Department of Community Medicine, University College Hospital, Ibadan, Oyo State, Nigeria; 2Department of Epidemiology and Biostatistics, University College Hospital, Ibadan, Oyo State, Nigeria

BACKGROUND: Resistance to cheap and long used antimalarials-chloroquine and sulphadoxine-pyrimethamine, led to recommendation that Artemisinin Based Combination Therapy (ACTs) be used in affected endemic countries. ACTs have been proven to have high efficacy and effectiveness. Improper use of these drugs may lead to resistance and ineffectiveness. Caregivers have a role in preserving this effectiveness by seeking and obtaining ACTs and using them appropriately. However, the use of this drug especially among under five children in Nigeria is still suboptimal. This study aimed at determining prevalence of ACTs drug use among caregivers of under five in Ibadan Northwest Local Government Area.

METHODS: A descriptive cross sectional survey using an interviewer-administered questionnaire was carried out among 478 caregivers selected using cluster sampling technique. Caregivers whose children had fever within two weeks prior to the survey were selected. Information was collected on use of ACTs, modes of prescription, sources of drugs and adherence to ACTs. Adherence to ACTs was assessed with reference to the duration of treatment and number of tablets taken. Data was analysed using descriptive statistics.

RESULTS: The mean age of the respondents was 30.8 ± 4.8. Many of the caregivers (69.4%) were between 25 and 34 years, 88.3% were married and most (72.0%) were Christians. More than half (60.9%) of the respondents had secondary education while (39.5%) were traders. A total of 162(38.5%) of caregivers reported they gave their children ACTs as their first line drug. Most of the children (75.3%) received the drug within 24 hours of onset of fever. About 36.4% mentioned health workers prescribed ACTs, 52.5% administered the drug based on self medication and 11.1% did so based on recommendation by neighbours, friends and relations. Many of the respondents (41.3%) bought the drugs from PPMVs. Adherence to ACTs was 56.5%.

CONCLUSION: Findings from this study showed that ACTs use among children under five is still low and self medication is a common practise. Appropriate use of ACT was moderate. This result showed that effort should be intensified on educating caregivers on malaria treatment and the need for adherence. The informal sector also needs to be strengthened.
P734: How does patient-provider communication around malaria rapid diagnostic testing affect patient perceptions of treatment? A qualitative study in western Uganda.

Robin Altaras¹, Anthony Nuwa², Agaba Bosco³, Elizabeth Streit¹, James K. Tibenderana⁴, Clare Strachan⁵
¹Malaria Consortium, Uganda ² National Malaria Control Programme, Ministry of Health, Uganda

BACKGROUND: Routine use of malaria rapid diagnostic tests (RDT) in the management of patients with fever represents a new approach in contexts with minimal exposure to diagnostic technologies. Successful scale-up of RDT use requires that patients accept testing and treatment based on RDT results and that providers treat according to test results. Patient reactions are important as perceived patient pressure or expectations have been shown to influence therapeutic decision making. We investigated how patient-provider communication around tests affects patient perceptions of treatment following RDT results.

METHODS: A qualitative study was conducted in a rural district in western Uganda, ten months after RDT introduction. 55 patients presenting with fever were observed during routine outpatient visits at 12 low-level health facilities. Observation focus was on communication practices around test purpose, results, diagnosis and treatment. All observed patients or caregivers were immediately followed up with in-depth interview. Analysis followed the ‘framework’ approach. Content analysis of observation data also used a summative approach.

RESULTS: Of all observed patients, 38 tested negative and 17 tested positive. Across both RDT-positive and negative patients, providers failed to consistently communicate the meaning of test results or inform the patient of a diagnosis. For patients who tested negative, about half were told they did not have malaria and given a minimal or unspecific explanation about another possible cause of illness, a third were told they did not have malaria but were not given any alternative explanation, and the remainder were not told anything about their test result. Although patients valued testing, they expressed frustration regarding the lack of communication on outcomes and reported a desire for more information. Among patients who tested negative, patient dissatisfaction with treatment appeared to be driven primarily by the absence of an alternative diagnosis and perceptions of not receiving adequate treatment.

CONCLUSION: Inadequate communication regarding test results and diagnosis influenced patient perceptions of treatment following testing. Patients have a right to health information and may be more likely to accept and adhere to treatment when they understand their diagnosis and treatment rationale. Findings emphasize the need to address communication practices in RDT training and supporting interventions.

P736: Malaria control in the South West of Madagascar: control of impact of Indoor Residual Spraying (IRS) and evaluation of vector population dynamic after IRS campaign with “Bendiocarb” (Carbamat)

Suzanantsoa A Z ¹, Rahararimanga R ², Ramaizafy L ³, Andriamarimanana A ⁴, Rakotomahafaly D ⁵, Ranaivo L ⁶, Ramarosandratana B ⁷, Ratsimbasona A ⁸
¹ National Malaria Control Program, ² Faculty of Medicine Antananarivo

BACKGROUND: Indoor Residual Spraying (IRS) is the cornerstone of vector control methods in Madagascar. In this study, we assessed the insecticide efficacy during IRS Campaign 2011-2012.

METHODS: This study was carried out from December 2011 to May 2012 (6 months) in Besakoa-Sakara, in the south west zone of the island. The insecticide used for malaria vector interventions were Bendiocarb WP 80%. To evaluate its efficacy, bioassay was conducted following World Health Organisation standard methods. Adult wild female of Anopheles gambiae s.l. susceptible to carbamat, caught at the locality, were used for the test. Bioassay was performed using plastic cones containing mosquitoes attached to treat surface. To evaluate the dynamic of population, human landing mosquitoes were collected during the night and by pyrethrum spray catches for endophilic mosquitoes. Mosquitoes caught were identified and later, were tested in laboratory for the presence of parasite and probably Kdr resistance gene.

RESULTS: The average of human bite rate per night of An gambiae s.l. showed high decrease from 3.06 in December before spraying to 0.22 in January, one month after IRS. A tendency to become exophilic has been noticed with An gambiae s.l in this locality (HBR per night outdoor superior than indoor). Bendiocarb spraying still has a residual effect after 96 days of treatment, with mortality rate 80%.

CONCLUSIONS: IRS is necessary to reduce the density of vector population. Therefore, this strategy remains an efficacy method for malaria vectors control.

P735: Malarial anaemia and severity in apparently healthy primary school pupils in urban and semi-urban settings in the Mount Cameroon Region

Irene U N Sumbelé¹, Helen K Kimbi¹, Malalaka Nwepoh¹, Judith K. Anchang-Kimbi¹, Emmaculate Lumi¹, Yannick Nana¹, Lucy M. Ndip², Henry Njome¹, Leopold G. Lehman³
¹ Department of Zoology and Animal Physiology, Faculty of Science, University of Buea, P.O. Box 63, Buea, SWR, Cameroon; ² Department of Microbiology and Parasitology, Faculty of Science, University of Buea, P.O. Box 63, Buea, SWR, Cameroon; ³ Emerging Infectious Disease Laboratory, Faculty of Science, University of Buea, P.O. Box 63, Buea, SWR, Cameroon; ⁴ Department of Animal Biology, Faculty of Science, University of Douala, P.O. Box 2701, Douala, Cameroon

BACKGROUND: Severe malarial anaemia can be a major cause of morbidity and mortality. While many of Africa’s health problems are common to both urban and rural environments, the epidemiology of some diseases can differ. This study examines the influence of urbanisation on the prevalence and severity of malarial anaemia (MA) in healthy primary school pupils.

METHODS: A cross-sectional study was conducted among 727 school pupils aged between four and 15 years living in an urban (303) and semi-urban (424) settings in the Mount Cameroon region. The investigative methods included the use of questionnaire, clinical evaluation and laboratory investigations. Blood sample collected from each child was used for the preparation of blood films for detection of malaria parasites and assessment of malaria parasite density as well as full blood count determination using an automated haematology analyzer. Based on the haemoglobin (Hb) measurements, pupils with malaria parasitaemia (MP) of any density were stratified into the following categories: MA (Hb <11g/dl), mild MA (Hb of 8 – 10.9g/dl); moderate MA (Hb of 6.1 – 7.9g/dl) and severe MA (Hb ≤ 6g/dl).

RESULTS: Out of the 727 pupils examined, 72 (9.9%) had MA. The prevalence (95% confidence interval, CI) of MA was significantly higher (χ² = 36.5, P <0.001) in pupils in the urban (17.8%, CI = 16.2 – 29.1%) than those in the semi-urban areas (4.2%, CI = 2.7 – 6.7%). Although the prevalence of the various categories of MA were comparable (P >0.05) in pupils in both settings, majority of the MA cases were mild (88.9%), with moderate (5.6%) and severe (5.6%) MA occurring in pupils from the urban set up only. In a multilinear regression analysis, MA was significantly associated with age (P = 0.005) and parasitaemia (P = 0.002) only.

CONCLUSIONS: Age and malaria parasitaemia density are the fundamental factors contributing to the occurrence of MA in healthy school pupils. Even though the occurrence of MA was higher in pupils in the urban setup than the semi-urban, the level of urbanisation was of limited influence in its occurrence.
Malaria and typhoid are two diseases of public health importance that have similar symptomatology and epidemiology and are associated with significant morbidity, mortality and economic loss. Individuals in areas endemic for both diseases are often at risk of contracting both of these diseases, either concurrently or in acute sequence. Individuals in areas endemic for both diseases are often at risk of contracting both of these diseases, either concurrently or in acute sequence.

**BACKGROUND:** Malaria and typhoid are two diseases of public health importance that have similar symptomatology and epidemiology and are associated with significant morbidity, mortality and economic loss. Individuals in areas endemic for both diseases are often at risk of contracting both of these diseases, either concurrently or in acute sequence. Individuals in areas endemic for both diseases are often at risk of contracting both of these diseases, either concurrently or in acute sequence.

**METHODS:** Key informant interviews were conducted with selected (male) household heads and (female) caregivers in net recipient households, and selected community health workers, in three districts in western Uganda two years post distribution. Villages were purposively sampled to provide for diversity in socio-economic, geographic, and demographic settings. 74 interviews were conducted. The enquiry incorporated the collection of ‘most significant change’ (MSC) stories to capture context specific aspects of both positive and negative change over time. Data analyses followed the ‘framework approach’ and a participatory analysis process for the MSC stories. Positive and negative deviance characteristics which affected net use over the long-term were identified and mapped.

**RESULTS:** Factors that determine use of LLINs in the long-term include subjective factors (knowledge/perception/experience), and objective (practical) factors. The former include perceived and actual benefits, past experience with net use, social support, influence of the household head or caregiver, and net preferences. The practical factors include continued availability or condition of nets, visibility of mosquitoes and seasonal factors, and net replacement possibilities. Net use appeared to be more consistent amongst settled urban and rural communities, compared to fishing, pastoralist and refugee/immigrant communities. The long-term changes reported through the MSC stories related to health, economic and psycho-social benefits felt at the individual, household and community level. A range of characteristics relating to past, current and intended net use behaviour were identified for both positive and negative deviance.

**CONCLUSIONS:** BCC campaigns should emphasise consistent use of nets throughout the year and effective net maintenance, and build on an enhanced contextual understanding of the factors which promote sustained net use. ‘Positive deviants’ have the potential to play a valuable role in the promotion of ongoing net use in communities.

**P740: Delineation of the phenotype and function of cells that infiltrate the placenta during placental malaria in Cameroonian women: implication of monocyte subsets in malaria pathogenesis**

**BACKGROUND:** A great diversity of immune cells infiltrates the placenta during malaria pregnancy. Monocytes have been described to be the major population of immune cells that infiltrate the placenta during pregnancy. There is a paucity of data regarding the sequential involvement of different monocyte subsets in malaria pathology. The aim of this work is to determine the phenotypic and functional properties of immune cells during placental malaria. Here we show data on monocyte subpopulations specifically addressing their phenotype, function and recruitment in placental malaria.

**METHOD:** Placental, cord and peripheral blood were collected from pregnant Cameroonian women at delivery after they were tested for either Malaria or HIV. Mononuclear cells were purified as well as monocyte enrichment. Cells were stimulated with malaria antigens for functionality assay. Staining with fluorochrome-conjugated monoclonal antibodies was carried out on either whole blood or on purified mononuclear cells. Stained cells were acquired with an 8-color Flow Cytometer and data was analyzed using the flowJo software.

**RESULTS:** Analyses were carried out on samples collected from 21 delivering women amongst which 1 Placental malaria/HIV dually infected, 7 Placental Malaria infected women, and 2 peripheral malaria infected women. The rest of the women were all negative and used as negative controls. We observed a general increase in the monocyte population that infiltrate the placenta during pregnancy with an increase in the intermediate and the non-classical subsets.

**CONCLUSION:** This preliminary data indicate that, malaria could alter the frequency of monocyte subsets infiltrating the placenta. The intermediate and the non-classical monocytes are the most affected. We will pursue the characterization of these subsets with respect to malaria pathogenesis.
P741: Can a complex intervention focusing on malaria case management at public health centres improve health outcomes of community children in Uganda? Results from the ACT PRIME cluster-randomised trial

Florenc Nanyak1, Edith Mbabaazi1, Catherine Maitike-Sebuguzi1, Deborah Diliberto1,2, Levi Mugenyi1, Simon P. Kigozi1, Grant Dorsey2,4, Eleanor Hutchinson2, Clare I.R. Chandler2, Moses Kamya1,4, Sarah G. Staedke1,2

1 Infectious Disease Research Collaboration, Kampala, Uganda; 2 London School of Hygiene and Tropical Medicine, UK; 3 University of California, San Francisco, USA; 4 Makerere University, Kampala, Uganda

BACKGROUND: In Uganda, inadequate services in the public healthcare sector limit appropriate fever case management and delivery of good quality care, and are argued to contribute to the lack of progress on malaria control. Evidence of the impact of improving public health services on population-level malaria indicators is needed. We are conducting a cluster-randomised trial in Tororo, a high malaria transmission area, to assess whether a complex intervention delivered at government-run health centres improves appropriateness of antimalarial treatment and health outcomes of children.

METHODS: Twenty health centres were randomized; 10 to the intervention and 10 to control. The intervention, which was rolled-out in May-June 2011, includes training in fever case management and use of RDTs, patient-centred services, health centre management, and provision of RDTs and artemether-lumefantrine when stock runs low. As part of the evaluation, we conducted annual cross-sectional surveys in children randomly selected from each cluster to assess the impact of the intervention on health indicators, including parasitaemia and prevalence of anaemia (haemoglobin <11.0 g/dL). The target sample size for each survey was 8766, including 4383 children under five, and 4383 aged 5-15 years.

RESULTS: Three surveys have been completed (Baseline: Dec 2010 to June 2011, First repeat: Jan-May 2012, and Final: Jan-April 2013). At baseline, prevalence of parasitaemia was higher in older children in both study arms (Under-fives: 57% control, 58% intervention; 5-15 years: 72% control, 72% intervention). Preliminary results from the first repeat survey, in an analysis adjusted for cluster-level prevalence of parasitaemia at baseline, age, gender and ITN use, suggest that prevalence of parasitaemia decreased in both age groups (Under-fives: 43% control, 44% intervention; 5-15 years: 52% control, 54% intervention), although there were no statistically significant differences between the study arms.

CONCLUSIONS: Our preliminary results suggest that the intervention may not have a significant impact on health outcomes of community children after one year. However, the decrease in prevalence of parasitaemia in the study area is notable, suggesting other contextual factors may be contributing. Analysis of the final survey data is on-going, and full results will be presented.

P742: Impact of introducing RDTs at public health centres on ACT use in a high malaria transmission area of Uganda: results from the ACT PRIME cluster randomised trial

Levi Mugenyi1, Florence Nanyak1, Emmanuel Ssemmondo1, Catherine Maitelki-Sebuguzi1, Deborah Diliberto1,2, Simon P. Kigozi1, Heidi Hopkins1, Grant Dorsey2,4, Moses Kamya1,4, Clare I.R. Chandler2, Sarah G. Staedke1,2

1 Infectious Disease Research Collaboration, Kampala, Uganda; 2 London School of Hygiene and Tropical Medicine, UK; 3 University of California, San Francisco, USA; 4 Makerere University, Kampala, Uganda

BACKGROUND: In 2010, the World Health Organization released new guidelines for malaria diagnosis and treatment recommending that all suspected cases be confirmed by a parasitological test, where possible, before treating. Since then, rapid diagnostic tests (RDTs) for malaria have been promoted as the solution to the overuse of valuable antimalarials in low-resource settings. However, in practice, introducing RDTs is not simple: the ideal approach to optimise use of RDTs, including how to target delivery and support health worker behaviour change, is not clear.

METHODS: The PRIME cluster-randomised trial is taking place in Tororo, a high transmission setting. Twenty government-run health centres were randomized to either continue with standard care (controls) or to receive an intervention, including training in fever case management and use of RDTs, patient-centred services, health centre management, and provision of RDTs and artemether-lumefantrine (AL) when stocks run low. As part of the evaluation, we collected out-patient data using a modified version of the Health Management Information Systems (HMIS) register completed by health workers.

RESULTS: Over two years, 438,257 patient visits (225,686 intervention, 212,571 control) were recorded. Preliminary results suggest that use of RDTs was higher in patients attending intervention health centres (117,286 [52%] intervention, 15,287 [7%] control), with variation between health centres (19-83% intervention, 0.3-17% control). The proportion of RDTs reported as positive was high in both arms (70% intervention, 59% control). Nearly all patients diagnosed with malaria were prescribed AL in both arms, but the proportion of malaria diagnoses confirmed by a positive RDT was substantially higher in the intervention (67% intervention, 7% control). Overall, the proportion of patients prescribed AL in the intervention (112,828 [50%]) was only slightly lower than in the control (113,496 [53%]). Appropriate treatment of malaria (proportion of RDT-positive patients prescribed AL) was high in both arms (93% intervention, 95% control).

CONCLUSIONS: Our preliminary results suggest that use of RDTs at intervention health centres improved confirmation of malaria diagnoses but did not substantially impact on prescription of AL, or appropriateness of treatment, which is likely attributable to the high prevalence of infection in this setting. Full results will be presented.

P743: Malaria receptivity of mosquitoes: Seasonality, spatial distribution and host choice of anopheline mosquitoes in southern Zambia

Limonty Simubali1, Jennifer Steenhoorn1,2, Harry Hamapumbu3, Sungano Mharakurwa2, Douglas E. Norris1

1 Macha Research Trust, Choma, Zambia; 2 The Malaria Research Institute, Department of MMI, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

BACKGROUND: Malaria prevalence in Macha has reduced significantly over the past decade, with parasite prevalence currently below 1%. It is important, however, to establish the receptivity of mosquitoes, the potential of the local mosquito population to spread disease. In addressing this, the spatial and temporal densities and biting preferences of primary and secondary vectors need to be established.

METHODS: In Macha, CDC light traps were set in randomly selected households from January 2012 to June 2013. Mosquitoes were identified to genus, and species using morphological keys. All female anophelines underwent DNA extraction. Identities of An. gambiae or An. funestus complex specimens were confirmed using a diagnostic PCR. For samples for which no PCR product was visualised, an ITS2 rDNA PCR was used for species identification. All female anophelines underwent a multiplexed PCR to detect the origin of the blood meal and therefore calculate human blood indices.

RESULTS: 952 female anophelines were collected during the study period, from 462 traps across 365 households giving a mean catch of 2.06 Anophelines per trap per night. Catches ranged from 0 to 178 anophelines per trap per night. Households with anophelines appeared to be clustered. Over 60% of the catches were morphologically identified as An. gambiae s.l of which the majority were An. arabiensis. PCR-confirmed An. arabiensis had fed solely on humans. At least 9 other species were identified using molecular techniques.
CONCLUSIONS: Despite substantial reduction in malaria in Macha, large numbers of anophelines can be found and spatial and temporal heterogeneity in vector density was evident. The main vector appears to be An. arabiensis which feeds preferentially on humans. Several other species have been recorded, demonstrating the value of molecular methods for species identity. Further studies are required to look at mosquito infectivity and age to further establish receptivity. Entomological surveillance must be maintained to monitor any increase in dominant or secondary vectors.

P744: Evidence of reduced protective efficacy of bednets against anophelines for school-aged children in the highlands of western Kenya

Mary Cooke1, Jennifer Stevenson1,2, Samuel Kahindi1, Chrispin Owaga3, Elizabeth Ayoma4, Robin Oriango1, Chris Drakeley5, Jonathan Cox1
1 Faulty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, U.K; 2 Centre for Global Health Research, Kenya Medical Research Institute/Centers for Disease Control and Prevention, Kisumu, Kenya

BACKGROUND: The effectiveness of bednets for malaria control relies on the assumption that the majority of vectors feed indoors and at times when people are under a net. It is important to establish whether this is true and to estimate the level of individual protection a net may confer. This study aimed to record the time and location of both vector and human activity to estimate the protective efficacy of bednets.

METHODS: Indoor and outdoor light trap collections were carried out from June 2011 to July 2012 in a village in the highlands of western Kenya, to calculate vector man biting rates (MBRs). Times of house-entry and exit and net usage of residents were captured using watches and questionnaires. Individual hourly exposure to vectors indoors and outdoors was calculated. Reduction in vector exposure was calculated for net users. The true protective efficacy (P*) for this population was estimated, and compared between age groups.

RESULTS: Catches of Anopheles funestus and An. arabiensis were significantly higher indoors than outdoors however significant peaks of activity were recorded as early as 19:30 outdoors and 18:30 indoors. Net use was calculated to reduce exposure from 1.3 bites per night (95% CI:1.2-1.3) to 0.63 (95% CI:0.60-0.67). P* for the population over the study period was calculated as 51% (95% CI:50%-53%) against these vectors. P* varied significantly with age (Kruskal-Wallis x²=147, 18 d.f., p<0.0001) with those above 5 years experiencing lower P* than younger children and infants.

CONCLUSION: Bednets continue to reduce MBRs and their universal distribution is likely to have had a community-wide effect in reducing vector populations in this village. However, against remaining vectors, the true protective efficacy of nets was estimated as 51% due to vector activity when people are not asleep. Lower P* was demonstrated for individuals above 5 years of age. In this area the highest parasite rates have been reported in school-aged children. Whilst reduced net usage is likely to be the greatest contributing factor, increased vector exposure outside times of net use may also play a part. This highlights the need to tailor appropriate control measures for this age-group.

P746: Malaria control in the South West of Madagascar: control of impact of Indoor Residual Spraying (IRS) and evaluation of vector population dynamic after IRS campaign with “Bendiocarb” (Carbamat)

Suzanatsa Z1, Rahararimanga 1, R., Rasamizafy L1, Andriamiarimanana A2., Rakotomahafy D1, .Ranaivo L1, Ramarosandratanana B1, RatSIMBSAOSA A1-2.
1 National Malaria Control Program, 2 Faculty of Medicine Antananarivo

BACKGROUND: Indoor Residual Spraying (IRS) is the cornerstone of vector control methods in Madagascar. In this study, we assessed the insecticide efficacy during IRS Campaign 2011- 2012.

METHODS: This study was carried out from December 2011 to May 2012 (6 months) in Besakoa-Sakaraha, in the south west part of the Island. The insecticide used for malaria vector interventions were Bendiocarb WP 80%. To evaluate its efficacy, bioassay was conducted following World Health Organisation standard methods. Adult wild female of Anopheles gambiae s/l were used for the test. Bioassay was performed using plastic cones attached to the insecticide treated wall and mosquitoes from the locality. To evaluate the dynamic of population, human landing mosquitoes were collected during the night, and by pyrethrum spray catches for endophilic mosquitoes. Mosquitoes caught were identified and later, were tested in laboratory for the presence of parasite and probably Kdr resistance gene.

RESULTS: The average of human bite rate of An gambiae s/l showed high decrease from 3.06 in December to 0.22 in January, one month after IRS. Species became exophagic. Four months after, bendiocarb spraying still has a residual effect with mortality rates 80%. CONCLUSIONS: IRS is necessary to reduce the density of vector population. Therefore, this strategy remains an efficacy method for malaria vectors control.

KEYWORDS: Malaria; Vector control; Indoor Residual Spraying; An gambiae s/l; Bendiocarb; Madagascar.

CONTACTS: zileralice@yahoo.fr.

P747: Toxicological evaluation of combination therapy of artemisinin derivative (artether and lumefantrine) with ivermectin in male albino rats (rattus novergicus)

Emmanuel T IDOWU1, Olubunmi A OTUBANJO2, Abdurrahmon B. TALABI2, Gideon C. ALIMBA2
1 Department of Zoology, University of Lagos, Akoka, Lagos State, Nigeria.; 2 Department of Zoology, University of Ibadan, Oyo State, Nigeria.

BACKGROUND: Combined therapies in the co-endemic treatment of parasitic infections are ongoing in sub-Saharan Africa. Artemisinin based Combination therapy (ACTs) is the drug of choice recommended by World Health Organization in the treatment of malaria while annual rounds of ivermectin (IVR) administration is ongoing in areas of Africa where onchocerciasis is endemic. Combined therapies pose potential drug toxicity, rare adverse events and complications.

METHODS: Behavioural biochemical and histopathological effects of artemisinin derivative ACT and IVR administered using normal and double human therapeutic doses (HTDs) in male albino rats were investigated. Rats were exposed daily to IVR for 15 days while, ACT exposure was undertaken for 3 days prior to rats sacrifice. The doses of these drugs were calculated based on the mean average weight of animals assigned to the group and administered orally. The estimations of L-asparate amino transferase (AST), L-alanine amino transferase (ALT), alanine phosphatase (ALP), total albumin, total protein and cholesterol were determined using the automated chemistry analyzer. Histopathological alterations of the liver and kidney were also assessed.

RESULTS: The result showed no significant loss or gain of body weight (p>0.05) of the animals in all the treated groups. Biochemical assessment also showed no significant alteration (p>0.05) in values of biochemical parameters including ALT, AST, ALP, Total protein, cholesterol, creatinine, total bilirubin and urea analyzed compared to the control. Histopathological damage of the kidney was minimal, however mild to moderate congestion of the liver were recorded in rats exposed to HTD of IVR and HTDs of IVR+ACT.

CONCLUSION: The findings of this study validate previous findings that co-administration of IVR+ACT is safe and the two drugs can be co-administered where the diseases are co-endemic.
P748: Correlations between weather and malaria incidence in the centre region of Burkina Faso.

Mu J Chen¹, Szu Y Yeh², Huey J Su¹, Tianhoun S Edouard³,⁴
¹Department of Environmental and Occupational Health, National Cheng Kung University, Taiwan, Republic of China (Taiwan); ² Ministry of health / Burkina Faso

BACKGROUND: Previous studies have demonstrated that weather variations may affect malaria occurrence in humans. Yet, only limited evidence could substantiate the relationship at the national and sub national level in Burkina Faso, where malaria causes a massive number of deaths every year. This study aims to assess the association between weather variations and malaria incidence in the centre region of Burkina-Faso at West Africa.

METHODS: Study aggregated 6 weather factors (monthly maximum temperature, minimum temperature, mean temperature, cumulated precipitation, maximum and minimum relative humidity) and monthly incidence of severe malaria and uncomplicated malaria for the six districts of the centre region of Burkina-Faso from 2006 to 2010. Spearman’s rank correlation coefficient designated the significant lagged effects and Generalized Additive Model (GAM) under Poisson regression was used to calculate the associations between weather variations and malaria incidence and to establish the relative importance of each factor at each of the two (2) seasons (dry and wet) of the year.

RESULTS: Results indicated that monthly cumulated precipitation, maximum and minimum related humidity were positively correlated with severe and uncomplicated malaria incidence. Temperature (maximum, minimum and mean) was negatively correlated with malaria incidence. Each 30 mm increase of cumulated precipitation has been linked to relative risk (RR) up to a 25.5% (95% CI: 25.5%–29.1%; p<0.0001) malaria incidence. A 1% increase in relative humidity showed an increased RR as high as 3.2% (95% CI: 3.1%–3.3%; p<0.0001) for malaria.

CONCLUSIONS: Findings suggest that weather factors can be of great potential to be considered an effective predictor of malaria incidence in the centre region of Burkina Faso. These predictions might be useful for targeted public health actions against malaria.

P749: Comparison between light microscopy results and PCR method in diagnosing Plasmodium vivax in Eastern Sudan

Albadawi Abdelbagi Talhaj1, Sedigheh Zakeri2, Samira Abdelrahman3 and Bakri Y.M. Nour3
Faculty of Med. Laboratory Sciences University of Gezira Sudan1, Malaria and Vector Research Group (MVGR) Biotechnology Research Center (BRC), Pasteur Institute of Iran, Tehran, Iran2, Blue Nile National Institute for Communicable Disease (BMNICO) University of Gezira – Sudan3.

BACKGROUND & OBJECTIVES: Although polymerase chain reaction (PCR) is a new technique in the diagnosis of malaria with very high accuracy; light microscopy is still conventional diagnostic method used in Sudan. In this study we compare the accuracy of light microscopy with the results of PCR as gold standard.

METHODS: The blood samples were collected from 75 febrile cases in Eastern Sudan diagnosed by light microscopy and the slide were checked by experts microscopists. DNA samples were processed by PCR to amplify species-specific sequences of 18ss subunit ribosomal ribonucleic acid (18ssrRNA) genes of Plasmodium vivax and P. falciparum.

RESULT & CONCLUSION: The results showed that the positive slides for P. vivax based on microscopy were 66/75 (88%) P. vivax mono-infection 3/75 (4%) were mixed infection of P. vivax and P. falciparum. 4/75 (5.3%) were not give DNA, 2/75 (3.7%) were P. falciparum. Nested PCR has useful in the detection of mixed infection than microscopy.

P750: Pooled deep sequencing of Plasmodium falciparum parasitemias: an efficient and scalable tool to quantify prevailing malaria drug-resistance genotypes

Steve M Taylor1,², Christian M Parabek3, Nash Aragam4, Billy E Ngasola5, Andreas Mårtensson6,⁷, Steven R Meshnick8, Jonathan J Juliano4
¹Department of Epidemiology, Gillings School of Global Public Health, University of North Carolina, Chapel Hill; ²Division of Infectious Diseases and International Health, Duke University Medical Center, Durham, NC; ³Department of Genetics, School of Medicine, University of North Carolina, Chapel Hill; ⁴Division of Infectious Diseases, School of Medicine, University of North Carolina, Chapel Hill; ⁵Department of Parasitology, Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania; ⁶Infectious Diseases Unit, Department of Medicine Solna, Karolinska University Hospital, Karolinska Institute, Stockholm, Sweden; ⁷Division of Global Health, Department of Public Health Sciences, Karolinska Institute, Stockholm, Sweden

Molecular surveillance for drug-resistant malaria parasites requires efficient, timely, and scalable methods in order to provide actionable data. Genotyping parasite populations using second-generation sequencing may provide these data efficiently. We designed and validated a protocol to quantify the frequencies of mutant alleles associated with sulfadoxine-pyrimethamine resistance in Plasmmodium falciparum genes dhfr and dhps in mixed parasitemias using 454 sequencing. We applied this new protocol to field isolates collected from a cohort of 50 Tanzanian children with uncomplicated falciparum malaria, and compared it on accuracy and cost to standard genotyping methods that employ Sanger sequencing with or without statistical inference of allele frequencies. In validation experiments with a mixture of parasite strains 3D7 and V1/S, the 454 sequencing protocol accurately quantified dhfr and dhps allele frequencies. Using Sanger sequencing with statistical inference, the frequencies of mutant alleles in dhfr were 78.9% (dhfr51I), 80.5% (dhfr59R), 86.2% (dhfr108N), and 0 (dhfr164L); mutation frequencies in dhps were 58.5% (dhps437G), 51.1% (dhps540E), and 1.1% (dhps581G). 454 sequencing of pooled DNA generated 91,157 and 92,638 reads of dhfr and dhps, respectively, which estimated mutant allele frequencies of 70.9% (dhfr51I), 84.6% (dhfr59R), 90.2% (dhfr108N), 0 (dhfr164L), 55.1% (dhps437G), 57.9% (dhps540E), and 6.2% (dhps581G); these estimates were highly correlated (r>98%) with frequencies estimated by traditional methods. 454 sequencing obviated most molecular steps in traditional sequencing methods, and because of this would be cost-saving to generate allele frequencies for parasite population sizes larger than 50. This genotyping method based upon second-generation sequencing can efficiently and reproducibly estimate parasite allele frequencies within populations of P. falciparum. This method would be rapid and cost-effective for molecular epidemiologic studies of parasite genotypes associated with transmission, vaccine efficacy, and drug resistance.

P751: Using Malaria Control Programme for Health Systems Strengthening: the Case of Senegal

Ibrahim Soce Fall1, Moussa Thierno2, Mady Ba3, Sylla Thiam4
1African Medical and Research Foundation, Dakar; 2Programme National de Lutte contre le Paludisme Senegal, Fann Rue Aime Cesaire Dokar; 3African Medical and Research Foundation, Nairobi Kenya

BACKGROUND: Reinforcing health systems aims to achieve improved outcomes of interventions to deal with major health challenges such as HIV, tuberculosis and malaria. This is vital for making progress towards universal access to health care and meeting the Millennium Development Goals. Knowing that most health systems in malaria-endemic countries are not very strong enough to deal with these challenges, The World Health Organisation champions Health Systems Strengthening
P752: Insecticide resistance in Anopheles gambiae population from RD Congo

Thierry L. Bobanga, Solange E. Umesumbu, Billy S. Kinyu, Olivier A. Fataki, Alain S. Mandozo, Celestin N. Nsibu

1 Department of Tropical Medicine, Faculty of Medicine, University of Kinshasa, DR Congo 2 Malaria National Control Program, Kinshasa, DR Congo 3 National Institute for BioMedical Research Kinshasa, DR Congo; 4 Department of Pediatrics, University of Kinshasa, Kinshasa, DRC

BACKGROUND: Anopheles resistance in Africa is recorded in many countries. Control methods against malaria currently depend among other on deploying insecticide-treated nets, and vector resistance to insecticides is one of the main limitations for the use of these treated materials.

METHODS: The study was carried out from October 2010 to January 2011 in 9 sites Standard WHO insecticide susceptibility tests were carried out with 4% DDT, 0.75% permethrin and 0.05% deltamethrin, and carbamates (bendiocarb 0.1% and propoxur 0.1%). Mortality rates and knockdown times (kdt50 and kdt95) were determined and the effect of pre-exposure to synergists partially restored insecticide knockdown effect and increased mortality rates, suggesting a role of detoxifying enzymes in increasing mosquito survival upon challenge by pyrethroids. All of 250 An. gambiae s.l samples from all sites were An. gambiae s.s. In regarding molecular forms, samples from different sites were homogenous. In Bandundu Province all analyzed samples were M form. In opposite, samples from North Kivu Province, Katanga Province and Equateur Province were S form. The kdr gene distribution of was very high at Kapolowe, Bolenge cite, Secli Wendji and Bongonde. The lowest frequencies were observed in Goma and Butembo with 0.1 and 0.2 respectively. The average frequencies were observed at Kikwit and Bandundu City respectively 0.48 and 0.53.

CONCLUSION: The resistance is increasing in the DRC, detoxification enzymes are involved in this resistance in addition to the kdr gene. Vector control tools should reflect the resistance in the country.

P753: Treatment of asymptomatic carriers of Plasmodium falciparum with artemether–lumefantrine: Impact on the prevalence of anemia

Alfred B. Tiong1, Alphonse Ouedraogo2, Christine Remy3, Kamal Hamed4

1 Centre National de Recherche et de Formation sur le Paludisme, Ouagadougou, Burkina Faso; 2 Novartis Pharma AG, Basel, Switzerland; 3 Novartis Pharmaceuticals Corporation, East Hanover, NJ, USA

BACKGROUND: Asymptomatic carriers (ACs) of Plasmodium falciparum act as a parasite reservoir, and contribute to the disease burden as they do not seek treatment for their infection and have an increased morbidity due to anemia1–4.

OBJECTIVE: To compare the effect of systematic treatment of asymptomatic carriers of Plasmodium falciparum with artemether–lumefantrine (AL) on hemoglobin (Hb) levels and anemic status, with those without treatment

METHODS: In a 12-month, single-center, controlled, parallel, cluster-randomized study conducted in 18 villages in Burkina Faso, inhabitants of villages were randomized (1:1) to intervention and control arms. They participated in four community screening campaigns (CSC1–4). The first 3 community screening campaigns (CSC1–3) included treatment of ACs in the intervention arm and were conducted approximately one month apart before the rainy season. The fourth screening campaign (CSC4) was conducted after the rainy season, which marked the end of the study at 12 months.

RESULTS: The change in Hb level in all asymptomatic carriers aged >6 months from Day 1 to Day 28 of CSC1 was +0.53 g/dl (from 11.81 to 12.33 g/dl) in the intervention arm vs. -0.21 g/dl (from 12.06 to 11.86 g/dl) in the control arm (p<0.001). During the same period, the proportion of asymptomatic carriers aged >6 months to <5 years with anemia (mild, moderate or severe) in the intervention arm decreased by 31.1% (from 75.7% to 44.6%), compared with a decrease of 4.7% (from 76.3% to 71.6%) in the control arm. After 12 months, the proportion of asymptomatic carriers with anemia was reduced in both arms.

CONCLUSION: Systematic screening and treatment of asymptomatic carriers of P. falciparum with AL at the community level can reduce the prevalence of anemia in children in the short term, although the difference between control and intervention arm was not sustained at 12 months.


P754: Fulani show decreased susceptibility to Plasmodium falciparum infection vs Mossi: Data from a community-wide screening and treatment of asymptomatic carriers in Burkina Faso

A. B. Tiong1, S. B. Sirima1, K. Hamed2

1 Centre National de Recherche et de Formation sur le Paludisme, Ouagadougou, Burkina Faso; 2 Novartis Pharmaceuticals Corporation, East Hanover, NJ, USA

BACKGROUND: A difference in susceptibility to Plasmodium falciparum infection between the two major ethnic groups, Fulani and Mossi, has been reported.

OBJECTIVES: To conduct a post-hoc analysis comparing the susceptibility to Plasmodium falciparum infection between Fulani and Mossi.

METHODS: Post-hoc analysis was conducted with respect to the outcome...
P755: Biochemical characterisation of metabolic enzymes associated with pyrethroid resistance in *Anopheles gambiae* s.s. In South Western, Nigeria.

Tola, M.1, Jolaoso, A.O.2, Oduola, A.O.1, Olojede, J.B.1, Adeogun, A.O.1, Amoo, A.O.J.1, Awolola, T.S.1

1Molecular Entomology and Vector Control Research Laboratory, Nigerian Institute of Medical Research, Lagos, Nigeria. 2University of Ilorin, Kwara, Nigeria. 3Obalisk Onabania University, Ogun, Nigeria.

**BACKGROUND:** Insecticide resistance is generally mediated by behavioral, metabolic or genetic factors and usually results from one or more of three different mechanisms: reduction in insecticide penetration, an increased metabolism of insecticide by metabolic enzymes and or modification of the insecticide target site.

**RESEARCH HYPOTHESIS:** To investigate the role of metabolic enzymes in DDT/Pyrethroid insecticide resistance.

**METHODOLOGY:** Wild-collected larva *anopheline* mosquitoes (from 3 different sites in Sagamu, Ogun State) were reared in the laboratory and were morphologically identified to belong to the *Anopheles gambiae* sensu lato. Molecular techniques (PCR) were used for species identification. Emerging 2-3 days adult mosquitoes were exposed to paper impregnated with deltamethrin (0.05%) and DDT (0.04%) insecticides following WHO test procedure. Biochemical assays were then used to determine the relative levels of detoxifying enzyme systems including monoxygenases and glutathione-S-transferases.

**RESULT:** Of the 1,436 mosquitoes morphologically identified as *Anopheles gambiae* s.l, 206 were processed for molecular identification using PCR. 86% of these were molecularly identified as *Anopheles gambiae* s.s., 8% as *Anopheles arabiensis*. Post exposure mortality in the *Anopheles gambiae* populations exposed to DDT and deltamethrin was 15% and 85% respectively. Biochemical analysis revealed high level of monoxygenase (>60% of samples tested, n = 93, p < 0.05) but a very low level of glutathione S-transferase (11.8% of samples tested, n = 93, p > 0.05) compared to the reference Kismu susceptible strain of *Anopheles gambiae* s.s.

**DISCUSSION AND CONCLUSION:** Increased levels of monoxygenase were correlated with resistance to deltamethrin across samples reared from wild-caught *Anopheles gambiae* s.s. The fact that kdr-based resistance mechanism had earlier been reported in the *Anopheles* from the same locality is worrisome because the presence of both resistance mechanisms could have important implications in resistant management.

P756: The anti-plasmodial activity of *Carissa edulis* and its constituent phytochemicals

Festus M. Tolo1,2,3 and Jeremiah Waweru1

1Kenya Medical Research Institute (KEMRI), Nairobi, Kenya; 2Institute of Tropical Medicine and Infectious Diseases (ITROMID), Nairobi, Kenya.

**BACKGROUND:** Malaria remains one of the leading causes of morbidity and mortality in the tropics. It is estimated to represent 2.3% of the overall global disease burden and 9% in sub-Saharan Africa. The parasite species of *Plasmodium falciparum* is the most causative agent of malaria in human. Current statistics indicate that there has been a resurgence of this disease in recent years, the main reasons being attributable in part to the widespread development of resistance to most anti-malarial drugs and the cost of newer anti-malarials. There is, therefore, need to identify new anti-malarial agents. We report on extract of a Kenyan medicinal plant, *Carissa edulis* (Forsk.) Vahl (Apocynaceae) and phytochemicals isolated from it, which have shown significant anti-plasmodial activity.

**METHODS:** The extracts/phytochemicals were examined by the *hypoxanthine* assay, an *in vitro* semi-automated micro-dilution assay technique that measures the ability of the extracts/compounds to inhibit the incorporation of [G-H] hypoxanthine into the malaria parasite (Chloroquine sensitive (D6) or Chloroquine resistant (W2) strains) as a measure of activity.

**RESULTS:** The extracts exhibited an IC50 at 3.9 ± 0.32 µg/ml (D6) and 8.9 ± 0.25 µg/ml (W2) p ≤ 0.05 control Vs test by Student’s t-test at concentrations not cytotoxic, CC50 of 2800.8 µg/ml. Two compounds isolated from the extract indicated an IC50 at between 4.27±0.00 - 66.42 ± 4.99 µg/ml for both Chloroquine sensitive and resistant strains of parasite with reasonable selectivity index (>2.9).

**CONCLUSIONS:** The total extract/phytochemicals of the plant has reasonable anti-plasmodial activity. Further evaluation of this activity in suitable mammalian models and human while using the isolated compounds as biological makers for quality assessment in formulation, would lead to the realization of an affordable complimentary anti-malarial for the people in the tropics.

**FUNDING SOURCE:** WHO-TDR project ID A30707: An investigation of the safety, efficacy and chemistry of herbal medicines used traditionally for treatment of malaria

Sarah Touger1, Andrea G. Mann,1 the ACTwatch Group,2 Yozoume Ye,3 Idriissa A Kourgueni,4,5 Rebecca Thomson,1,4 John H. Amuasi,3,4,5 Ruilin Ren,1 Barbara A. Willey,1 Daniel Ansong,4,5 Katia Bruxvoort,4,5 Graciela Diap,1 Charles Festo,1 Boniface Johannes,1 Admirabilis Kaloleo,1 Oumarou Malam,1 Blessing Mberu,1 Salif Ndiaye,1 Samy Blay Ngahou,1 Moctar Seydou,1 Mark Taylor,1 Marilyn Wamukoya,1 Fred Arnold,1 Kara Hanson,1 Catherine Goodman1

1London School of Hygiene and Tropical Medicine, Keppeell Street, London, UK, WClE 7HT; 2Population Services International (PSI), Malaria & Child Survival Department PO Box 43640 Nairobi, Kenya; 3ICF International, 11785 Beltsville Drive, Calverton, MD, USA; 4Centre International d’Études et de Recherches sur les Populations Africaines (CIERP), Niamey, Niger; 5Institut National de la Statistique, 182 Rue de la Siraba, BP 3416, Niamey, Niger; 6Ifakara Health Institute, Plot 453, Kiko Avenue Mikocheni, Dar es Salaam, PO Box 78 373, 7University of Minnesota School of Public Health, Mayo Memorial Building, 420 Delaware Street S.E., Minneapolis, MN 55455, USA; 8Komen Anoyke Teaching Hospital (KATH), PO BOX KS 1934, Kumasi, Ghana; 9 Kwame Nkrumah University of Science & Technology, Department of Child Health, School of Medical Sciences; 10 Drugs for Neglected Diseases initiative, 15 Chemin Louis-Dantur, Geneva, 1202, Switzerland; 11African Population and Health Research Center, AFHRC Campus, 2nd Floor, Mango Close, Off Kisora Road, PO Box 10787-00100, Nairobi, Kenya; 12 Centre de Recherche pour le Développement Humain (CRDH), Dakar, Senegal
BACKGROUND: The Affordable Medicines Facility - malaria (AMFm) aimed to improve access to quality-assured artemisinin combination therapies (QAACTs) and reduce use of monotherapies. It subsidized QAACTs at the factory gate for distribution through existing supply chains. This was complemented by manufacturer negotiations to reduce prices and supporting interventions, including recommended retail prices (RRPs). AMFm was implemented in eight national-scale pilots in sub-Saharan Africa, with over 340 million treatments ordered by January 2013. An independent Evaluation reported large improvements in QAACT availability, price and market share in six of the eight pilots. AMFm has remained controversial, provoking debate about whether subsidies are passed onto consumers or absorbed by the supply chain.

METHODS: We assessed the extent to which the subsidy was passed onto the consumer using price and mark-up data from outlet surveys conducted for the independent Evaluation (based on the ACTwatch outlet survey methodology). Nationally representative surveys of antimalarial stockists were conducted at baseline (2009/10) and follow-up (2011) in each pilot. Data from 19,625 private for-profit outlets were used to calculate changes between baseline and endline in prices and mark-ups for QAACTs, which were compared with those on other antimalarials.

RESULTS: We found that QAACT price decreased by US$1.28 to US$4.34 and absolute retail markups on QAACTs decreased by US$0.31 to US$1.03, in six of the eight pilots. Prices and markups on other antimalarial classes also changed over the evaluation period, but not to the same extent. Despite the reductions in QAACT prices and absolute markups, prices and markups on non-artemisinin therapies remained lower than those on QAACTs in all but two pilots. We further examined the degree of price transmission by presenting the association of prices with RRPs, and found that RRPs acted as a price floor. Moreover, we found evidence of further scope for price reductions in all but two pilots, and that the wholesale prices that retailers face may be a limiting factor to further price reductions.

CONCLUSION: The evidence presented here will help countries decide whether private sector subsidies are likely to be effective in reducing consumer prices, and what supporting mechanisms are needed to optimize subsidy transmission.

P758: Impact of artemisinin based combination therapy (ACTs) repeated treatment on the prevalence of Plasmodium falciparum Drug resistance molecular markers (Pfcr7 and Pfmdr1).

Aliou Traoré, Demba Dembélé, Bakary Sidibé, Sekou Touré, Sekou Koumaré, Amadou Togo, Sanogo Kassim, Doumougo Ogbara and Abdoulaye Dijimdé

ACTs are currently used as the malaria first-line treatment in most endemic countries. The aim of this study was to access the impact of repeated treatment with AS+AQ and AR-L on Pfcr and Pfmdr1, in a 3 years randomized clinical trial in Bougoula (Mali). We used WHO 28-day standard in-vivo protocol. Overall 521 blood spoted filter papers were analyzed; mutations frequencies on Pfcr and Pfmdr1 genes were compared before and after intervention. In the AS+AQ arm we observed a base line frequency of 41.6% against 6.2%, 18.2%, 7.1% and >93% in the second, third and fourth episodes of malaria. For the Pfcr76T gene we observe a besline frequency of 58.9% against 88% during the first episodes and >93% in the second, third and fourth episodes of malaria. For the Pfmdr1-86Y gene we observe a baseline frequency of 41.6% against 6.2%, 18.2%, 7.1% and 0% on Pfmdr1-86Y gene for episodes 1, 2, 3 and 5 respectively. Concerning Pfcr76T gene the base line frequency was 58.9% against 59.1% and 88.8% for episodes 1, 2 and 3 respectively. This study demonstrate that there is a significant increase in Pfmdr1-86Y, and Pfcr76T mutants after treatment with AS+AQ and a significant decrease of Pfmdr1 mutations after treatment with AR-L. Despite the presence of artemisinin, the CTAs select the molecular markers of resistance to the partner molecule.

KEYWORDS: Malaria, Repeated treatment, Molecular markers, Drug resistance, and Plasmodium falciparum.

P759: Towards release control programs of the malaria mosquito Anopheles gambiae: Can heterotic supermales solve the reproductive performance deficiencies of laboratory-produced males?

Nikru E. Ekechukwu*, Rowida Baeshen* and Frédéric Triplet*
Centre for Applied Entomology and Parasitology, School of Life Sciences, Keele University, UK

BACKGROUND: The success of vector control strategies aiming to decrease disease transmission via the release of sterile or genetically-modified male mosquitoes critically depends on mating between laboratory-reared males and wild females. Mosquito colonization and laboratory maintenance lead to profound genetic and phenotypic changes that may negatively affect male mating performance.

METHODS: We compared, the sperm length, and accessory gland and testes size in male progeny from field-collected females and laboratory strains of Anopheles gambiae sensu stricto colonized from 1 to over 25 years ago. Next, exploiting the principle of heterosis, we produced ‘supermales’ by crossing the KI and Mopti strains respectively colonized 25+ and 7 years ago. The potential fitness advantage of supermales was further demonstrated through detailed fitness experiment of group and individual male reproductive success.

RESULTS: The results show that sperm length steadily decreases with the age of mosquito colonies due to inbreeding, whilst selection for laboratory conditions leads to larger testes but smaller accessory glands. In group mating cages, no apparent differences in female insemination rates and fecundity were found between supermales and males from their inbred parental strains. However, the sperm of supermales transferred to females was twice as large and significantly more active than that of older strains. Finally, individual male reproductive success studies revealed that males from the old inbred KI strain achieved higher female insemination rates than Mopti males and supermales but were frequently infertile. In contrast, females mated with supermales produced significantly more eggs than when mated with inbred ones, and this ultimately resulted in a 4.2-fold increase in the mean number of larvae fathered by supermales compared to the inbred KI strain.

CONCLUSIONS: These results validate the use of heterosis for creating males with improved reproductive success from inbred mosquito lines and have important implications for malaria control strategies relying on male mosquito releases.

P760: Characterisation of bacterial diversity in the midguts of wild Anopheles gambiae mosquitoes and the impact of natural midgut bacterial communities on Plasmodium falciparum sporogonic development

T Tsapi
LRP/OCEAC

Malaria remains the most impactful vector-borne disease worldwide and a number of methods used to control the disease are focus on combined interventions, among them the insecticide-treated nets (ITNs) and treatment with effective antimalarial drugs. Despite these methods some resistance have been observed and due to unavailability of an effective vaccine, a recent studies now focus on tripartite interactions between vectors, parasite and the vector's intestinal microflora at the molecular level have revealed complexities that can drastically affect immune responses and Plasmodium densities in mosquitoes. Now the influence of environmental factors on the Plasmodium transmission success have been study, we present here the diversity of natural midgut bacteria at different stage of Anopheles gambiae population in four localities in Yaounde Cameroon. Bacterial communities of wild...
Anopheles gambiae mosquitoes was recovered using a conventional culture technique on MacConkey medium and sequencing using the 16S rRNA gene. Interestingly, the results gut community revealed that the enterobacteriaceae family was dominant in all developmental stage and the main genera were Escherichia-shigella and Serratia with 55% and 38% respectively in adult female mosquitoes. This diversity was previously described except here where we report the presence of Delfia genus in Anopheles mosquito; the genus Enterobacter was found at larval stage and adult males and this is in contrast with others studies. We next measured the effects of these natural bacterial isolates to Plasmodium falciparum infection prevalence and intensity over multiple infectious feedings using a meta-analysis. Our investigations to verify the potential role of field isolated bacteria shown that the prevalence and intensity of Plasmodium falciparum infection was drastically reduced when mosquitoes were first challenged with Pseudomonas stutzeri, Serratia marcescens and Escherichia coli whereas Enterobacter sp has no detectable effect which is contrast with the recent study. The details of natural mosquito gut and their effects on natural Plasmodium falciparum are now study but the mechanisms used by bacteria remain poorly elucidated. Deciphering microbe-pathogen interactions remains the challenge and may offers new perspectives to control malaria transmission.

**P761: The Process and Influences on the Annual Operational Planning (AOP) and Budgeting Processes in Kenya’s Health Sector**

**Benjamin Tofa**1, Sassy Molyneux2,3, Catherine Goodman12

1KEMRI-Wellcome Trust Research Programme, Kilifi, Kenya; 2Centre for Tropical Medicine, University of Oxford, UK; 3London School of Hygiene and Tropical Medicine, UK

**INTRODUCTION:** Operational planning in public sectors has been considered as an important tool for translating government sector policies and strategic objectives into day to day operational activities. For a long time, public sectors in developing countries, including the health sector, have had a problem of misalignment between policy, planning and budgeting. The Medium Term Expenditure Framework (MTEF) process was introduced and widely adopted as a tool to help with this alignment. The Ministry of Health (MoH) in Kenya adopted MTEF in the early 2000s and in 2005 introduced the Annual Operational Plan (AOP) as a way of implementing the sector strategic plan, and of aligning planning and budgeting.

**METHODS:** Through a qualitative approach involving document reviews, participant observation and key informant interviews, this study sought to critically examine the AOP and budgeting processes at national level in Kenya’s health sector; and factors influencing these processes. The policy analysis triangle was used as a framework for analysis.

**FINDINGS:** The Kenyan health sector is still struggling to achieve planning and budgeting alignment, and has no structured way of balancing national and peripheral level priorities during planning. Several factors have contributed to challenges in implementing the AOP process as designed in the policy and strategic documents. These factors include poor stakeholder participation particularly by non-governmental actors, inadequate leadership and coordination by the MoH, and the prevailing political environment within the Ministry of Health and the broader government. There is however lots of optimism among many key actors within the sector; and a conviction that if these challenges are addressed, the planning and budgeting would be improved.

**CONCLUSION:** The inadequacies in the planning process may have consequences for both resource mobilization and the achievement of the health sector strategic objectives. Strategies to enhance operational planning could include greater flexibility in planning tools used at the periphery, strengthening mechanisms for stakeholder engagement, integration of the ministry’s planning and budgeting functions under one unit; and better coordination and political leadership on the planning process, particularly during times of political transition.

**P762: Evaluation of Olyset Plus, a permethrin / piperonyl butoxide combination net for control of pyrethroid resistant mosquitoes in Tanzania**

Patrick K. Tungu1,2, Robert C. Malima1,2, Lauren A. Wright1,3, Natacha Protobopou1,2, Benard Batengana1,3, Joseph B. Myamba1,3, Matt J. Kirby1,4, Stephen M. Magesa1,2, William N. Kisinza1,3 and Mark W. Rowland1,3

1National Institute for Medical Research, Mwanza, Tanzania; 2London School of Hygiene and Tropical Medicine, WC1E 7HT, London, UK; 3Pan-African Malaria Vector Research Consortium; 4RTI International, Global Health Division, Nairobi, Kenya

Combination mosquito nets incorporating two unrelated insecticides or insecticide plus a synergist are designed to control insecticide-resistant mosquitoes. Olyset Plus is a long-lasting combination net incorporating permethrin and a synergist piperonyl butoxide. An experimental hut trial was conducted in NE Tanzania to compare the efficacy of Olyset Plus against Olyset Net (long-lasting insecticidal net incorporating permethrin only) and a conventional treated net treated with permethrin at 500mg/m². The hut trials were tested against free flying pyrethroid-susceptible Anopheles gambiae s.l. The results showed that, un washed Olyset Plus and Olyset Net induced very high mortality (>97%) and after 20 washes of the two products, Olyset Plus induced significantly higher mortality (90.5% vs 74.4%) and blood feeding inhibition (100% vs 87.5%) than Olyset Net. In laboratory tunnel test against pyrethroid-resistant Cx. quinquefasciatus the unwashed Olyset® Plus induced higher mortality (74.6%) compared to unwashed Olyset Net (0%) and bloodfeeding inhibition (68% vs 32%). Over twenty washes, there was a decline in mortality of both products in the tunnel test. Percentage mortality of unwashed Olyset Plus versus Olyset Net in cone test against resistant mosquitoes was as follows: An gambiae susceptible: 100 vs 100%; An gambiae kdr east: 100 vs 66%; An gambiae Muleba: 95 vs 25%; An arabiensis Moshi: 96 vs 0%. As a long lasting net and tool to control pyrethroid resistant mosquitoes, Olyset® Plus has the potential to be an effective against a range of pyrethroid resistant Anophelines.

**P763: Plasmodium knowlesi infection in INDIA: differential drug resistance pattern of Plasmodium species**

Rupesh Tyagi1, Manoj Das2, Shiv Singh3 and Yagya Sharma4

1Department of Biotechnology, All India Institute of Medical Sciences, New Delhi, India; 2National Institute of Malaria Research, Dwarka, New Delhi, India; 3G.B. Pant Hospital, Port Blair, Andaman and Nicobar, India

**BACKGROUND:** Plasmodium knowlesi is a monkey malaria parasite but it can also infect humans. It can cause very high parasitemia in humans leading to anaemia and other complications. The human P. knowlesi infections had been reported from several Southeast Asian countries excluding India, but its drug susceptibility profile in mixed infection cases remains unknown.

**METHODS:** We have checked 445 malaria patient samples for the Plasmodium knowlesi infections. The chloroquine resistance transporter (CRT) and dihydrofolate reductase (DHFR) genes of P. knowlesi and other Plasmodium species were sequenced from the clinical isolates obtained from malaria patients living in Andaman and Nicobar Islands, India. The merozoite surface protein1 and 18S rRNA genes of P. knowlesi were also sequenced from these isolates.

**RESULTS:** Among 445 samples analyzed, only 53 of them had Pl knowlesi specific gene sequences. While three of 53 cases (5.66%) had P. knowlesi mono-infection, rests were co-infected with P. falciparum (86.79%, n=46) or P. vivax (7.55%, n=4), but none with P. malariae, or P. ovale.

**CONCLUSIONS:** There was discordance in the drug resistance associated mutations among co-infecting Plasmodium species as the P. knowlesi isolates contained the wild type sequences of CRT and DHFR but the respective P. falciparum genes had mutations at the
key amino acid positions associated with higher level of chloroquine and antifolate drug resistance. The mutation pattern indicates that the same patient, having mixed infection, may be harboring the drug sensitive \textit{P} \textit{knowlesi} and a highly drug resistant \textit{P} \textit{falciparum} parasite. Thus, larger human population in Southeast Asia may be at the risk of \textit{P} \textit{knowlesi} infections than reported so far. The different drug susceptibility genotypes of \textit{P} \textit{knowlesi} than its co-infecting \textit{Plasmodium} species in mixed infections would help malaria control programs to formulate the appropriate strategy to control this disease.

**P764: Exploring ownership, use and expenditure on different malaria preventive tools among different socioeconomic groups in Southeast Nigeria**

Nkolika Uguru

**BACKGROUND:** In Nigeria, malaria is responsible for 60% of outpatient visits and the leading cause of under-five mortality while also placing a heavy economic burden on the country. Given the level of disease burden and economic impact on the population in the country, it is essential that malaria control interventions are cost effective and affordable. Inequity in malaria prevention tools has been demonstrated across many sub-Saharan African countries including Nigeria. This will reduce the level of coverage needed to eradicate malaria. This study therefore assessed the current level of ownership, utilization and expenditure on different malaria preventive methods among different SES groups to determine if there are any residual inequities.

**METHODS:** A cross-sectional survey using semi-structured interviewer administered questionnaire was administered to 500 households systematically selected from two villages in Enugu state, south-east Nigeria. Information was collected on ownership, utilization and expenditure on various malaria preventive methods. Information was also collected on the per capita household food expenditure and assets ownership of respondents to determine their socio-economic status.

**RESULTS:** There was high ITN ownership (73%) and utilization (71.2%), 40% utilization was by children under 5. No significant inequity was found in ownership and utilization of bed nets. Utilization of other malaria preventive tools like aerosol spray, window and door netting, indoor residual spray was more in the higher SES group ($p<0.05$). The poorer SES group used more traditional prevention means like drinking of herbs, smoke, bush clearing. It was also observed that the higher SES group spent more on other preventive methods than the poorer SES groups.

**CONCLUSION:** The ownership and utilization of insecticide treated nets was similar across SES groups because of the free bednet scheme in the region. The lower SES groups have a disparity in the use of other malaria preventive tools because of the cost implications. Therefore strategies to deliver other malaria prevention methods need to be put in place by the government to ensure equity in all malaria prevention tools and improve on malaria eradication agenda.

**P765: Perception of Malaria Diagnosis using RDT by Proprietary Patent Medicine Vendors:** Evidence from a pilot study in six states of Nigeria

Jennifer Anyanti, Chinaza Ujuju, Chinedu C. Okoli, Benjamim C Uzochukwui

**Society for Family Health, Abuja, Nigeria**

**BACKGROUND:** Nigeria is one of the developing countries where private drug shops still play a vital role in disease management including malaria. Patients with fever cases are most likely to first seek treatment from these private drug shops referred to as Proprietary Patent Medicine Vendors (PPMVs) in Nigeria. WHO recommends parasite based diagnosis before malaria treatment; hence it is important to explore the perception of PPMVs about conducting malaria Rapid Diagnostic test (mRDT), in their outlets.

**RESEARCH QUESTION:** What is the PPMVs perception about conducting Malaria mRDT in their outlet?

**METHODODOLOGY:** Qualitative study using focus group discussion (FGD) was conducted with PPMVs involved in a pilot study to explore their perception about conducting mRDT for clients seeking treatment for malaria. These PPMVs selected from six states of Nigeria were first trained and deployed to their various states to conduct malaria RDT for clients seeking treatment for fever cases at their outlets. Eleven FGDs were subsequently conducted with the PPMVs.

**RESULTS:** Findings show that PPMVs perceived confirmatory diagnostic with mRDT as easy, fast, safe and accurate method of confirming malaria illness prior to treatment especially in rural areas where microscopic diagnosis may not be available. RDT would prevent over administration of anti-malaria through repeated treatment of undiagnosed illness in rural communities. It would also enhance better client counselling before malaria treatment by the PPMVs. The PPMVs also perceived RDT by PPMVs as a useful tool to enlighten the communities that all fever cases are not malaria. They also affirmed that scaling up malaria diagnosis through PPMV signifies willingness of the government to eradicate Malaria

**CONCLUSIONS:** PPMVs in Nigeria have a positive perception about conducting malaria diagnosis using the RDT. They are aware of the benefits malaria RDT services portend for effective malaria diagnosis and case management and demonstrated willingness to provide mRDT diagnosis in their communities if given the opportunity.

**P766: Presumptive treatment of childhood malaria in 3rd tier hospitals in southeast Nigeria; whom to blame?**

Maduka D. Ughasoro, Chinedu C. Okoli, Benjamim C Uzochukwui

1. Department of Paediatrics, University of Nigeria Enugu Campus, Nigeria; 2. Department of Surgery, Nnamdi Azikiwe Teaching Hospital, Nnewi, Nigeria; 3. Department of Community Medicine, University of Nigeria Enugu Campus, Nigeria.

**BACKGROUND:** Presumptive treatment of childhood-malaria (PTCM) is common in tertiary hospitals in Nigeria. Delayed laboratory result is mostly blamed with little attention on patients and health-care-providers roles. The aim of this study is to determine patients, health-care-providers and laboratory attributes that sustain PTCM in Southeast Nigeria.

**METHODS:** Qualitative data were collected by two focus-group discussions for parents/guardians, and 10 in-depth interviews; 6 doctors and 4 laboratory scientists in two tertiary hospitals. Results: FGDs: All participants agreed to malaria test. Majority accept to come back for full treatment at later date, provided that some form of treatment is commenced. Majority affirmed that doctors rarely explain their prescriptions, but are more interested with their children improvement.

**PROVIDERS IDIs:** All accept to the practice of PTCM. Reasons were: 1) Malaria is endemic and should be suspected and treated. 2) Microscopy takes 2 days to be available; parents want immediate treatment for their children, thus delay may lead to self-medication. 3) Relying on result for decision to treat creates the impression of incompetent. 4) Rapid diagnostic test kits (RDTs) are not available in the clinics and even if deployed there is doubt on its reliability. 5) Patients except those seen early must have wasted time before being reviewed, so wasting more time on investigation does not worth it. 6) Parents always request for drugs, whenever informed that treatment will succeed lab. result. 7) Attempt to withhold malaria treatment may be feasible in suspected uncomplicated malaria, if severe malaria; antimalarial-treatment has to start immediately.

**Laboratory scientists’ IDIs:** 1) Malaria test is done in batches, and it takes 24 hours to be ready, thus malaria microscopy cannot be an urgent test. 2) Request of malaria test with other investigations in the same form, contribute to the delay. 3) RDTs have not been deployed to the laboratory facilities.
CONCLUSIONS: Parents are willing-to-accept antipyrhetics, multivitamins e.t.c. for uncomplicated cases and intravenous fluid, blood transfusion, oxygen e.t.c for children with features of severe cases, on day zero, for antimalarial drugs to be added later. Doctors should accept and use RDTs in clinics to confirmed malaria cases on day zero.

P767: Schistosoma and microfilaria infection are associated with an increase of antibodies to Plasmodium falciparum sexual stage antigens

Ulysses Ateba-Ngoa 1,2, Teun Bousema 4,6, Will Roefen 1, Bertrand Lell 1,3, Ayola Akim Adeginka 1,2, Peter G. Kremsner 1,2, Maria Yazdanbakhsh 1,4, 1Centre de Recherches Médicales de Lambaréné, BP : 118, Lambaréné, Gabon, 2Institut für Tropenmedizin, Universität Tübingen, Wilhelmstraße 27 D-72074 Tübingen, Germany; 3Department of Parasitology, Leiden University Medical Center, Albinusdreef 2 2333 ZA Leiden, The Netherlands; 4Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; 5Department of Immunology and Infection, London School of Hygiene and Tropical Medicine, London, United Kingdom;

Helminth infections appear to be a potential confounder in immunological studies on Plasmodium falciparum. However, despite an increasing body of evidence documenting the effects of helminth on malariometric indices it is still unclear whether a concurrent helminth infection can augment the susceptibility of the human host to gametocyte which could consequently increase malaria transmission. To address this we conducted a prospective study in Lambaréné (Gabon), an area where helminth and malaria are highly endemic. We hypothesized that helminth infected subjects will have a higher rate of gametocyte carriage and a higher antibody response to gametocyte antigens. Schistosoma haematobium and microfilaria infection were determined by urine filtration and by the Leucoconcentration technique, respectively. Gametocyte carriage was determined by microscopy and was used as a marker of current exposure to Plasmodium falciparum (Pf) sexual form. Antibody response to Pfs gametocyte antigens Pfs230 and Pfs48/45 was assessed by ELISA. All malaria and helminth infected subjects were treated at inclusion and their infectious status and antibody response was assessed again 6 weeks following treatment. Overall, a total of 287 subjects were included in our study. Among them, 229 (81%) had either Schistosoma or microfilaria infection while 52 (19%) carried Pf sexual forms. Gametocyte carriage was not different between helminth infected and uninfected subjects (19% vs 20%, p=0.46) regardless the species. The proportion of responders to Pfs48/45 was significantly higher in Schistosoma (76% vs 57%; p=0.004) and microfilaria infected subjects (80% vs 68%; p=0.038) compared to uninfected subjects. The proportion of responders to Pfs48/45 significantly decreased following treatment of S. haematobium (74% vs 63%; p<0.001) and/or malaria (78% vs 60%; p<0.001) at the pre and post treatment time points, respectively. In contrary, no differences were found for the antibody response against Pfs230. These results imply either an increased cumulative exposure of helminth infected subjects to Pf gametocytes or a better ability to produce antibody able to block gamete fertilization in the mosquito gut. In either case the implication for malaria control program can be important.

P768: Exploring ownership, use and expenditure on different malaria preventive tools among different socioeconomic groups in Southeast Nigeria

Nkolia Ugaru
Health Policy Research Group, College of Medicine University of Nigeria

BACKGROUND: In Nigeria, malaria is responsible for 60% of outpatient visits and the leading cause of under-five mortality while also placing a heavy economic burden on the country. Given the level of disease burden and economic impact on the population in the country, it is essential that malaria control interventions are cost effective and affordable. Inequity in malaria prevention tools has been demonstrated across many sub-Saharan African countries including Nigeria. This will reduce the level of coverage needed to eradicate malaria. This study therefore assessed the current level of ownership, utilization and expenditure on different malaria preventive methods among different SES groups to determine if there are any residual inequities.

METHODS: A cross-sectional survey using semi-structured interviewer administered questionnaire was administered to 500 households systematically selected from two villages in Enugu state, south-east Nigeria. Information was collected on ownership, utilization and expenditure on various malaria preventive methods. Information was also collected on the per capita household food expenditure and assets ownership of respondents to determine their socio-economic status.

RESULTS: There was high ITN ownership (73%) and utilization (71.2%), 40% utilization was by children under 5. No significant inequity was found in ownership and utilization of bed nets. Utilization of other malaria preventive tools like aerosol spray, window and door netting, indoor residual spray was more in the higher SES group (p<0.05). The poorer SES group used more traditional prevention means like drinking of herbs, smoke, bush clearing. It was also observed that the higher SES group spent more on other preventive methods than the poorer SES groups.

CONCLUSION: The ownership and utilization of insecticide treated nets was similar across SES groups because of the free bednet scheme in the region. However there was a disparity between the use of other malaria preventive tools because of the cost implications. Therefore strategies to deliver other malaria prevention methods need to be put in place by the government to ensure equity in all malaria prevention tools and improve on malaria eradication agenda.

P769: Evaluating the impact of malaria interventions in Nigeria

Ndubue K Ukohe; Azra Ghani; María-Gloria Basáñez
Department of Infectious Disease Epidemiology, Imperial College, London, United Kingdom

BACKGROUND: Malaria control has intensified globally with targets set by the World Health Assembly; the Roll Back Malaria Partnership and the Millennium Development Goals. These targets aim for the short-term goal of reducing the burden of malaria until it is no longer a public-health problem, and the long-term goal of reducing the global incidence to zero by progressively eliminating the disease in endemic countries. Goals are becoming more ambitious as countries record reductions in malaria incidence in the last ten years. In spite of this progress made, Africa still bears the enormity of the global burden of the disease, with Nigeria accounting for a quarter of the disease burden in the continent. A review of evaluation studies on malaria interventions show that the link between intervention scale up and decreasing trends in malaria incidence and deaths in Nigeria remains unclear.

METHODS: This lack of clarity between intervention scale up and decreasing trends in malaria incidence underscores the need for this project. Data on prevalence, morbidity and mortality were extracted systematically from baseline studies of malaria transmission in Nigeria from the year 2000 onwards. These data was geo-located, classified by state, and statistically analysed. Intervention coverage data on mosquitosets, Artemisinin-based combination therapy rates and Intermittent Preventive Treatment were collated and evaluated. Descriptive analysis was undertaken on the intervention coverage data to characterise the scale up and to uncover spatial variation in interventions uptake. Preliminary results informed the design of a survey to understand the impact of intervention scale up by region, and gaps between expected and observed impact. This survey focused on areas in which impact has been achieved and those in which further impact could be made to understand barriers to intervention effectiveness.

RESULTS: Whilst the final results of this work is being aggregated, the main objective is to provide evidence-based recommendations to the
OBJECTIVE: This research compares the different structures of malaria-endemic areas and those areas which have controlled the problem by combining quantitative and qualitative techniques and using macro-, meso- and micro-analysis

METHODLOGY: the level of analysis used in the present study, including the number of observations by analysis level. Structural analysis from 1960-2004 considered primary and secondary bibliography. 1998-2004 situational analysis included primary and secondary information from national, department, municipal and household levels

Results: This study was aimed at understanding of malaria transmission and control in Colombia with a socio-cultural and systemic perspective in order to inform policy and control strategy on how the disease burden can be reduced through a systemic and societal understanding and control strategies that are adequate and acceptable for local populations as well as the socio-political anatomy of the departments/regions.

The micro analyses at the municipal, village and household level correlated the findings of macro and meso level. The individual analyses shown in terms of health, education, incomes, and social welfare precarious indicators. All those indicators correlated with the persistence of malaria and other communicable diseases of poverty. The economic development models pursued in the country, region and localities are strongly associated with the level of malaria and more generally with low levels of well-being as evidenced in the overall diseases burden, low coverage of health interventions and consequently limited access to health services, education, and development activities.

P772: Investigation of the inhibitory effect of 1,4-diamino-2-butane on Plasmodium falciparum parasites

Riette van Biljon, Dina le Roux, Jandeli Niemand, Prof LynMarie Birkholtz
Department of Biochemistry, University of Pretoria

BACKGROUND: Polyamines are involved in a variety of important cellular functions like cell differentiation and division, macromolecule synthesis and regulating gene expression. Polyamines are also a promising target in malaria parasites as they are present in much higher levels in the highly proliferating parasites than in normal erythrocyttes. In this study, the antiproliferative effect of 1,4-diamino-2-butane (DAB), a putrescine analog, was investigated on Plasmodium falciparum 3D7 parasites.

METHODS: Proliferation inhibition assays were conducted in order to determine effective concentrations of DAB on the intraerythrocytic P. falciparum parasites. Subsequent investigation to better understand its antiproliferative activity included the production of reactive oxygen species and inhibition of recombinant AdoMet/ODC activity. Interactions between DAB and another polyamine biosynthesis inhibitor, difluoromethylornithine, were studied using isobole analysis and the ability of exogenous putrescine to reverse the antiproliferative effects of DAB was also investigated.

RESULTS: The IC 50 of DAB in P. falciparum 3D7 was determined to be 95.7 μM ± 8.5 μM (n=6±SEM). When intraerythrocytic parasites were treated with DAB in the presence of putrescine, a statistically significant (P< 0.05; n=3) reversal of the inhibition of DAB was observed after 72 h. According to the isobologram data (n=3), DAB and DFMO show an additive interaction with an FIC value of 0.5, tending towards antagonism. In the presence of DAB, the ODC domain retained 15.1 ± 4.1% of its activity while AdoMetDC retained 104.6 ± 14.9% of its activity. DAB therefore inhibits the activity of the recombinant P. falciparum ODC domain but not the AdoMetDC domain. A correlation was seen between the H2O2 control and the DAB treated parasite, both having higher fluorescence values than the untreated parasites. The difference between treated and untreated was not significant at P>0.05.

CONCLUSIONS: DAB was found to have an anti-proliferative effect on P. falciparum 3D7. Investigating its mechanism of action identified the ODC domain of the AdoMet/ODC enzyme as its primary target in the parasite.
P773: The antimalarial properties of citronellol- and geraniol-containing insect repellents

Robyn L van Zyl RL, Babita Cangi, Lizanne Conradie
Pharmacology Division, Department of Pharmacy and Pharmacology, Faculty of Health Sciences, University of Witwatersrand, South Africa; WITS Malaria Institute for Malaria, South Africa

BACKGROUND: Mosquito repellents used in the prevention of a malaria infection contain within their formulations, volatile essential oils from plants such as, Cymbopogon nardus (citronella), Cymbopogon citratus (lemon grass), Melissa officinalis (melissa) and/or Pelargonium odoratissimum (geranium). These aromatic plants are traditionally used by patients with clinical symptoms such as “fever” or “flu-like” associated with a malaria infection. The volatile, lipophilic terpenes contained within these complex oils are capable of crossing dermal layers into the systemic circulation and as such the question arises: can these volatile, lipophilic compounds effect the in vitro growth of P. falciparum?

METHODS: Commercially available essential oils of the aforementioned plants were analysed by GC/MS for the major constituents. The antimalarial properties of the essential oils and constituents were screened against the chloroquine-sensitive 3D7 strain of Plasmodium falciparum using the tritated hypoxanthine incorporation assay. The compounds were also evaluated for their ability to induce haemolysis, inhibit β-haematin formation and scavenge DPPH free radicals.

RESULTS: All essential oils possessed in vitro antimalarial activity with IC50 values ranging between 0.97 ± 0.09 and 4.88 ± 1.00μg/ml. The oils were active against the whole parasite, but did not do so by inhibiting β-haematin formation or inducing membrane instability of the red blood cell host. Trans-geraniol, a major constituent of all the oils possessed promising antimalarial activity (0.89 ± 0.09μg/ml), as did citronellol (1.66 ± 0.23μg/ml) and citral (0.37 ± 0.03μg/ml). None of the essential oils could scavenge free radicals.

CONCLUSIONS: Essential oils used as insect repellents have the potential to inhibit intra-erythrocytic parasites by inhibiting haemozoin formation or interfering with the redox status within the parasitized erythrocyte. However, further investigation is required to determine the transdermal dose required to achieve a therapeutic effect.

P774: In vitro evaluation of the effect of Newboldia laevis on the susceptibility of Plasmodium falciparum to chloroquine

Vanda Koko1, Olivia A Achoudou1, Kenneth Eyong2, Eugenie Madiesse1, Palmer Netongo1, Aristid H E Mbange1, Jean P Chedjou1, Fidelis Cho-Ngwa1 and Wilfred F Mbacham1
1Laboratory for Public Health Research and Biotechnologies, Biotechnology Center, UfJ, Cameroon; 2Department of Organic Chemistry, University of Yaounde I, Cameroon; 3Department of Biochemistry, University of Buea, Cameroon

BACKGROUND: Chloroquine was considered to be the most useful and widely used antimalarial drug due to its effectiveness, tolerance and reasonable cost but resistance to chloroquine emerged and spread rapidly leading to its withdrawal from the market in most malaria-endemic countries. Studies have shown that this observed tendency may be reversed in combination with resistance reversals or its effects potentiated with drugs that may act in synergy. Newboldia laevis commonly known as African border tree is reported to have anti-inflammatory, anti-oxidant, antimicrobial and antimalarial properties and let us to determine the effect of extracts from N. laevis on the sensitivity of P. falciparum field isolates

METHODS: Field isolates of P. falciparum collected from febrile patients were subjected to in vitro susceptibility testing together with chloroquine and the leaf/roots methanolic and ethanolic extracts from N. laevis (NL-1, NL-2, NL-3, NL-4 and NL-5). Five percent parasitemia were cultured in RPMI-1640 culture medium, supplemented with AlbuMAX II in the presence of NaHCO3 and HEPES for 72 hours. Schizonts were quantified by examination of thick blood films under the light microscope. To determine the genetic mutations predisposing to resistance, DNA was extracted from blood spots on filter paper, PCR/RFLP was used to determine pfcrt K76T mutation alongside the diversity markermsp-2. Time-Kill curves obtained were used to determine IC50 values.

RESULTS: Of the twelve P. falciparum isolates, 5 were chloroquine sensitive (CQS) with an IC50 ranging from 0.1023ng/ml to 0.1823ng/ml compared to chloroquine resistant (CQR) with IC50 from 10.3 -17ng/ml. Extracts from root bark (NL-1) showed the highest activity when combined with chloroquine, with an IC50 isolob change from 16.63mg/ml to 2.55mg/ml. Msp-2 genotyping showed the presence of 6 alleles and the K76T mutation proportion were 5/12 CQS, 4/12 CQR and 2/12 CQS+CQR.

CONCLUSION: We conclude that extracts showed a synergistic effect with CQ on the CQR isolates. An extract of N leavis NL-1(from root bark) could possibly serve as source for antimalarial candidates for use in combination with CQ.

P775: Treating febrile children in sub-Saharan Africa: evidence from national household surveys in Madagascar, Nigeria and Uganda.

Stephen Poyer1, Megan Littrell2, Kathryn O’Connell2, Tanya Shewchuk2, Vamsi Vasireddy1
1Population Services International (PSI), Nairobi, Kenya; 2PATH – Program for Appropriate Technology in Health; 1Independent consultant

BACKGROUND: Appropriate case management is a key control target for all childhood illnesses. The focus of indicators collected from population-based surveys is often restricted by illness, with little examination of polypharmacy or the use by caregivers of multiple sources of advice and treatment. Understanding how caregivers respond to an illness episode in its entirety merits further investigation.

MATERIAL AND METHODS: Nationally-representative household surveys focused on treatment-seeking behaviour for fever among children under five were conducted in 2012 in Madagascar, Nigeria and Uganda as part of the ACTwatch program. Detailed information on treatment-seeking behaviour was collected, including where advice and treatment was sought, and diagnostic services and medicines received at each source. Unlike standard population-based surveys the ACTwatch questionnaire uses an audit mechanism that enables brand and active ingredient details to be recorded from available medicine packages, reducing the likelihood of recall bias. Treatment indicators are tabulated by country, and cross-tabulated by urban and rural location.

RESULTS: In all three countries caregivers most commonly first sought treatment for a child’s fever at home (Madagascar 44%, Nigeria 48%, Uganda 61%). Treatment was first sought from the informal private sector for 20% of fevers in Madagascar. Additional source indicators will present the number of external sources visited and the source mix. Results will be presented on the proportion of children receiving single and multiple medicines and the distribution of medicine types received from each source. For example, in Uganda 16% of children received both an antimalarial and antibiotic during their fever episode.

CONCLUSIONS: Treatment-seeking for childhood illness is a dynamic process, frequently involving multiple treatments that are often sourced from several providers. This complex picture is masked by the standard approach to reporting population-based indicators. The information presented in this work has the potential to inform programming and health promotion campaigns.
P776: Detection of Single Nucleotide Polymorphisms in Drug Resistance Genes of Plasmodium falciparum by High Resolution Melt Curve Analysis

Martin Wahome1,2, Beth Mutai1, Sabah Omar3 & John Waitumbi1
1Walter Reed Project, United States Army Medical Research Unit – Kenya/ Kenya Medical Research Institute, Kisumu, Kenya; 2Institute of Tropical Medicine and Infectious Diseases, Jomo Kenyatta University of Agriculture & Technology, Nairobi, Kenya; 3Centre of Geographical Medicine Research Coast, Kenya Medical Research Institute, Kilifi, Kenya

BACKGROUND: The detection of single nucleotide polymorphisms (SNPs) is key in monitoring emergence and spread of antimarial drug resistance.

The traditional methods for detection of SNPs are expensive, requiring either sequencing or allelic discrimination by qPCR. We describe a non-probe real time PCR (RT-PCR) method for detecting SNPs in drug resistance genes of P. falciparum based on melt curve analysis post amplification.

METHODS: 102 clinical samples with confirmed malaria diagnosis of P. falciparum were analysed for SNPs at the following drug resistance gene loci: PfCRT (codon 76), Pfdfr1 (codons 184, 1034 and 1042), PfPdh (codons 436 and 581) and Pfdhr (codons 16, 22, 51, 59 and 108) using RT-PCR that incorporates SYTO 9 dDNA binding dye in a high resolution melt (PCR-HRM) master mix (Applied Biosystems, US), followed by melt curve analysis. Melting was performed from 60-95°C at 0.5% ramp rate. Performance of the assay was compared to PCR-RFLP for PfCRT and probe based qPCR for the other loci. Discordant samples (24) were sequenced and data used to determine assay sensitivity and specificity.

RESULT: Of the 102 samples analyzed, 78 (77%) had 100% concordance to the two methods. Compared to probe based qPCR, HRM concordance at Pfdhrs 436 was 99% and at 581, 100%, at PfPdhv strength concordance was 100% at 16 and 22, 98% at 51, 80% at 59 and 93% at 108, at Pfdhr1 concordance was 76% at 184, 97% at 1034 and 100% at 1042. In comparison to RFLP-PCR, HRM showed concordance of 93% at PfCRT 76. The implication of these data sets will be presented and discussed.

CONCLUSION: High Resolution melt offers a simple, viable and effective alternative for the detection of SNPs in Plasmodium falciparum drug resistant loci and can be easily adapted and expanded to investigate other genes with single nucleotide point mutations.

P777: Insecticidal properties of local plants use against Anopheles gambiae malaria vector in Burkina Faso, West Africa.

Wangrawa W.D.1,2, Badolo A.1,2, Guelbeogo M.3, Nebie R. C. H.3, Sagnon N.Y.1,2, Sanon A.3
1Laboratoire d’Entomologie Fondamentale et Appliquée- UFR SVT Université de Ouagadougou, 03 BP 7021 Ouagadougou, Burkina Faso; 2Centre National de Recherche et Formation sur le Paludisme(CNRFP), Burkina Faso; 3Institut de recherche en Sciences Appliquées et technologie(IRSAT), Burkina Faso

BACKGROUND: Malaria remains a serious public health problem killing mostly in Africa. It is estimated to 225 millions of cases and 781 000 deaths the burden of malaria in 2009. Malaria control is still largely not characterized. This study aims to deeply search for the novel is-ncRNAs in Plasmodium falciparum.

METHODS: Total RNAs from intraerythrocytic P. falciparum 3D7 were extracted. A specific is-ncRNAs library was constructed by size fragmentation and depletion of highly expressed rRNAs and U1-U6 snRNAs. Illumina/ Solexa paired-end sequencing technology was applied to sequence to library. Bowtie was used to map the sequencing reads to the P. falciparum 3D7 genome. Novel is-ncRNAs were obtained after known ncRNAs were filtered. Conservation and structure features of the novel is-ncRNAs were characterized by bioinformatics analysis. The expression of the novel is-ncRNAs were confirmed by RT-PCR and northern blotting assays. The expression profiles of the selected is-ncRNAs were detected by qRT-PCR.

RESULT: A total of 1,159 novel is-ncRNAs, including antisense ncRNAs, intergenic and intronic is-ncRNAs, in the parasite were identified. Bioinformatics analysis indicated that the intergenic is-ncRNAs were shown to be the least conserved among eight different Plasmodium species and antisense ncRNAs were the most conserved. Seven potentially novel classes of ncRNAs and two novel internal motifs were discovered from the intergenic is-ncRNAs. Sixty-two out of 80 randomly chosen novel is-ncRNAs were successfully validated by RT-PCR and six were further confirmed by northern blotting. All 13 selected novel is-ncRNAs showed significant differences of expression profiles between the early and late intraerythrocytic developmental stages and 11 out of the 13 molecules showed an obvious higher expression in the early stage than in the late stage. The expression levels of four antisense ncRNAs were shown to be co-regulated with that of their cis-encoded sense RNAs by qRT-PCR.

CONCLUSIONS: This study provides indispensable information to the whole noncoding transcriptome of the parasite and will help further function study of the novel is-ncRNAs in the intraerythrocytic development of P. falciparum.

P778: Systematic analysis and identification of novel is-ncRNAs in Plasmodium falciparum

Chunyan Wei1, Zhensheng Wang1, Tengfei Xiao1, Lianhui Zhang1, Heng Wang1
1Department of Microbiology and Parasitology, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences and School of Basic Medicine, Peking Union Medical College, 59 Dong Dan San Tiao, Beijing, 100005, China
2Laboratory of Bioinformatics and Noncoding RNA, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China

BACKGROUND: Intermediate-size (50-500nt) non-coding RNAs (is-ncRNAs) have been recognized as important players during the development of several eukaryotic organisms. The parasitic protozoa Plasmodium falciparum is the most deadly Plasmodium species that cause human malaria. Although identification of ncRNAs in P. falciparum has been conducted in several studies, the whole profile of is-ncRNAs is still largely not characterized. This study aims to deeply search for the novel is-ncRNAs in Plasmodium falciparum.

METHODS: Total RNAs from intraerythrocytic P. falciparum 3D7 were extracted. A specific is-ncRNAs library was constructed by size fragmentation and depletion of highly expressed rRNAs and U1-U6 snRNAs. Illumina/ Solexa paired-end sequencing technology was applied to sequence to library. Bowtie was used to map the sequencing reads to the P. falciparum 3D7 genome. Novel is-ncRNAs were obtained after known ncRNAs were filtered. Conservation and structure features of the novel is-ncRNAs were characterized by bioinformatics analysis. The expression of the novel is-ncRNAs were confirmed by RT-PCR and northern blotting assays. The expression profiles of the selected is-ncRNAs were detected by qRT-PCR.

RESULTS: A total of 1,159 novel is-ncRNAs, including antisense ncRNAs, intergenic and intronic is-ncRNAs, in the parasite were identified. Bioinformatics analysis indicated that the intergenic is-ncRNAs were shown to be the least conserved among eight different Plasmodium species and antisense ncRNAs were the most conserved. Seven potentially novel classes of ncRNAs and two novel internal motifs were discovered from the intergenic is-ncRNAs. Sixty-two out of 80 randomly chosen novel is-ncRNAs were successfully validated by RT-PCR and six were further confirmed by northern blotting. All 13 selected novel is-ncRNAs showed significant differences of expression profiles between the early and late intraerythrocytic developmental stages and 11 out of the 13 molecules showed an obvious higher expression in the early stage than in the late stage. The expression levels of four antisense ncRNAs were shown to be co-regulated with that of their cis-encoded sense RNAs by qRT-PCR.

CONCLUSIONS: This study provides indispensable information to the whole noncoding transcriptome of the parasite and will help further function study of the novel is-ncRNAs in the intraerythrocytic development of P. falciparum.
P779: Unraveling the Greater Mekong mystery: A comprehensive picture of the antimalarial market in Myanmar

Chris White,1 Tin Aung,2 Hnin Su Su Khin,1 Zaw Win1, Vamsi Vasireddy2, Stephen Poyer2, Tanya Shawcuck1
1 Population Services International (PSI), Myanmar, Yangon, Myanmar; 2 ACTwatch, Population Services International (PSI), Nairobi, Kenya

BACKGROUND: Myanmar is the largest country in mainland South-East Asia with a population of 59.78 million. Malaria remains a leading cause of morbidity and mortality in Myanmar. Despite considerable progress over the past 20 years in reducing the disease burden, malaria is a re-emerging public health problem due to climatic and ecological changes, development of multi-drug resistant Plasmodium parasite, and development of insecticide resistant vectors. In order to monitor trends in the availability, price, and volume of antimalarial medicines, Population Services International (PSI) Myanmar adopted the ACTwatch methodology and conducted nationally representative outlet surveys (OS) in 2012 and 2013.

METHODS: A nationally representative sample of all private outlets with the potential to sell or provide antimalarial medicines to a consumer was taken through a census approach in 61 wards in the urban domain and 65 village tracts in the rural domain, giving a total of 122 wards and 130 village tracts across Myanmar. The cluster was defined as wards in urban and village tract in rural areas. Sampling was conducted using a two-stage probability proportion to size (PPS) cluster design, with the measure of size being the relative cluster population. Baseline OS was conducted in 2012 and endline in 2013.

RESULTS: Data was collected from a total of 3658 outlets. Among outlets stocking antimalarials on the day of interview in 2012, patterns of antimalarial availability differed by type of outlet – the highest being across health workers (76.6%) and private health facilities (66.8%). Pharmacies and general retailers rarely stocked Atemoin Combination Therapy (ACT) but very high proportion of pharmacies (85%) and general retailers (80%) stocked oral artemisinin monotherapy. Health workers reported the highest stock of first-line quality assured ACT (FAACT) (76%). Besides availability of antimalarials in 2012, the presentation will discuss trends in availability and price of antimalarials, particularly focusing on ACTs and monotherapies.

CONCLUSIONS: Continued use of artemisinin monotherapies has shown to be a cause of resistance to artemisinin. Understanding the complete market picture of availability and price of antimalarials in Myanmar has potential to improve access to FAACT and regulate the availability of monotherapies, thereby strengthening the malaria control efforts in the region.

P780: Health workers experiences and perceptions of the impact of clinical trials on health care services in rural Coastal Kenya

Angwenyi Vibian1, Nancy Mwangome1, Patricia Njuguana1, Sassy Molyneux1,2 and Caroline Jones1,3

1 Health Systems Research Department; KEMRI/Wellcome Trust P.O. Box 230-80108, Kilifi-Kenya; 2 Clinical Trials Facility; KEMRI/Wellcome Trust P.O. Box 230-80108, Kilifi-Kenya; 3 The Ethox Centre, Department of Public Health, University of Oxford Old Road Campus, Headington, Oxford, OX3 7LF, UK; 4 The Centre for Clinical Vaccinology and Tropical Medicine, Nuffield Department of Medicine, Oxford University, Old Road Campus, Headington OX3 7LI, Oxford, UK

BACKGROUND: Clinical trials, particularly in low-income settings involve huge financial investments, often linked to existing health infrastructure, to support study related activities. Limited evidence exists of the impact on routine health care of these inputs or of potential benefits or harms to the health system they might generate. An EDCTP funded multi-country project was established to evaluate the impact of clinical trials (eICT) on health systems in sub-Saharan Africa. This paper reports on perceptions and experiences of health workers involved in a vaccine trial delivered through three rural health facilities in one partner country, Kenya.

METHODS: Data collection involved human resource audit (N=14); health facility audits (N=3); in-depth interviews with government employed health facility staff (with and without direct roles in the trial) and trial clinicians (N=14); and focus group discussions with community based fieldworkers employed by the trial (N=3). Data from the audits were managed using MS Excel to generate descriptive summaries. Qualitative data were managed in NVivo 8 and analysed using a framework approach.

FINDINGS AND DISCUSSION: Facilities involved in this trial received clinical care support, refurbishment, and an increased number of skilled staff. Government health facility staff appreciated training; salary top-ups; transport assistance for supplies and referrals; and an improved working environment. Trial staff reported benefitting from various trainings and increased skills and experience in running and managing trials. Immediate concerns about the trial were related to payment differentials between trial staff and health facility staff providing patient clinical care. Concerns were also expressed that once the trial ended there would be a return to drug shortages and more congestion at facilities leading to increased workload and low staff morale.

CONCLUSION: Trials benefit staff and contribute to improvements in routine health service provision especially in resource constrained settings. However, concerns arise with regards to the potential implications for staff and the functioning of health facilities when trial resources and support are withdrawn. Trial teams need to consider developing exit strategies with health ministry/stakeholders, identifying types of inputs that could be left behind, discussing ways of addressing potential problems and communicating plans to communities and facility staff.

P781: High prevalence of asymptomatic malaria in urban settings in Douala, Cameroon

Léopold Gustave Lehman,1 Jeanne Dina Mfon Pribo2, Calvin Tonga1, Hervé Nyabeyue Nyabayeyue1, Larissa Koudjip Nono1, Arlette Linda Ngapmen Yamadjí1, Lafontaine Kangam1, Antoine Mouangue1, Adolphe Dikoum Mbongo1, Loick Pradel Kojom Foko1, Sorelle Wakam Nobou1, Peguy Assomo Ndemb1, Nicolas Nolia1, Isabelle Matip Mbou4

1 University of Douala, Douala, Cameroon; 2 Cameroon Business Coalition Against Malaria, Tuberculosis and HIV/AIDS (CCA/SIDA), Douala, Cameroon; 4 The Ethox Centre, Department of Public Health, University of Yaoundé I, Yaoundé, Cameroon

BACKGROUND: Malaria remains a major health problem in Cameroon with 38% of consultations and 24% of deaths. The negative economical impact of malaria has encouraged a new approach targeting companies and communities through indoor spraying and distribution of Long Lasting Insecticidal Nets (LLINs). 2600 inhabitants of six neighborhoods, 829 workers of three enterprises were interviewed and screened with a mass diagnostic method based on malaria rapid blood test using pre-stained slides for fluorescence microscopy (CyScope®, Partec GmbH, Germany). Alongside, 785 children were screened in five schools. All positive cases were treated on the spot.

RESULTS: The prevalence of malaria in the 4212 participants was 37.23%, most of the infected persons (79.81%) being asymptomatic. The prevalence of malaria infection in enterprises, communities and schools was 24.49%, 38.81% and 45.47% (Chi²=83.1, p<0.0001) respectively. Children aged less than five years recorded the highest prevalence (41.09%, Chi²=28.9, p<0.0001). Only 38.18% of the 3641 respondents possessed a LLIN of which 31.73% were damaged. The average coverage was 2.24±1.23 pers/net in households of this group. Damaged LLIN was significantly associated with increased malaria infection (Chi²=7.82, OR=0.74[0.60-0.91]; p<0.005). Owners of LLIN were less affected (36.83%
P782: Binding partners of putative SWIB domain proteins in *Plasmodium falciparum*

Warren A. Vieira1,2, Pierre M. Durand1,2 and Thérèse L. Coetzter1,2,3

1Wits Research Institute for Malaria
2Department of Molecular Medicine and Haematology, University of the Witwatersrand
3National Health Laboratory Service, Johannesburg, South Africa.

**BACKGROUND:** Malaria infections, mainly due to *Plasmodium falciparum*, result in over six hundred thousand deaths a year. Programmed cell death (PCD) in metazoan cells involves an array of proteins, including MDM2 which binds to p53 via its SWIB domain and promotes the degradation of p53. Although it is hypothesized that *P. falciparum* undergoes PCD, no molecular participants for PCD have been identified in the parasite. Bioinformatics analysis has revealed two putative homologues of the MDM2/SWIB protein in *P. falciparum* (PF0910w and PF3D7_0611400) and this study aims to assess their molecular functions.

**METHODS:** Genomic DNA was extracted from cultured 3D7 *P. falciparum* parasites and used for the amplification of the SWIB domains of these two genes for directional insertion into the pGEX-4T-2 vector. Recombinant GST-fusion proteins were expressed in *E. coli*, purified by affinity chromatography and assessed by SDS-PAGE and immunoblotting, using an anti-GST-HP conjugate. The recombinant proteins were used for biopanning against a *P. falciparum* phage display library. The entire PF0910w gene was directionally inserted into the pARL2-GFP vector and used for the creation of transgenic *P. falciparum* parasites and localisation of the fluorescently–tagged protein.

**RESULTS:** Biopanning the recombinant proteins revealed several binding partners. PF0910w interacted with a conserved protein of unknown function – PF3D7_1303400; while PF3D7_0611400 bound to a component of the inner membrane complex (PF3D7_1003600), a putative serine-threonine protein kinase (PF3D7_1356800) and a putative 40S ribosomal protein (PF3D7_1342000). The fluorescently tagged PF0910w protein did not localise to the nucleus in the transgenic parasites as expected, but to a round structure next to the nucleus. The localization of the protein did not alter in response to heat stress, a PCD trigger for the parasites.

**CONCLUSIONS:** The data suggest that these MDM2/SWIB proteins may fulfil a different role in *P. falciparum* and may participate in novel pathways. Further work will be conducted to identify the organelle to which the PF0910w protein is localizing, and additional biopanning and bioinformatics experiments will assist in constructing an interacting protein network for these MDM2/SWIB proteins.

P783: Assessment of the malaria control programmes impact in Madagascar using fifteen serological markers of *Plasmodium falciparum* and *Plasmodium vivax* simultaneously integrated in a single MAGPIX®-Luminex magnetic beads-based multiplex assay

Kesteman T1, Ravaoarisa E1, Guillotte M1, Perraut R1, Mercereau-Puijalon O1, Rogier C1, Randrianarivelosia M1, Vigan-Womas E1

1Institut Pasteur de Madagascar, Unité de Parasitologie, 101 Antananarivo, Madagascar; 2Institut Pasteur de Paris, Unité d’Immunologie Moléculaire du Parasi, Paris, France; 3Institut Pasteur de Dakar, Unité d’Immunologie, Dakar, Sénégal

**BACKGROUND:** In Madagascar, the implementation of artemisinin-based combination therapies and malaria control interventions globally led to a decrease of malaria burden. However, recently malaria recrudescence was documented in some areas. Efforts to achieve malaria elimination need to be aligned with these changes of malaria epidemiology through the development of efficient methods to monitor the impact of malaria control programmes. The malaria infection rate could be evaluated by the measurement of immune responses at the population level. Here, we investigated the potential of a multiplex immunoassay based on the novel MAGPIX®-Luminex technology, to assess the humoral immune responses against fifteen antigens of *Plasmodium falciparum* and *Plasmodium vivax* simultaneously detected in a single assay.

**METHODS:** A cross-sectional study was carried out at the population level in 64 sites covering all the malaria epidemiological patterns in Madagascar. The sero-prevalence of antibodies (Ab) against a panel of pre-erythrocytic and erythrocytic recombinant proteins or peptides derived from 10 *P. falciparum* specific proteins (MSP1, AMA1, CSP, GLURP, SALSA, LSA1-41, LSA1-J, LSA3, PFE039W-DBL1a, and PFE039W-DBL3X-Var2CSA domains), 4 *P. vivax* specific proteins (MSP1, PVDBP, CSP-VK210 and CSP-VK247) and a peptides specific for the *Anopheles gambiae* saliva protein gSG6 (saliv1) was analysed with a magnetic bead-based immunoassay using the MAGPIX®-Luminex system.

**RESULTS:** The malaria MAGPIX®-Luminex multiplex assay developed here was reproducible and required a small volume of serum. Preliminary results obtained from a cohort 2,286 individuals (children and adults) living in areas with different transmission intensities show high prevalences (> 50%) of Ab against *P. falciparum* AMA1, MSP1, GLURP, LSA1-41 and PFE039W-DBL1a, antigens. We also found a significant prevalence of anti-PvDBP antibodies (56.4%) highlighting the importance of *P. vivax* transmission in Madagascar.

**CONCLUSION:** These results indicate that the epidemiological situation of malaria in Madagascar, in low as well as in high transmission conditions, can be efficiently monitored using *P. falciparum* and *P. vivax* antigens simultaneously combined in a single MAGPIX®-Luminex magnetic beads-based multiplex serological assay. The sero-prevalence of these plasmodial antigens in the whole population is useful for the assessment of the effectiveness of malaria control programmes in order to guide the future malaria elimination strategies.

P784: Sero-epidemiology of *Plasmodium falciparum* rosetting: Acquisition of PfEMP1 domain- and surface-reactive antibody is not associated with the acquisition of functional rosette disrupting antibodies.

Bouille Mikab1, Guillette Micheline1, Hessé Audrey1, Touré-Balde Alissatou1, Richard Vincent1, Tall Adama1, Trape Jean-Francois1, Mercereau-Puijalon Odile1, Vigan-Womas Emmanuel1

1Institut Pasteur de Paris, Unité d’Immunologie Moléculaire des Parasites, CNRS URA 2581, Paris, France; 2Institut Pasteur de Dakar, Unité d’Immunologie, Dakar, Sénégal; 3Institut Pasteur de Dakar, Unité d’Épidémiologie, Dakar, Sénégal; 4Institut de Recherche pour le Développement (IRD), URMITE, Dakar, Sénégal

**BACKGROUND:** In this study, we have capitalised on the longitudinal cohort study carried out in Dieblo Senegal, a holoendemic setting, to investigate the capacity of *Plasmodium falciparum* infected red blood cells (IRBCs) to bind uninfected red blood cells (IRBCs) is associated with severe malaria in African children. It involves specific interaction between RBC receptors and a subgroup of the clonally variant *P. falciparum* Erythrocyte Membrane Protein 1 (PfEMP1) adhesins, with RBC binding competence being assigned to the N-terminal DBL1a domain. Targeting rosette formation using small soluble inhibitors or specific antibodies are viewed as interesting strategies against severe malaria. To this end, we need to better understand the specific immune responses acquired in endemic area.

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**RESULTS:** In this study, we have capitalised on the longitudinal cohort study carried out in Dieblo Senegal, a holoendemic setting, to investigate the capacity of *Plasmodium falciparum* infected red blood cells (IRBCs) to bind uninfected red blood cells (IRBCs) is associated with severe malaria in African children. It involves specific interaction between RBC receptors and a subgroup of the clonally variant *P. falciparum* Erythrocyte Membrane Protein 1 (PfEMP1) adhesins, with RBC binding competence being assigned to the N-terminal DBL1a domain. Targeting rosette formation using small soluble inhibitors or specific antibodies are viewed as interesting strategies against severe malaria. To this end, we need to better understand the specific immune responses acquired in endemic area.

**CONCLUSION:** These results indicate that the epidemiological situation of malaria in Madagascar, in low as well as in high transmission conditions, can be efficiently monitored using *P. falciparum* and *P. vivax* antigens simultaneously combined in a single MAGPIX®-Luminex magnetic beads-based multiplex serological assay. The sero-prevalence of these plasmodial antigens in the whole population is useful for the assessment of the effectiveness of malaria control programmes in order to guide the future malaria elimination strategies.
the dynamics of antibody (Ab) acquisition in a cohort of: i) 32 children on a yearly basis during their first 10y of life, ii) 19 mothers during the last trimester of pregnancy and iii) 43 male adults (> 18y) enrolled in the follow-up study. Ab to the various recombinant PfEMP1-VaO domains were assessed by ELISA. The monovariant Palo Alto VarO parasite culture was used to monitor iRBC surface reactive antibodies by flow cytometry and functionally active Ab in rosette disrupting assays.

RESULTS: Results show variable sero-conversion kinetics in children. Reactivity to DBL1α, and the Head (DBL1α-CIDRy) domain readily increased after 2 years and reached the adult level before 5 years of age, whereas antibodies to the downstream domains were acquired later, some not reaching the adult level at 10y. VarO-iRBC surface reactivity strongly correlated with presence of antibodies reacting with DBL1α and the Head domain. However, acquisition of domain- and surface-reactive antibody was not associated with acquisition of functional rosette-disrupting or rosette-inhibiting antibodies, with less than 15% of anti-rosetting antibodies seroprevalence in the whole population (children and adults).

CONCLUSIONS: These results indicate that Ab reacting with rosette-forming parasites are acquired early in life, with domain-specific kinetics of Ab responses. They outline an unexpected dissociation between surface-reactive and anti-rosetting antibodies. Work is in progress to explore involvement of surface reactive Ab in other functional assays such as osponisation/phagocytosis of the iRBCs.


Thomas KESTEMAN1,2, Solofoniana A. RAFALIMANTSOA1,2, Harimahefa RAZAFIMANDIBY1,3, Herinaina H. RASAMIMANANA1,2, Vaomalala RARAHIMANGA1, Daudet RANDRIANASOLO1, Solo H. RAJAOBARY1, Marie-Cleemence RAKOTOARIVONY1, Arsene RATSIMBASOA1,2, Louise RANAIVO1, Mirasoa RANDRIANOMANANA1, Jocelyn RATOVONJIALO1, Suzanantsoa A. ZILERA1, Inès VIGAN-WOMAS2, Nohal ELISSA1, Laurence RANDRIANAOLO1, Alyssa FINLAY3, Milijaona RANDRIANARIVELOJOIA3, Christophe ROGIER1

1 Institut Pasteur de Madagascar; 2 URMITE (UMR 6236), Université Aix-Marseille II; 3 DVSE, Ministère de la Santé; 4 PNLH, Ministère de la Santé; 5 Faculté de Médecine, Université d’Antananarivo; 6 US Centers for Disease Control and Prevention; 7 Institut Pasteur de Paris

BACKGROUND: The malaria burden in Madagascar dropped down last decade, largely due to scale-up of control measures. Nevertheless, a significant rise in cases was observed in the surveillance system in February, 2012 in Vatovavy Fitovinany and Atsimo Atsinanana regions. These are located in South-Eastern Madagascar, where malaria is considered as hyperendemic and the population is supposed to be protected by its acquired immunity against Plasmodium. A preliminary analysis of data from health structures showed that the rise had begun in December, 2011. A multidisciplinary team was build up as to identify causes of the outbreak. The objective was to pinpoint factors associated with the risk of infection with Plasmodium sp., and to evaluate the effectiveness of control measures.

METHODS: In March, 2012 we conducted a cross-sectional case-control study in 20 randomly selected villages (clusters). We included approx. 22 households per cluster. The investigation involved the rapid diagnostic testing of all >6 month-old members of households, a questionnaire about socio-demographic data and exposure to malaria control measures, observation of housings, remote sensing of environmental and climatic conditions, and a survey of health systems. Forty bed nets (2 per cluster) have been sampled in order to evaluate their condition and the remanence of their insecticidal activity. We collected and analyzed Anopheles vectors in 2 sites.

RESULTS: All 20 randomized clusters have been studied; 1615 members of 440 households have been sampled and investigated. The mean Plasmodium infection rate was 26% (3-46%, depending on clusters) and the mean bed net use on the day before survey was 72% (32-93%, depending on clusters). The prevalence of Plasmodium sp. infections was higher in 6-14 year-old children (OR=1.5 as compared with 0-5 year-old), in rural areas (OR=6.4), in individuals not having slept under a bed net the night before (OR=1.9), and in people living in areas where shortages in antimalarial drugs of >1 month in the last 6 months have been reported (OR=1.4). Rainfalls were increased as compared with 3 last rainy seasons. Vectors collected were sensitive to pyrethroids. Nearly all insecticide-treated nets (ITN) collected showed a decreased insecticide activity and loss of physical integrity.

CONCLUSIONS: Increased rainfalls, rapidly declining insecticide activity of ITNs, a decrease in immunity against Plasmodium (possibly consecutive to control measures), and shortages in antimalarial drugs (possibly due to underestimation of needs) are responsible for, or have contributed to, the outbreak observed in South-Eastern Madagascar in 2011-2012. Malaria control measures should be strengthened in this region despite the apparent decline in malaria endemicity that has been observed during the previous years.

P786: Myth Buster: High sensitivity and specificity of clinical microscopy in rural health facilities in western Kenya under an EQA program.

Rebecca Wafula1, Edna Sang1, Olympia Cheruiyot1, Angeline Aboto1, Diana Meya1, Wendy Prudhomme O'Meara2,3,4
1 Academic Model Providing Access to Healthcare, Eldoret, Kenya; 2 Ministry of Health, Uasin Gishu County, Kenya; 3 School of Public Health, Moi University College of Health Sciences, Eldoret Kenya; 4 Duke University School of Medicine, Durham, NC; 5 Duke Global Health Institute, Durham NC

BACKGROUND: Microscopic diagnosis of malaria is a well-established and inexpensive technique that has the potential to provide accurate diagnosis of malaria infection. However, it requires both training and experience. Although it is considered the gold-standard in research settings, the sensitivity and specificity of routine microscopy for clinical care in the primary care setting has been reported to be unacceptably low. For example, in Tanzania and Kenya, sensitivity of about 70% and specificity of 47-61% of clinical microscopy compared to expert readings has been observed (Kaham-Maroo 2011, Zurovac 2006). As part of a cluster-randomized trial to improve diagnosis and treatment of malaria, we established a monthly external quality assurance program to monitor the performance of clinical microscopy in 18 rural health centres in western Kenya.

METHODS: One laboratory technician from each facility was sent to a two-week training at the Malaria Diagnostic Centre of Excellence in Kisumu, Kenya. Starting in October 2012, all slides prepared in the facility were archived by the staff and collected each month by the field team. Thirty slides per facility per month are randomly selected and reread by the study microscopist who is blinded to the facility results. Specificity and sensitivity is calculated by comparing the results recorded in the laboratory register to that of the study microscopist. The discrepant slides are re-read by a third research microscopist.

RESULTS: Technicians improved their microscopy sensitivity and specificity by an average of 18 percentage points following the two-week training. During the first six months of EQA, facilities in nine high transmission areas had an average sensitivity of 96% and specificity of 90% while nine low transmission facilities recorded 100% sensitivity and 82% specificity. Facilities with low specificity had equipment in poor condition. Excluding those facilities, average specificity was 89%. Low transmission facilities recorded the most improvement in specificity with 46 percentage points following the two-week training.

CONCLUSION: Maintaining high quality malaria diagnosis in high-volume, resource-constrained health facilities is possible. Clinicians can have confidence in laboratory results particularly for ruling out malaria as a cause of illness.
**P787: Detection of Single Nucleotide Polymorphisms in Drug Resistance Genes of Plasmodium falciparum by High Resolution Melt Curve Analysis**

**Martin Wahome**<sup>1</sup>, **Beth Mutai**<sup>1</sup>, **Sabah Omar**<sup>2</sup> & **John Waitumbi**<sup>2</sup>

<sup>1</sup>Walter Reed Project, United States Army Medical Research Unit – Kenya/ Kenya Medical Research Institute, Kisumu, Kenya; <sup>2</sup>Institute of Tropical Medicine and Infectious Diseases, Jomo Kenyatta University of Agriculture & Technology, Nairobi, Kenya; <sup>3</sup>Centre of Geographical Medicine Research Coast, Kenya Medical Research Institute, Kilifi, Kenya

**Email:** martwah@gmail.com, Beth.Mutai@usamru-k.org, somaru@kilifi.kemri-welcome.org; john.waitumbi@usamru-k.org

**BACKGROUND:** The detection of single nucleotide polymorphisms (SNPs) is key in monitoring emergence and spread of antimalarial drug resistance. The traditional methods for detection of SNPs are expensive, requiring either sequencing or allelic discrimination by qPCR. We describe a non-probe real time PCR (RT-PCR) method for detecting SNPs in drug resistance genes of *P. falciparum* based on melt curve analysis post amplification.

**METHODS:** 102 clinical samples with confirmed malaria diagnosis of *P. falciparum* were analysed for SNPs at the following drug resistance gene loci: PfCRT (codon 76), PfMDR1 (codons 1034 and 1042), PfPdh (codons 436 and 581) and PfDHFR (codons 16, 22, 51, 59 and 108) using RT-PCR that incorporates SYTO<sup>9</sup> dsDNA binding dye in a high resolution melt (PCR-HRM) master mix (Applied Biosystems, US), based qPCR for the other loci. Discordant samples (24) were sequenced and performed HRM analysis. Melting was performed from 60-95°C at 0.5% ramp rate. Performance of the assay was compared to PCR-RFLP for PfCRT and probe based qPCR for the other loci. Discordant samples (24) were sequenced and data used to determine assay sensitivity and specificity.

**RESULT:** Of the 102 samples analyzed, 78 (77%) had 100% concordance to the two methods. Compared to probe based qPCR, HRM concordance at PfPdh 436 was 99% and at 581, 100%; at PfDHFR concordance was 100% at 16 and 22, 98% at 51, 80% at 59 and 93% at 108, at PfMDR1 concordance was 76% at 184, 97% at 1034 and 100% at 1042. In comparison to RFLP-PCR, HRM showed concordance of 93% at PfCRT 76. The implication of these data sets will be presented and discussed.

**CONCLUSION:** High Resolution melt offers a simple, viable and effective alternative for the detection of SNPs in *Plasmodium falciparum* drug resistant loci and can be easily adapted and expanded to investigate other genes with single nucleotide point mutations.

**P788: Fetal haemoglobin and β<sub>s</sub>-globin gene cluster haplotypes in sickle cell anaemia patients from coastal Kenya**

**Alexander W. Macharia**, **George Mochamah**

<sup>1</sup>Kenya Medical Research Institute, Centre for Geographic Medicine Research Coast/Wellcome Trust Collaborative Program, Kenya

**INTRODUCTION:** Foetal haemoglobin (Hbf) remains the single most important factor associated with reduced morbidity and mortality in patients with Sickle cell anaemia (SCA). The variation in Hbf levels has been associated with a number of genetic variants amongst them are the 5’ β<sub>s</sub>-globin gene cluster haplotypes that are associated with varying disease severity, and named after regions where they are most prevalent; Bantu, Benin, Indian-Arab and Senegal. The prevalence of these haplotypes and their relation to Hbf levels in SCA populations in Kenya is poorly described. The aim of this observational study was to determine the prevalence of 5’ β<sub>s</sub>-haplotypes and Hbf levels in a cohort of children attending an outpatient clinic at the Kilifi District Hospital.

**METHODS:** In this study we included 134 children (mean age 8.78 ± 5.47 years) attending routine clinical management of SCA at the Kilifi District Hospital. Hbf levels were quantified using high performance liquid chromatography and the β-globin gene cluster haplotypes determined using restriction fragment length polymorphism on 8 polymorphic sites.

**SUMMARY OF PRELIMINARY RESULTS:** The Bantu haplotype was predominant haplotype at a homozygous frequency of 87% and accounted for the second haplotype in all the heterozygotes. The frequency of the Bantu haplotype in the 268 chromosomes analyzed was 93% with the rest of the β<sub>s</sub>-chromosomes being mainly of the atypical type apart from one patient who had the Benin haplotype. Mean Hbf level was 7.31±7.0%.

**DISCUSSION AND CONCLUSION:** In this population the Bantu haplotype remains the major haplotype accounting for 93% of the δ<sub>s</sub>-haplotypes across the coast of Kenya. There is little or no recombination with haplotypes from other regions as the Benin haplotype was the only haplotype identified amongst the geographically defined β<sub>s</sub>-haplotypes. The mean Hbf level of 7.3% confirms SCA patients with Bantu haplotype have a threshold below 10% that has previously been shown to confer protection against disease severity.

**P789: Estimates of the risk of placental infection and burden of low birthweight attributable to *P. falciparum* malaria in Africa in 2010.**

**P.G.T. Walker**<sup>1</sup>, **F. O. ter Kuile**<sup>2</sup>, **T. Garske**<sup>3</sup>, **C. Menendez**<sup>4</sup> & **A.C. Ghani**

<sup>1</sup>NIC Centre for Outbreak Analysis & Modelling, Department of Infectious Disease Epidemiology, Imperial College London, London, United Kingdom; <sup>2</sup>Child and Reproductive Health Group, Liverpool School of Tropical Medicine, Liverpool, UK; <sup>3</sup>Kenya Medical Research Institute—University of Oxford-Wellcome Trust Collaborative Programme, Kenyatta National Hospital Grounds, Nairobi, Kenya; <sup>4</sup>Barcelona Centre for International Health Research, Hospital Clinic-Universitat de Barcelona, Barcelona, Spain

**Pfalciparum** infection during pregnancy leads to adverse outcomes including low birthweight (LBW). Women acquire immunity to malaria in pregnancy over consecutive pregnancies and are most susceptible during their first pregnancy. Using a model of the parity-dependent immunity acquisition and estimates of the geographical distribution of fertility and *P. falciparum* transmission, we estimate the number of women who could be expected to experience placental infection in the absence of pregnancy-specific interventions in Africa. By fitting our model to patterns of excess LBW risk in women experiencing placental infection in Kilifi, Kenya and Ifakara, Tanzania, we then estimated the burden of malaria-attributable LBW. Without pregnancy-specific protection we estimate that, in Africa in 2010, 11.4% (95% Cr 10.7-12.1) million pregnancies would have experienced placental infection at some stage of gestation, accounting for 41% of the total 27.6 million live-births. Combining this with our estimated relationship between placental infection and LBW, we found the potential LBW burden due to placental malaria was 900,000 (95% Cr 530,000-1,240,000) LBW deliveries per year. The time at which the placenta becomes susceptible to infection, around the end of the first trimester, is a key period when we estimate 65% (95% Cr 61%-70%) of the potentially infected pregnancies first experience infection. Primigravidae experience a disproportionate 39% (95% Cr 33%-46%) of the total potential placental malaria-attributable LBW burden. These are the only contemporary estimates of the distribution of risk and associated LBW burden of malaria in pregnancy in Africa. They suggest that the risk of placental infection across Africa in unprotected women remains large. Prevention of malaria pre-conception or very early in pregnancy is predicted to have a major impact upon reducing LBW, particularly in primigravidae. Lifetime risk of LBW changes gradually with transmission, highlighting the need to maintain protection as transmission falls and the incremental benefit of malaria elimination.
**P790: Insecticidal properties of local plants use against Anopheles gambiae malaria vector in Burkina Faso, West Africa.**

WANGARA W.D.1,2, BADOLLO A1,4, GUELBOGO M.2, NEBIE R. C. H.1, SAGNON N.T.1,5, SANON A.1

1Laboratoire d’Entomologie Fondamentale et Appliquée - UFR SVT Université de Ouagadougou, 03 BP 7021 Ouagadougou, Burkina Faso, (email: a.badollo@gmail.com); 2Centre National de Recherche et Formation sur le Paludisme(CNRFP), Burkina Faso; 3Institut de recherche en Sciences Appliquées et technologie(IRSAT), Burkina Faso.

**BACKGROUND:** Malaria remains a serious public health problem killing mostly in Africa. It is estimated to 225 millions of cases and 781 000 deaths the burden of malaria in 2009. Malaria control is still heavily dependent of insecticides for vector control but the emergence of resistance to insecticides in Malaria vector population can jeopardize this control effort. The main objective of this study was to evaluate ovicidal, larvicidal properties of essential oil of local plants, namely, *Ocimum canum*, *Hyptis suaveolens*, *Hyptis spicigera* and *Lantana camara* extracts on *Anopheles gambiae* and their inhibiting activity of acetylcholinesterase activity (AChE).

**METHODS:** Young branches with leaves were collected from local plants and the extraction of essential oil was processed. Eggs and third-fourth stage larvae were used for bioassay tests based on WHO protocols. Different concentrations have been used for each plant essential oil and results have been assessed as hatchability rate after 48 hours exposure and larvae mortality after 24 hours and the lethal concentration calculated for each essential oil. The inhibition effect of essential oils on acetylcholinesterase activity has been evaluated using adaptation of the spectrophotometric method of Ellman.

**RESULTS:** All tested essential oils exhibited ovicidal and larvicidal activities. The LD50 and LD90 lethal doses value observed were 53.59 and 170.89 ppm respectively on eggs for essential oil. On larvae, the LD50 and LD90 values of this oil were 61 and 125 ppm respectively. The high inhibitory activity was observed with *O. americanum* and *H. suaveolens* essential oil whose IC50 were 0.21 and 0.55 µg/ml respectively.

**CONCLUSION:** Our results highlighted that essential oils of these plants have a potential as insecticides for malaria vector control and can be considered as an interesting source of natural and ecofriendly substances for vector control.

**P791: Pharmacovigilance Electronic Reporting System: A Rapid Response Drug Monitoring Platform**

Jayesh Pandit1, Edward Abwao2, Steven W. Macharia3, Christine Wasunna4

1Department of Pharmacovigilance, Pharmacy and Poison’s Board, Nairobi, Kenya
2International Technical and Education Center for Health, Nairobi, Kenya
3Centre for Clinical Research, Kenya Medical Research Institute, Nairobi, Kenya

**BACKGROUND:** The Pharmacovigilance Department at the Pharmacy and Poison’s Board (PPB), the National Drug Regulatory Authority in Kenya, receives suspected adverse drug reaction (ADR) and suspected poor quality medicinal products reports from all over the country. The subscribers are able view the electronic evaluation of their reports; print multiple copies for their records e.g. patient files; maintain copies of their submitted reports and be able to monitor the reporting trends, a feature that is useful for medicine and therapeutic committees (MTCs) in the various counties. At PPB, patient safety remains the core business. Despite the lack of adequate resources and appropriate structures to systematically support the detection and prevention of adverse drug reactions (ADRs) and other medicine-related problems, developing countries such as Kenya have no option but to strengthen the monitoring program, the Pharmacovigilance Electronic Reporting System (PV ERS).

**METHODOLOGY:** The ADRs are entered into a data base, Vigiflow™, developed by World Health Organization. During the 2011-2013 reporting period, 6297 suspected adverse drug reaction reports had been received at the National Pharmacovigilance Centre. As at May 2013, information from a total of 4685 (74.4%) reports have been entered into the Vigiflow™ database. Vigiflow™ is a web-based Individual Case Safety Report (ICSR) management system that is specially designed for use by national centres in the WHO Medicines Safety Programme.

**RESULTS:** 92% of the reports are ARVs related, Antibiotics 3.4% while Antimalarials consisted of 0.9%. Stavudine and Stavudine combination form the bulk of the reports at more than 70%.

**CONCLUSION:** The disease burden in Africa is not only pronounced due to lack of drugs but also presence of substandard drugs. The exact extent of the substandard, spurious or falsified medicines in Kenya is not well known. Monitoring both the quality and safety of the medicines after registration is important as most poor patients usually opt for over-the-counter medications since they can rarely afford to visit well-equipped hospitals which at times are far between. This means millions of patients risk health complications or even death due to use of poor quality medicines or drugs with adverse reactions. The PV ERS therefore will act as a way to monitor the quality of medicines already circulating in the Kenyan market. A client can report any suspected ADR or poor quality medicine using a computer or even the mobile phone. This report is then received immediately at the National Pharmacovigilance Centre which will then trigger action from PPB to prevent any more patients from being harmed.

**P792: Systematic analysis and identification of novel is-ncRNAs in Plasmodium falciparum**

Chunyan Wei1, Zhensheng Wang1, Tengfei Xiao2, Lianhui Zhang3, and Heng Wang1

1Department of Microbiology and Parasitology, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences and School of Basic Medicine, Peking Union Medical College, #5 Dong Dan San Tiao, Beijing, 100005, China; 2Laboratory of Bioinformatics and Noncoding RNA, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China

**BACKGROUND:** Intermediate-size (50-500nt) non-coding RNAs (is-ncRNAs) have been recognized as important players during the development of several eukaryotic organisms. The parasitic protozoa *Plasmodium falciparum* is the most deadly *Plasmodium* species that cause human malaria. Although identification of ncRNAs in *P. falciparum* has been conducted in several studies, the whole profile of is-ncRNAs is still largely not characterized. This study aims to deeply search for the novel is-ncRNAs in intraerythrocytic *P. falciparum*.

**METHODS:** Total RNAs from intraerythrocytic *P. falciparum* 3D7 were extracted. A specific is-ncRNAs library was constructed by size fractionation and depletion of highly expressed rRNAs and U1-U6 snRNAs. Illumina/Solexa paired-end sequencing technology was applied to sequence to library. Bowtie was used to map the sequencing reads to the *P. falciparum* 3D7 genome. Novel is-ncRNAs were obtained after known ncRNAs were filtered. Conservation and structure features of the novel is-ncRNAs were characterized by bioinformatics analysis. The expression of the novel is-ncRNAs were confirmed by RT-PCR and northern blotting assays. The expression profiles of the selected is-ncRNAs were detected by qRT-PCR.

**RESULTS:** A total of 1,159 novel is-ncRNAs, including antisense RNAs, intergenic and intronic is-ncRNAs, in the parasite were identified. Bioinformatics analysis indicated that the intergenic is-ncRNAs were shown to be the least conserved among eight different *Plasmodium* species and antisense RNAs were the most conserved. Seven potential novel classes of ncRNAs and two novel internal motifs were discovered from the intergenic is-ncRNAs. Sixty-two out of 80 randomly chosen novel is-ncRNAs were successfully validated by RT-PCR and six were further confirmed by northern blotting. All 13 selected novel is-ncRNAs showed significant differences of expression profiles between the early and late intraerythrocytic developmental stages and 11 out of the 13 molecules showed an obvious higher expression in the early stage than in the late stage. The expression levels of four antisense RNAs were shown to be correlated with that of their cis-encoded sense RNAs by qRT-PCR.

**CONCLUSIONS:** This study provides indispensable information to the whole noncoding transcriptome of the parasite and will help further
function study of the novel is-ncRNAs in during the intraerythrocytic development of P. falciparum.

P793: Comprehensive assessment of variation in the invasion ligand, eba175, by \textit{de novo} assembly of 600 worldwide field isolates of \textit{Plasmodium falciparum}

\textbf{Jason Wendler}¹, \textbf{Zamin Iqbal}², \textbf{Kirk Rockett}¹, \textbf{Philip Bejon}¹, \textbf{Dominic Kwiatkowski}³

¢Wellcome Trust Centre for Human Genetics, Roosevelt Drive, Oxford, OX3 7BN, UK; ²Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, UK.

\textit{Plasmodium falciparum} erythrocyte binding antigen-175 (\textit{EBA-175}) is an important ligand for merozoite invasion, and thus an attractive candidate antigen for a multi-component blood stage vaccine. Characterization of the worldwide sequence diversity in \textit{eba175} would contribute to efforts toward understanding parasite invasion and vaccine development, however this is a difficult problem because structural polymorphism in exon 1 confuses short-read aligners. Structural variation in field isolates with regard to the reference genome results in spurious sequencing coverage and unreliable ascertainment of SNPs and indels.

To access this variation we have developed a pipeline for parallel \textit{de novo} assembly of targeted genes from Illumina sequenced field isolates. We catalog more than 60 SNPs and describe the distribution of several structural variants that show evidence of balancing selection. For validation we have amplified and capillary sequenced tiling fragments across the genes of interest in a subset of samples using universally conserved primers. We have limited our initial efforts to samples that are clonal (i.e., homozygous in the region of interest), but we also describe our approaches and progress toward assembling mixed infections, as well as related work from our group.

P794: Malaria prevalence and performance characteristics of the Partec CyScope® in Koto Barombi, Southwest Cameroon

\textbf{Godlove Wepnie}¹, \textbf{Helen Kimbi}¹, \textbf{Judith Anchang-Kimbi}¹, \textbf{Bate Ayukchencangamba}¹, \textbf{Conica Njabi}² and \textbf{Leopold Lehman}²

¹Department of Zoology and Animal Physiology, Faculty of Science, University of Buea, P.O. Box 63 Buea, Buea, SWR, Cameroon; ²Department of Animal Biology, Faculty of Science, University of Douala, P.O. Box 2701, Douala, Cameroon

\textbf{BACKGROUND AND AIMS:} Malaria presents a diagnostic challenge to laboratories in endemic countries and delayed or inaccurate results contribute to high rates of morbidity and mortality. New rapid diagnostic techniques such as the Partec CyScope® (fluorescent microscope) have been developed to overcome the limitations of light microscopy. The aim of this work was to determine the prevalence of malaria and evaluate the performance characteristics of the Partec CyScope® using light microscopy as a gold standard in Koto Barombi, Southwest Cameroon.

\textbf{METHODS:} After recording demographic data on each child, capillary blood was collected for the preparation of thick and thin blood films for the assessment of parasite density and speciation respectively. Five \mu l of blood was placed on the dye-labeled portion of the CyScope® slide. A cover-slip was added and the slide was incubated for 1 minute and observed under the CyScope® for parasites. Performance characteristics of CyScope® were calculated.

\textbf{RESULTS:} A total of 215 children were recruited into the study. The overall prevalence of malaria was 18.98 % (41) and 41.2% (89) for light microscopy and Partec CyScope® respectively. The highest prevalence of malaria (41.76%, 38/91) was recorded in the 7-9 years age group while malaria (41.76%, 38/91) was recorded in the 7-9 years age group while the lowest (40.43%, 47/91) was recorded in the ≤6 years age group but the difference was not significant ($\chi^2 = 0.0323$, $P = 0.984$). Overall, 41 pupils were positive for malaria and 113 were negative for malaria using both diagnostic techniques. The sensitivity of the CyScope® was 68.3% (confidence interval, CI = 51.8% - 81.4%) while the specificity was 64.9% (CI = 57.3% - 71.9%). The accuracy and reliability index were 65.6% and 45.9% respectively.

\textbf{CONCLUSION:} The Partec CyScope® showed relatively high sensitivity as well as specificity and can therefore be a good diagnostic tool for malaria in areas that lack electricity since it can be battery operated.

P795: Enabling appropriate use of ACTs: Building the capacity of the private sector health workers in Nairobi, Kenya.

\textbf{Evelyn Wesangula}¹, \textbf{Dorothy Memusi}², \textbf{Jonathan Mbulu}³, \textbf{Richard Kolute}¹

¹Kenya Medical Training College, Nairobi, Kenya; ²Division of Malaria Control, Kenya; ³Mission for Essential Drug Supplies, Nairobi, Kenya

\textbf{INTRODUCTION:} Several determinants such as Unaffordable prices have limited access to quality assured medicines in most malaria endemic countries. The Affordable Medicines Facility- malaria (AmFm), by the Global Fund against Tuberculosis, AIDS and Malaria (GFATM) aimed at expanding access to the most effective treatment for malaria. Artemisinin based combinations (ACTs), to save lives and reduce the presence and use of less effective treatment options.

Access is not only measured by the availability of the medicines at the point of use, but the promptness and effectiveness of treatment offered. Improved availability of medicines needs to be matched with the ability of service providers to handle and appropriately diagnose, prescribe and dispense the correct treatment.

In 2012 the Division of Malaria control introduced the test before you treat policy. Rapid Diagnostic Test Kits (RDTs) were introduced in the public sector.

\textbf{AIMS:} Assessing the levels of knowledge on malaria case management among private sector health workers (HW) in Nairobi.

\textbf{METHODOLOGY:} Two sets of cross-sectional data were collected on a population of 500 private sector health workers from Nairobi. Pre-test/Post-studies done were analyzed for 141 randomly sampled participants to determine their levels of knowledge, attitudes and practices prior to training.

\textbf{RESULTS:} 85% of the HW had never attended any malaria case management training. 40% knew the correct frequency of administration of AL, 56% did not know the correct loading dose for quinine and 55% were aware of the second line of treatment. In diagnosis of malaria 55% could correctly report microscopy laboratory results while 59% had incorrect perceptions on the utility of RDTs immediately after treatment with AL. 51% did not know how to manage fever in under fives in accordance with the current guidelines.

\textbf{CONCLUSION:} Training private sector health workers is an essential milestone towards achieving increased access to malaria medicines and appropriate malaria case management. Provision of low cost rapid diagnostic test kits and further capacity building of private sector health workers in providing prompt point of care diagnosis and treatment is required.

P796: Translating health policy into practice: successes and challenges at implementation in Bungoma South district Western Kenya

\textbf{Evelyn Wesangula}¹

¹Kenya Medical Training College, Nairobi, Kenya

\textbf{BACKGROUND:} Malaria is one of the most common infectious diseases in the world with more than 40 percent of the world's population at risk and one of the greatest public health concerns especially in sub-Saharan Africa. In Bungoma South district, malaria is the leading cause of morbidity, accounting for 49 percent of the top ten diseases in the district. In 2006, Kenya implemented a new malaria treatment policy recommending the use of Artemether-Lumefantrine (AL) as the first line of treatment.

National guidelines on the diagnosis, treatment and prevention and job aids were developed and disseminated to health workers alongside in-
service training. The survey investigated if treatment of uncomplicated malaria conformed to national malaria treatment guidelines in Kenya.

METHODS: Face to face interviews for 31 health workers routinely performing consultations at out-patient departments in 17 health facilities were conducted. Data on health facility inventory control practices and stock status was retrospectively collected from records available. Outcome measures: availability of antimalarial drugs on the survey day, stock-outs in past six months, presence of job aids, health worker’s exposure to in-service training on AL and access to new national malaria treatment guidelines.

RESULTS: 35 percent of the health facilities had access to job aids and current treatment guidelines, 76 percent of the health workers had been trained on malaria case management. AL was almost universally available in all the health facilities. All facilities had recorded stock outs of AL six months prior to the survey and the duration of the stock outs was substantial lasting two months on average.

CONCLUSION: Treatment practices in uncomplicated malaria after policy change, did not fully conform to the national malaria treatment guidelines. Targets set for key implementation indicators by the division of malaria control, in terms of availability of recommended drug and training of health workers, had not been fully achieved. If the government does not ensure uninterrupted supply of recommended treatment, high quality focused training and appropriate patient education, and if provider prescription practices do not fully conform to the recommended treatment guidelines, the major potential public health benefits of AL may not be realized.

P797: A combined analysis of immune responses, antibody kinetics and vaccine efficacy from Phase II trials of the RTS,S malaria vaccine

Michael White1, Philip Bejon2,3, Ally Oluto4, Jamie Griffin4, Kalifa Bojang5, John Lusingu5, Naha Salim5, Salim Abdulla5, Nekoye Otsyula6, Selidji Agnandji6, Kwaku Poku Asante6, Seth Owusu-Agyei6, Emmanuel Mahama6,7, Turi Abugynega6, Daniel Ansong6, Jahit Sacarlil7, John J. Aponte2,3, Azra Ghanim1

1MRC Centre for Overseas Analysis and Modelling, Imperial College London, London, UK
2KEMRI-Wellcome Trust Research Programme, Kenya Medical Research Institute, Kilifi, Kenya
3Centre for Clinical Vaccinology and Tropical Medicine, University of Oxford, UK
4Medical Research Council Laboratories, Fajara, The Gambia
5National Institute for Medical Research, Tanga Centre, Tanga, Tanzania
6Ifakara Health Institute, Bagamoyo, Tanzania
7Kenya Medical Research Institute, and US Army Medical Research Unit-Kenya, Nairobi, Kenya
8Medical Research Unit, Albert Schweitzer Hospital, Lambaréné, Gabon
9Institute of Tropical Medicine, University of Tübingen, Tübingen, Germany
10Kintampo Health Research Centre, Kintampo, Ghana
11School of Medical Sciences, Kumasi, Ghana
12Centro de Investigación en Salud de Managua, Managua, Mozambique
13Barcelona Centre for International Health Research (CRESIB), Universitat de Barcelona, Barcelona, Spain

BACKGROUND: The RTS,S malaria vaccine for preventing infection with the Plasmodium falciparum parasite has been extensively tested in Phase 2 trials in ten sites in six different African countries and is currently undergoing Phase 3 trials. RTS,S vaccination induces high antibody titres to the circumsporozoite protein (CSP) antigen which have been shown to be associated with protection from infection.

METHODS: By fitting mathematical models to individual-level data from over 5,000 participants in Phase 2 trials, we investigate (i) the determinants of RTS,S-induced anti-CSP antibody titres; (ii) the duration of the antibody response following vaccination; (iii) the association between anti-CSP antibody titre and protection from infection and episodes of clinical malaria; and (iv) estimate vaccine efficacy and cumulative cases averted in cohorts of children across a wide range of transmission settings as measured by entomological inoculation rate (EIR).

RESULTS: Several key findings are presented. (i) Anti-CSP antibody titres following three doses of RTS,S depend on age, adjuvant, co-administration with other vaccines, pre-vaccination anti-Hepatitis B surface antigen titres, and pre-vaccination anti-CSP titres. (ii) Following vaccination, the duration of antibody response can be described by a bi-phasic exponential decay model, with a short-lived component with half-life ≠ 2 months, and a long-lived component with half-life = 2 years. (iii) Anti-CSP antibody titres are significantly associated with protection, with a titre of 63 (95%, 42 – 87) EU/mL predicted to prevent 50% of infections. (iv) The magnitude and duration of efficacy against clinical malaria depend on transmission intensity, with efficacy estimated to decline to zero over 4 years in a setting with EIR = 30 infectious bites per year.

CONCLUSIONS: Simple baseline variables can predict anti-CSP antibody titres following RTS,S vaccination and their subsequent decay. With an understanding of the antibody kinetics, vaccine efficacy and cases averted can be predicted across a range of transmission settings.

P798: Malaria associated changes in Syncytiotrophoblast function

Winifrida B. Kidima1, Rose G. F. Leke2, James M. Burns3, and Diane W. Taylor4

1University of Hawaii at Manoa, Hawaii, USA; 2University of Yaoundé, Yaoundé, Cameroon; 3Drexel University, College of Medicine, Philadelphia, USA

BACKGROUND: Plasmodium falciparum infections during pregnancy increase the risk of women having poor pregnancy outcomes, including low birth weight (LBW) babies. P. falciparum infected erythrocytes (IE) sequester in the intervillous space (IVS) of the placenta leading an inflammatory response, creating pathology that may lead to poor neonatal outcomes. ST which line the IVS play a critical role in foetal development. It is not fully understood how PM changes ST functions. Therefore, the aim of this study was to elucidate the influence of PM on ST functions.

METHODS: In Cameroon, biopsies of 30 placenta were collected within 15 minutes of delivery, washed free of maternal blood, and foetal cells were placed in RNAlater. The biopsies contained foetal ST, stroma, macrophages and blood vessel endothelial cells. Microarray analysis was done on biopsies from 3 PM+ positive samples compared with a pool of 6 PM-negative placentas. To assess the nature of biological relationship between genes that were differentially expressed, the Pathway Miner Version 1.1 was used.

RESULTS: We identified metabolic, cellular and regulatory pathways that were significantly altered during PM. These include the insulin-like growth factor, insulin, and mammalian target for rapamycin signaling pathways. Others are placenta growth factor, tumor growth factor β, and epidermal growth factor signaling pathways. Several genes encoding proteins known to be important in LBW babies were also downregulated, for example, leptin, system A amino acid transporter and insulin-regulated glucose transporter gene. Several inflammatory pathways were dysregulated, including a pathway involved in prostaglandin synthesis regulation in which placental 11beta-hydroxysteroid dehydrogenase-2 enzyme which prevents maternal cortisol from reaching foetus compartment was downregulated, indicating compromised barrier role of ST during PM.

CONCLUSIONS: PM perturbs normal functioning of foetal cells, including changes that are potential risk factors that may lead to poor pregnancy outcome. The pathways that are altered can be used as targets for intervention against PM related outcomes.

Rose Lusinde¹, Mohamed Ali¹, Abdul-wahab Al-maafazy², Abdullah S. Ali³, Issa Garimo¹, Mahdi Ramsani¹, Shabbir Lalji¹, Jessica M. Kafuko¹, Osia Mwaipape⁴, Rita Willilo⁴, Jeremiah M. Ngondi⁴
¹RTI International, Dar es Salaam, Tanzania; ²Zanzibar Malaria Control Program, Ministry of Health, Zanzibar, Tanzania; ³United States Agency for International Development/President’s Malaria Initiative, Dar es Salaam, Tanzania.

BACKGROUND: In malaria transmission, rainfall, temperature and humidity are major natural risk factors affecting the life cycle, breeding and lifespan of the mosquito. Between 2006 and 2010, blanket indoor residual spraying (IRS) was undertaken in Zanzibar. Following declines in malaria cases, IRS was scaled down to focal spraying in malaria transmission hotspots. This study aims to determine the impact of climate on the spatial-temporal variation of malaria transmission for the period 2008 – 2012.

METHODS: Data on malaria were obtained from weekly record of confirmed cases of malaria from each health facility. Rainfall (mm), temperature (°C) and humidity (%) data were collected monthly at monitoring stations located in each of the 10 districts. Malaria cases were compiled for each period and incidence per 1000 population was calculated using service area population. Monthly climate data were calculated into averages for each period. ArcGIS software was used to plot all variables. Geographically Weighted Regression (GWR) was done to investigate associations between malaria incidence and each climatic factor. Data validation was undertaken by comparing values of climatic factors available in websites to those from TMA.

RESULTS: From 2008 to 2011, mean difference in incidence was 1.5 (95% CI: 1.0-2.0). Over the study period, mean difference in temperature was 11.8(95% CI: 6.7-16.9) rainfall 118.7 (95% CI:103.0-134.5) and humidity 34.8 (CI: 19.7 -49.9).Pearson’s product-moment correlation coefficients were 0.8 (95% CI: 0.6-1.0) rainfall 118.7 (95% CI:103.0-134.5) and humidity 34.8 (CI: 19.7 -49.9).Pearson’s product-moment correlation coefficients between incidence and temperature was 0.2, incidence and rainfall was -0.3 and between incidence and relative humidity was 0.2.

CONCLUSION: Malaria shows a great decline during the study period; however seasonal variations need to be thoroughly monitored. Knowing the spatial variability pattern of malaria will make targeting of IRS at this pre-elimination stage more timely, effective and efficient.

P800: Electronic data capture for indoor residual spraying (IRS) activities in Zambia: a pilot to map and monitor spray activities

Daniel Bridges¹, Chadwick Siakaala¹, Mulakwa Kamulilwo², Benjamin Kayungwa³, Benjamin Winters¹, Brian Chirwa¹
¹Akros, Lake Road, Lusaka, Zambia; ²Department of Public Health and Research, National Malaria Control Centre, Ministry of Health, Lusaka, Zambia; ³Zambia Integrated System Strengthening Program, Lusaka, Zambia; ⁴Presidents Malaria Initiative, Lusaka, Zambia

BACKGROUND: Indoor residual spraying (IRS) along with long-lasting insecticide treated nets (LLIN) form the mainstay of vector and subsequently malaria control throughout sub-Saharan Africa. Zambia has invested heavily in IRS over the past decade and boasts coverage levels in excess of 35% in urban/peri-urban settings contributing to a significant reduction in national malaria parasitemia from 22% in 2006 to 16% in 2010. Historically, spray data is collected on paper, which is then manually aggregated and entered into a spreadsheet for reporting. Multiple spreadsheets are manually combined to produce the final dataset. This system is labour intensive, prone to errors and limited in scope. To address this issue, an electronic data capture solution (mSPRAY) was developed and piloted in Chibombo district, Zambia, for rapid collection and dissemination of IRS data.

METHODS: IRS operators were individually equipped with a personal digital assistant (PDA) pre-loaded with the mSPRAY software that guides them through collection of all data elements including GPS coordinates for every structure, spray application details, LLIN usage and previous spray history. Validation rules built into the software ensured that only valid data was entered. Supervisors were able to review these data to increase accuracy. Periodically, datasets were exported for timely reporting to all level(s).

RESULTS: During the trial spray season, mSPRAY was able to provide regular feedback on overall performance. As a result it was able to identify a major shortfall in reaching total target structure coverage. Based on this data, operational changes were made and coverage was dramatically improved. mSPRAY also identified areas missed during spraying that were originally targeted, again allowing spray teams to revisit these overlooked areas.

CONCLUSIONS: In short, mSPRAY offers a robust and expanded data collection method allowing fine spatial mapping of spray activities to ensure that IRS applications are as effective and efficient as possible.

P801: Integrated Entomological Surveillance in Zambia: Implementation of a Phased Program for District Based Delivery through Environmental Health Technicians

Chadwick Sikala¹, William Lubembe¹, Musapa Mulenga¹, Mulakwa Kamulilwo¹, Anna M. Winters², Daniel Bridges², Benjamin Winters², Matthew Burns²
¹Department of Public Health and Research, National Malaria Control Centre, Ministry of Health, P.O. Box 32509, Lusaka, Zambia; ²Akros, Lake Road, Lusaka, Zambia

BACKGROUND: Zambia has witnessed a rapid expansion in delivery of insecticidal based interventions such as Indoor Residual Spraying and Long Lasting Insecticidal Nets. Despite intensification of vector control programming, entomological surveillance is conducted sporadically and is geographically limited in coverage. Until now, there has been no routine, decentralized government entrenched longitudinal surveillance system that monitors localized species prevalence and supports routine processing of specimens to measure entomological impact of vector control interventions.

METHODS: A conceptual framework based on phased delivery of individual components of an integrated entomological surveillance system has been designed with supporting tools for districts with ongoing vector control activities. Individual components of the program support training of new and existing recruits, utilization of a standardized field surveillance protocol, data management, intra- and inter-district program performance, species composition mapping, and vector bionomics output associated with local malaria transmission. Post-district specimen analyses and quality assurance audits are also conducted as part of the program. Nine Environmental Health Technicians who were trained for this program were selected to self-manage surveillance sessions in their respective sentinel sites with Community Health Worker assistance; based on their assessed training performance and their national representation.

RESULTS: All sentinel sites were proficient in adopting a standardized collection protocol over multiple months during the wet-season and in yielding specimen data for building localized spatial and temporal species maps and associated bionomics.

CONCLUSION: Findings highlight that the decentralized model of entomological surveillance is an achievable goal for national programs. Further exploration is required to address options that allow for nationally sustainable routes to multiply sentinel sites to ensure comprehensive spatial and temporal mapping of vector species and related parameters and assist the National Malaria Control Centre with evidenced based intervention selection and targeting.
P802: Community based malaria elimination efforts in Southern Zambia

Zunda Chisha; Daniel J. Bridges; Benjamin Winters; Busiku Hamainza; Mercie Mwanza; Mulakwa Kamulwiko; Sicthamba Wamulume; Duncan Earle; John Miller; Anna M. Winters; Akros, Lake Road, Lusaka, Zambia

Department of Public Health and Research, National Malaria Control Centre, Ministry of Health, Lusaka, Zambia; PATH Malaria Control and Evaluation Partnership in Africa (MACEPA), National Malaria Control Centre, Chainama Hospital College Grounds, Great East Road, Lusaka, Zambia

BACKGROUND: Progress in malaria control efforts in Zambia resulted in a drop in malaria parasitemia in children under five from 22% in 2006 to 16% in 2010. This success, however, is not uniformly distributed with certain areas of Zambia reporting resurgence in malaria cases while other areas, mainly Lusaka and Southern Provinces have reached sufficiently low levels of malaria transmission to warrant an in-country push towards malaria elimination. Given this level of progress, the Zambian Ministry of Health set a goal of achieving malaria elimination in five areas by 2015. This goal warrants the establishment of a robust malaria surveillance system with a high level of sensitivity to detect malaria infections at community level.

METHODS: The existing passive malaria surveillance system, which detects malaria cases at formal health facilities, has been enhanced by leveraging volunteer community health worker networks to detect hotspots of malaria transmission through follow up and screening of households in proximity of identified index cases. These enhancements have been termed “Step 3” and constitute the final stage of an innovative three-step sequence designed to measure the progress, and move towards malaria elimination. Through Step 3, over 690 community health workers from five districts of Southern Province received a 4-day refresher training in aspects of clinical presentation, testing using rapid diagnostic tests and treatment of uncomplicated malaria according to current Ministry of Health policy.

RESULTS: Through Step 3, over 20,000 RDTs have been administered. Average positivity rate during community testing has been 11.87. Reporting completeness from community health workers each month has averaged approximately 90%. Initial results show this program increases the sensitivity and timeliness of malaria surveillance such that malaria infections previously undetected by the routine passive surveillance system are now being identified and treated. Data are being monitored by district personnel for hotspot activity to guide interventions.

CONCLUSION: As Zambia targets malaria elimination, community-level malaria surveillance has been implemented to measure malaria burden and to detect and treat malaria infections that previously went undetected. The efforts, success and challenges faced during the implementation of this program in Zambia are highlighted.

P803: Multiple insecticide resistance detected in Anopheles funestus in Zimbabwe and Zambia*

Oliver Wood1,2, Richard Hunt1, Kwang Shick Choi1,2, Basil Brooke1,2, Lizette Koekemoere1,2, Maureen Coetzee1,2

1Wits Research Institute for Malaria, School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa; 2Vector Control Reference Laboratory, National Institute for Communicable Diseases (NICD), National Health Laboratory Service (NHLS), Johannesburg, South Africa; *This work is part of the Southern African International Centre of Excellence in Malaria Research (ICEMR) project funded by the NIH and lead by the Johns Hopkins Malaria Research Institute, Baltimore, USA.

INTRODUCTION: Malaria mosquito insecticide resistance surveys were conducted in 2013 at two southern African localities, Honde Valley, Eastern Zimbabwe bordering Mozambique, and Nchelenge, Northern Zambia on Lake Mweru. Both are holoendemic malaria regions and both have indoor spraying programmes (lambda-cyhalothrin and bendiocarb respectively). The aim of the survey was to determine the insecticide resistance profile of the main vector species in the areas.

METHODOLOGY: Mosquitoes were collected indoors using torches and handheld aspirators. Where possible, larvae were caught and reared to adults. WHO insecticide bioassays were conducted in the field. The mosquitoes were morphologically identified, and transported back to the laboratory for molecular confirmation. Pooled 2-5 day old offspring from live females were used in synergist exposures.

RESULTS: Anopheles funestus was the only malaria vector species found in the Honde Valley. The 454 exposed individuals showed high frequencies of resistance to the pyrethroids lambda-cyhalothrin and deltamethrin, as well as the carbamates bendiocarb and propoxur. They remained fully susceptible to DDT, dieldrin, malathion and fenitrothion. In Nchelenge the majority of the vectors were An. funestus with a few An. gambiae present. Resistance to deltamethrin and lambda-cyhalothrin were detected in the exposed 676 mosquitoes. While the few An. gambiae showed DDT and pyrethroid resistance, the An. funestus were susceptible to DDT, dieldrin, malathion and fenitrothion.

DISCUSSION/CONCLUSION: Multiple insecticide resistance at these two sites makes vector control much more difficult and a resistance management strategy is a critical requirement.

P804: Demand for artemisinin combination therapies at different price points: an empirical analysis of prices and product uptake at retail shops across 8 sub-Saharan African countries.

Woolsey AM1, Cohen JM2, Poyer S1, Moonen B3

1Clinton Health Access Initiative, Boston, MA; 2ACTwatch, Population Services International, Washington, D.C.

One of the goals of the Affordable Medicines Facility for malaria (AMFm) was to increase uptake of artemisinin combination therapies (ACTs) among consumers seeking malaria treatment in the retail sector. AMFm employed subsidies at distribution-entry points to make ACT retail prices more competitive with retail prices for non-ACT antimalarials, with the hope that this would shift consumer purchasing habits. However, as cheaper (though less effective) alternative antimalarials remain widely available, the effect that ACT retail price reductions have had on ACT uptake remains unclear. Using price and sales volume data from biannual surveys conducted in retail treatment access points across 8 sub-Saharan African countries (Benin, Democratic Republic of the Congo, Kenya, Madagascar, Nigeria, Uganda, Zambia, and Zimbabwe), we calculated the fraction of antimalarial sales that were ACTs and modeled this outcome as a function of mean ACT sales prices relative to mean sales prices of other available antimalarial drugs. Controlling for potentially confounding covariates such as local malaria prevalence, population density, outlet type and access point accessibility, we modeled an empirical relationship describing how ACT market share changes with retail price. Results confirm that lower prices were associated with greater uptake. While the fraction of retail antimalarial sales that were ACTs was low across all countries (less than 7%), ACT prices were 5.5x more expensive than non-ACTs in Nigerian shops that did not sell any ACTs during the 2011 survey period, but only 3.5x more expensive in shops that did sell ACTs. We then applied the derived relationship between price and ACT sales volume to survey-derived estimates of demand for antimalarial drugs across sub-Saharan Africa, estimating ACT demand at different price points. This information provides critical insights to retail market dynamics in sub-Saharan Africa that can be used by policy makers to shape current and future malaria treatment interventions.
P805: The importance and relevance of long-term archiving of electronic clinical research data

Lesley Workman, Clarissa Moreira, Karen I Barnes
Worldwide Antimalarial Resistance Network (WWARN)

BACKGROUND: To ensure long-term accessibility of data collected during clinical trials, archiving of electronic clinical research data on completion of a study is mandatory. In addition the value of sharing data has become clear, with pooling of data increasing statistical power to answer broad scientific and public health questions, and is increasingly required by funding agencies. Recently it has become evident that electronic data collected during clinical studies have often not been adequately achieved, resulting in the loss of valuable information.

METHODS: As part of a WWARN-led pooled pharmacokinetic/ pharmacodynamic pooled individual patient data analysis of Sulfadoxine-Pyrimethamine (SP) a literature search was conducted using PUBMED, Embase, and Google Scholar, to identify relevant studies. Researchers were invited to join this pooled analysis which aims to provide the necessary evidence to inform recommendations on SP dosage and use in key target populations.

RESULTS: SP had been administered and drug concentrations reported in 27 studies, including patients with uncomplicated P. falciparum malaria (n=1996), intermittent preventative treatment (n=230) and healthy volunteers (n=214). Following the response to the call for data contribution, 11/27 studies (41%) were submitted for inclusion. Of the 16 studies not submitted, there was a positive response from 10 researchers, but their data was not retrievable; reasons given included data being destroyed, lost, and the data had been stored on a “floppy disc”. For the remaining 6 studies there was no response to repeated invitations.

CONCLUSION: Pooled individual patient data analyses is an important tool for ensuring optimal use of available data, and relies on data sharing to answer important scientific and public health questions. In this study we have shown that from the 10/21 (48%) studies, with 647/1924 (34%) participants in which we received a response the data had not been adequately archived, resulting in valuable data being lost. To address this critical need, best practise guidance for archiving electronic clinical research data will be developed, tested and made available for download from the WWARN web-site.

P806: Malaria in adults of Dielmo (Senegal): decreasing clinical immunity sustain pre-elimination despite increasing pyrethroid resistance.

Amélie N. Wotodia1,2, Cheikh Sokhna1, Nafiassatou Diagne2, Adama Tall2, Fambaye Dieye1, Ngor Faye1, Jean-François Trape1
1 Institut de Recherche pour le Développement, URMI TE Dakar, Sénégal
2 Département de Biologie Animale, FST/ UCAD de Dakar

Institut Pasteur de Dakar, Unité d’Epidémiologie.

BACKGROUND: Malaria burden decreased significantly in recent years in Africa through the widespread use Artemisinin-based combination therapy (ACT) and long-lasting insecticide-treated nets (LLINs). However, pyrethroid resistance of A. gambiae is rapidly emerging, raising serious concerns about the future of malaria elimination efforts. This study investigated the evolution of malaria morbidity in adults of Dielmo (Senegal) before and after the implementation of LLINs.

METHODS: A longitudinal study was carried out in Dielmo where ACT and long-lasting insecticide-treated nets (LLINs) were introduced in 2006 and LLINs in July 2008. The presence of each person in the village was monitored daily. Thick smears were performed for all cases of fever. To assess malaria prevalence, thick smears were performed quarterly in all individuals.

RESULTS: The incidence of P. falciparum attacks decreased 5 fold the first and second year after LLINs implementation (0.15 attacks per adult per year in 2007 versus 0.03 in 2008/2009). The same trend was observed for malaria prevalence which decreased from 17.5% in 2007 to 4.4% and 1.8% in 2008 and 2009, respectively. However, a rebound of malaria morbidity occurred 2 years after LLINs implementation (0.11 and 0.15 attacks per adult per year in 2010 and 2011, respectively) when all new infections became symptomatic. The rebound ended at mid-2011 and malaria incidence in adults was only 0.05 attacks per adult per year in 2012 and prevalence 0.3%.

CONCLUSION: Since all patent infections were symptomatic from 2010 onwards, they were rapidly detected and treated. The efficient ACT treatment prevented replenishment of the parasite reservoir despite pyrethroid resistance in A. gambiae increasing from 7% in 2007 to 48% in 2010. The rapid decline of protective immunity, although a double edge sword as all infections become symptomatic, tends to sustain effectiveness of combining ACT and LLINs despite rapidly increasing pyrethroid resistance.

P807: Unexpected age-dependent high prevalence of asymptomatic Plasmodium carriages in areas of seasonal and unstable transmission settings in south-central Oromia, Ethiopia

Lemu Golossal1, 2, *, Nizar Enweji1, Berhanu Erko1 Gote Swedberg1, Abraham Asefa3
1Addis Ababa University, Aklilu Lemma Institute of Pathobiology, Addis Ababa, Ethiopia, 2Armauer Hansen Research Institute, Addis Ababa, Ethiopia, 3Uppsala University, Department of Medical Biochemistry and Microbiology, Uppsala, Sweden.

BACKGROUND: Following repeated exposure to infectious Anopheles mosquito bite, individuals living in regions where malaria transmission is high tend to develop acquired immunity, which enables them to remain asymptomatic while still carrying and transmitting parasites. But there is paucity of data on the presence of asymptomatic malaria carriages in areas of low or unstable transmission. The purpose of this study was to determine the existence and magnitude of asymptomatic Plasmodium carriages in Shalla district, West Arsi zone, Oromia region, Ethiopia.

METHODS: A total of 1091 individuals living in 317 homesteads were randomly selected and surveyed during the dry period, November 2012 through December 2012. All members of the randomly selected households aged ≥2 years were requested to provide a fingerprick blood sample after obtaining informed consent and/or assent. Malaria diagnosis was made by microscopy and Rapid Diagnostics Test (RDT).

RESULTS: The overall prevalence of asymptomatic malaria carriage was 5.0% (55/1091) (95% CI, 3.7%-6.3%) as diagnosed by microscopy. P. falciparum, P. vivax and mixed infections accounted for 45.4%, 40.0% and 14.5%, respectively. The prevalence of asymptomatic malaria as diagnosed by RDT was 8.2% (95% CI, 6.6%-9.9%) in which P. falciparum, P. vivax and mixed infections accounted for 66.7%, 25.6% and 7.8% of the infections, respectively. Using microscopy as the gold standard, the sensitivity of RDT for detecting asymptomatic Plasmodial infections was 85.5%. There was an agreement between RDT and microscopy (kappa= 0.6247, Z=28.84, P = 0.000). RDT had 88%, 81.8% and 75% positive predictive value for P. falciparum, P. vivax and mixed infections, respectively. The highest parasitaemia was observed in the age group ≤ 5 years. None of the study subjects aged ≥ 46 years had asymptomatic malaria. Asymptomatic malaria carriage was significantly associated with sex (X² = 6.13, p = 0.013) and age of the study subjects (X² = 28, P = 0.000). Of the 317 households surveyed, asymptomatic infections were identified in 63 (19.9%) of the households. Asymptomatic parasitaemia individual had 50.9%, 18.2% and 30.9% asexual, sexual and mixed stages (axexual and gametocytes), respectively. Parasite counts ranged from as low as 64 to as high as 74, 260 µl⁻¹.

CONCLUSIONS: Age-dependent high prevalence of asymptomatic carriers was observed in study areas where malaria transmission is seasonal and unstable, a phenomenon usually observed in areas of high malaria transmission settings. Although asymptomatic parasitaemia could likely be the result of repeated exposure over time (age being the surrogate marker), how clinical immunity developed and maintained in younger age groups in areas of low and seasonal transmission is unclear. But there are possible explanations for this to happen. The transmission level may be higher than previously thought using existing diagnostic tools and maybe these levels are sufficient to develop and maintain clinical immunity. Secondly, the parasite diversity may perhaps be extremely low in the study area that one only
needs to experience one or two infections to develop the immunity. Third, asymptomatic parasitaemia in adults may be at sub-microscopic levels so that microscopy and RDT failed to detect it. This could be explained by the fact that adults may have strong anti-parasite immunity that keep the parasitaemia below the threshold of microscopy and RDT detection compared to young age groups. These asymptomatic infections have been suggested to represent a significant source of parasites for local mosquitoes, maintaining residual malaria transmission. Thus, active survey may help to identify and treat the reservoir pools and reduce onward transmission of malaria using molecular techniques. Malaria control efforts should address those young age groups if attention are to be given to pinpoint asymptomatic carriers.

KEYWORDS: Asymptomatic malaria; unstable transmission; RDT; Microscopy; Plasmodium falciparum; Oromia, Ethiopia

P808: The impact of the expansion of urban vegetable farming on malaria transmission in major cities of Benin.

YAOULETON0 Anges, N’Guessan1 Raphael and AKOGBEYO1 Martin
1= CREC, 06 BP 2504, Cotonou-Benin.

BACKGROUND: Urban agricultural practices are expanding in several cities of the Republic of Benin. This study aims to assess the impact of such practices on transmission of the malaria parasite in major cities of Benin.

METHOD: A cross sectional entomological study was carried out from January to December in two vegetable farming sites in southern Benin (Houeyiho and Acron) and one in the northern area (Azérékè). The study was based on sampling of mosquitoes by Human Landing Catches (HLC) in households close to the vegetable farms and in others located far from the farms.

RESULTS: During the year of study, 71,678 female mosquitoes were caught by HLC of which 25% (17,920/71,678) were Anopheles species. In the areas surveyed, the main malaria parasite, Plasmodium falciparum was transmitted in the south by Anopheles gambiae s.s. Transmission was high during the two rainy seasons (April to July and October to November) but declined in the two dry seasons (December to March and August to September). In the north, transmission occurred from June to October during the rainy season and was vehicled by two members of the An. gambiae complex: Anopheles gambiae s.s. (98%) and Anopheles arabiensis (2%). At Houeyiho, Acron and Azérékè, the Entomological Inoculation Rates (EIRs) and the Human Biting Rates (HBRs) to Vegetable Farms (HCVF) than in those located far from the vegetable areas (HVF) (p < 0.05). However, there were no significant differences in HBRs or EIRs between HCFV and HVF during the rainy seasons at these sites (p > 0.05). The knock-down resistance (kdr) mutation was the main resistance mechanism detected at high frequency (0.86 to 0.91) in An. gambiae s.l. at all sites. The ace-1 R mutation was also found but at a very low frequency (< 0.1).

CONCLUSION: These findings showed that communities living close to vegetable farms are permanently exposed to malaria throughout the year, whereas the risk in those living far from such agricultural practices is limited and only critical during the rainy seasons. Measures must be taken by African governments to create awareness among farmers and ultimately decentralize farming activities from urban to rural areas where human-vector contact is limited.

P809: Faecal antibody response, age variation in antibody responses and histopathological changes in mice immunized with Lactococcus lactis expressing a malaria parasite protein

Surangi G.Yasawardene1 and Ranjan Ramasamy2
1Faculty of Medical Sciences, University of Sri Jayewardenapura, Sri Lanka; 2Institute of Medicine, Universiti Brunei Darussalam, Brunei Darussalam.

BACKGROUND: The gram positive food grade bacterium Lactococcus lactis is a potential vehicle for delivering immunogens to the mucosal immune system. Mucosally delivered vaccines are known to activate the local mucosal immune system to elicit protective secretory IgA antibodies and systemic antibody response.

METHODS: Plasmodium falciparum merozoite surface antigen2 (PfMSA2) was expressed in recombinant Lactococcus lactis in a form that was partially covalentlyanchored to the peptidoglycan of the cell wall (MSA2CP). Recombinant L. lactis strain was delivered oro-nasally for mucosal immunization to Balb/c mice of ages 1wk (neonates), 6wk (young adults) and more than 25 wks (old adults). Non-recombinant Lactis was used as control. The serum and faecal antibody response was investigated by ELISA using recombinant MSA2 as antigen and by immunofluorescence assay. Histopathological changes in gut associated lymphoid tissue were investigated.

RESULTS: Antibodies in the faecal pellets were detectable after oro-nasal immunizations by IFA. Serum IgG anti-MSA2 response was significantly higher in young adult Balb/c mice, after oronasal delivery, compared to old mice and neonates. Antibodies elicited in young mice reacted with native MSA2 in the surface of P. falciparum merozoites in an immunofluorescence assay. Enlargement of mesenteric lymph nodes and increased lymphatic infiltration of the lamina propria were noted in both recombinant and non recombinant L. lactis immunized mice. The gastrointestinal tract was otherwise normal in oronasally immunized mice. The spleen showed periartherosial lymphoid aggregations in immunized mice.

CONCLUSIONS: Recombinant L. lactis is a suitably safe vector for subunit vaccines. Ora-nasal immunizations give rise to detectable faecal antibodies. The foreign proteins expressed in L. lactis can be used in nasal or oral vaccination procedures to elicit protective secretory antibodies in the gut. The antibody responses to recombinant L. lactis were markedly weaker in extremes of age. The histological changes of spleens of older mice support weak antibody response seen. These findings are relevant for further developing L. lactis to deliver vaccines mucosally for use in humans of different ages.

P810: External competency assessment can improve quality of malaria microscopy in Africa

1African Medical and Research Foundation (AMREF), Nairobi, Kenya 2Medical Care Development International (MCDI), Washington, USA 3Research Institute for Tropical Medicine (WHO Collaborating Centre), Alabang, Philippines, 4WHO Regional Office for Africa, Harare, Zimbabwe.

BACKGROUND: Malaria is the leading cause of morbidity and mortality in sub-Saharan Africa responsible for 90% of the annual global burden. Accurate diagnosis of malaria is important to ensure correct case management. Studies have shown that microscopy in field conditions has a sensitivity of 68.6% and specificity of 61.5%. WHO AFRO and AMREF introduced a competency assessment course for malaria microbiologists in Africa based on the model approved by WHO.

METHODOLOGY: A five day competency assessment comprising theory and laboratory practical sessions were conducted as recommended by WHO. Pre and post course practical evaluations were used consisting of 16 and 55 well characterized slides respectively. Twenty slides were negative, ten had Plasmodium falciparum parasites with densities between 80-200 parasites/µL, and ten slides had P. malariae, P. vivax and mixed parasite species. Fifteen slides containing P. falciparum were used to assess parasite quantification.

RESULTS: One hundred and ninety eight microscopists have participated from 19 countries since 2009. Overall, parasite detection rates ranged from 80-88% (mean 8%, 95% CI 5.7 - 9.8%; p<0.0001); species identification ranged from 40% to 59% (mean 19%, 95% CI 15.0 - 21.9%; p<0.0001). Parasite quantification ranged from 26 to 35% (mean 9% 95% CI 5.4 - 12.4%; p<0.0001). Sensitivity ranged from 80% to 90% (mean 10%, 95% CI 6.9 - 12.4%; p<0.0001), while specificity ranged from 83 to 88% (mean 5% 95% CI 2.6 - 8.8%; p<0.0001). Overall 9 participants obtained Expert Level One, 31 obtained Level 2, 26 obtained Level 3 and
P811: Efficacy and tolerability of artesunate-amodiaquine compared to artemether-lumefantrine in the treatment of uncomplicated Plasmodium falciparum malaria in Côte d’Ivoire.

Yaya W 1,2, PMC Kiki-Barro 1,2, KF Kassi 1,2, V Djohan 1, A Konaté 1,2, H Vanga-Bosson, KE Angora 1,2, EIH Menan 1,2

1Department of Parasitology and Mycology, Faculty of Pharmacy, Abidjan, Côte d’Ivoire; 2Malaria Research and Control Center, NIPH, Abidjan, Côte d’Ivoire; 3Laboratory of Parasitology and Mycology, CeDReS, Abidjan, Côte d’Ivoire.

BACKGROUND: The treatment of malaria with ACTs has been freely provided in public hospital of Côte d’Ivoire since 2010. Two years after, this study was carried out to evaluate the efficacy and tolerability of artesunate-amodiaquine (ASAQ) versus artemether-lumefantrine (AL) in the treatment of uncomplicated Plasmodium falciparum malaria in Côte d’Ivoire.

METHODS: It was a multicentre open randomized clinical trial of 3-days treatment of ASAQ against AL for the treatment of 2 parallel groups of patients aged 2 years and above and suffering from non complicated falciparum malaria in three malaria sentinel sites of Côte d’Ivoire. The endpoints were Adequate Clinical and Parasitological Response (ACPR) at day 28, the clinical and biological tolerability. The WHO 2003 protocol was used.

RESULTS: A total 300 patients were enrolled: 151 in the ASAQ arm and 149 in the AL arm. The data analysis involved the 289 subjects who correctly followed the protocol, i.e. 143 (49.5%) in the ASAQ group and 146 (50.5%) in the AL group. The corrected ACPR was 99.3% in each group. More than 94% of patients did no longer present fever 48 hours after treatment. Approximately 78% of the people in the ASAQ group had a parasite clearance time of 48 hours or less compared to 81% in the AL group (p=0.496). The clinical tolerance was good in both groups. Anaemia was significantly higher at D4 in the two groups compared to D0. More than 94% of patients did no longer present fever 48 hours after treatment.

CONCLUSIONS: ASAQ was as effective and well-tolerated as AL in the treatment of uncomplicated falciparum malaria. These ACTs can be continued to be used to treat uncomplicated falciparum malaria in Côte d’Ivoire.

Keywords: malaria, Plasmodium falciparum, artesunate-amodiaquine, artemether-lumefantrine, Côte d’Ivoire.

P812: In vitro antiplasmodial activity of new benzimidazole and imidazopyridine chalcones

W Yaya 1,2, M Ouattara 1,2, EIH Menan 1,2, M Chendjou 1,2, A Djéré 1,2, KD Tano 1,2, D Sissouma 1,2, AD Yapi 1,2

1Laboratory of Parasitology and Mycology, Faculty of Pharmacy, Félix Houphouët Boigny University of Cocody-Abidjan; 2Laboratory of Therapeutic Chemistry, Faculty of Pharmacy, Félix Houphouët Boigny University of Cocody-Abidjan; 3Malaria Research and Control Center, NIPH, Abidjan-Côte d’Ivoire; 4Laboratory of Parasitology and Mycology, CeDReS, Hospital University Center of Treichville, Côte d’Ivoire; 5Laboratory of Structural Organic Chemistry, Faculty of Science, Félix Houphouët Boigny University of Cocody-Abidjan

BACKGROUND: Resistance to antimalarial drugs is a serious health concern. We carried out this study to determine the in vitro susceptibility of Plasmodium falciparum to new benzimidazole and imidazopyridine chalcone.

METHODS: Eight new chalcones to benzimidazole or imidazopyridine support have been designed according to the concept of pharmaco juxtaposition of bioactive entities. The evaluation of the in vitro antiplasmodial activity of the synthesized compounds was performed according to the technique of Rieckmann microtest followed by ELISA assay for the determination of the production of antigen HRP2 related parasite growth.

RESULTS: All chalcones tested showed significant antiplasmodial activities against the chloroquine-susceptible isolates with IC50s ranging from 11.02 to 2.27 µg/ ml. Their antiplasmodial profiles were better on chloroquine-resistant isolates with IC50s ranging from 1.96 to 0.22 µg/ml.

CONCLUSION: Development of new chalcones to vector pharmacophore benzimidazole and imidazopyridine with significant antiplasmodial activities including the chloroquine-resistant isolates opens pathways of investigation towards the development of new antimalarial drug candidates.

KEY WORDS: Malaria, Plasmodium falciparum, chalcone, antimalarial drug.

P813: Assessment of Stability of Plasmodium falciparum Field Isolates During Long-term In vitro culture

Redemptah Yoda,1 Charles Okudo,1 Agnes Cheruiyot,1 Cheruiyot Jelagat,1 Dennis Juma,1 Luiser Ingasia,1 Angela Achieng4 Ben Andagalu,1 Hoseah Akala,1 and Edwin Kamau.1

1Global Emerging Infections Surveillance (GEIS) Program, United States Army Medical Research Unit-Kenya (USAMRU-K)/Kenya Medical Research Institute (KEMRI) - Walter Reed Project, Kisumu and Nairobi, Kenya; 2Maseno University, Department of Zoology P. O. Box Private Bag Maseno.

BACKGROUND: In vitro Plasmodium falciparum culture is a sustainable technique for understanding parasite-host biology using reference clones and field isolates, for guiding treatment and control strategies. Temporal studies show that parasite characteristics in natural environment are transient, due to intra-parasite factors, within host ecosystem and climate. Although findings from clones and cultured field isolates are critical for inferring in vivo responses, stability of isolates during culture is understudied hence construing the role of culturing process in study observations. In this study, we assessed in vitro and genetic profiles for each sample in even and subsequent generations in culture.

METHODS: P. falciparum day zero field samples were analyzed for ex vivo susceptibility to a panel of drugs which included amodiaquine, artemether, arteisinim, chloroquine, dihydroartemisinin, mefloquine and piperaquine by Malaria SYBR Green I assay. Duplicate number of the sample was maintained in drug-free tissue medium continuous culture for 90 days and tested for in vitro susceptibility against the panel of drugs wherever a 3%-8% parasitemia was attained. Cultures were collected and genetic analysis done for samples collected on day 0, and on every 7th day thereafter until around 90th day or until the culture crushed. MSP1/2 and GLURP analysis were done to assess clonal stability of the culture overtime. Additionally, genetic polymorphisms of selected multidrug resistance gene 1 (PFMDR1) and chloroquine resistance transporter gene (PFCRT) alleles were analyzed for each generation of the cultures.

PRELIMINARY RESULTS: The IC50 of cultures varied per drug between day zero and subsequent days. Notably, Arteisinim increased from 3.76 to 10.51 µM from day0 to day90, chloroquine was stable (77.45 to 86.69 µM) whereas piperaquine decreased from 240.30 to 78.25 µM from day0 to day90. The PFMDR1 gene profile at codons 86 and 184 were significant at the first generation. The patient was not stable, with alternating profiles seen to appear, disappear and/or re-appear over generations. Data analysis on population structure is still on-going.

Conclusion: For the first time, we have shown phenotypic and genotypic variability of falciparum cultures across generations. Understanding key parasite factors, responsible for these patterns in field isolates and reference clones would be useful to accurately describe responses under study.
P814: Transmission blocking activity of Azadirachta indica and Guiera senegalensis extracts on Anopheles coluzzii mosquitoes.

Rakiswende S Yerbangha1,2, Leonardo Lucantoni3, Robert K Ouedraogo1,2, Dari F Da3, Franck A Yao1, Koudraogo B Yaméogo1,4, Thomas S Churcher5,6, Giulio Lupidi1,8, Orazio Tagliatela-Scalifi1,2, Louis Clement Gougna4, Anna Cohuet7, George K Christophides1, Jean Bosco Ouedraogo3 and Amnette Habluetzel2

1Institut de Recherche pour le Développement, Unité MINEVEC (IRD 224 - CNRS 5290-UM1-UM2), BP 64501, Montpellier Cedex 5 34394, France. 2Department of Life Sciences, Imperial College London, United Kingdom. *Corresponding author: Tel.: +326 71484866. Email: vsrserge@yahoo.fr (RS. Yerbangha)

Targeting the stages of the malaria parasites responsible for transmission from the human host to the mosquito vector is a key pharmacological strategy for malaria control. Research efforts to identify compounds that act against these stages have significantly increased in recent years. However, at present, only two drugs are available, namely primaquine and artesunate, which reportedly act on late stage gametocytes. In this study, we assessed the antimalarial effects of 5 extracts obtained from the neem tree Azadirachta indica and Guiera senegalensis against the early vector stages of Plasmodium falciparum field isolates in an ex vivo assay. Gametocytaemic blood was supplemented with the plant extracts and offered to Anopheles coluzzii females by membrane feeding. Transmission blocking activity was evaluated by assessing oocyst prevalence and intensity on the mosquito midguts. Initial screening of the 5 plant extracts at 250 ppm revealed transmission blocking activity in two neem preparations. In 250 and 70 ppm the commercial extract NeemAzal® completely blocked transmission and at 60 ppm mosquitoes were uninsected in 4 out of 5 replicate groups of mosquitoes. Mosquitoes fed on the ethyl acetate phase of neem leaves at 250 ppm showed a mean oocyst number per midgut of 0.63 (CI 0.19 - 1.06) compared to 6.67 (CI 4.42 - 8.92) in controls, while the ethanol extract from the same plant part did not exhibit any activity. No evidence of transmission blocking activity was found using G. senegalensis ethyl acetate extract from stem galls. The results of this study highlight the potential of antimalarial plants for the discovery of novel transmission blocking molecules, and open up the potential of developing standardized transmission blocking herbal formulations as malaria control tools to complement currently used antimalarial drugs and combinations treatments.

KEYWORDS: Plasmodium falciparum, gametocytes, sporogonic stages, plant extracts, transmission-blocking drugs.

P815: Vector diversity and malaria transmission in Ndop, North West Region Cameroon

1National Reference Unit for Vector Control, the Biotechnology Center, University of Yaoundé 1. 2Department of Biochemistry, University of Yaoundé 1. 3National Malaria Control Programme.

BACKGROUND: Malaria is endemic in Ndop. There is the need to simultaneously use different control measures to fight this disease. Knowledge of vectors involved in the transmission of malaria using evidence collected in the field are required to make decisions about the combination of control measures and improved malaria control strategies. This work aims to study the vector fauna and the role of existing Anopheles species involved in malaria transmission in Ndop.

METHODS: In three neighborhoods within the Ndop health area (Backvit, Mbaful and Mbapishi), a series of longitudinal entomological surveys were conducted during one year (2011). Anopheles mosquitoes were collected through human landing night catches and identified morphologically. Four consecutive indoor and outdoor night catches were carried out in each zone per month. Molecular identification (PCR) was carried out for An. gambiae s.s. Infectivity with Plasmodium falciparum was detected by Enzyme-linked immunosorbent assay (ELISA).

RESULTS: A total of 3,972 anopheles was collected, belonging to 09 species: Anopheles gamibae, An. ziemanni, An. chrystyi, An. implexus, An. maculipalpis, An. nii, An. funestus, An. tenebrosus and An. pharoensis. An. ziemanni was the predominant species in this area (93.19%) and had the highest infection rates, followed by An. gambiae (4.7%). Overall the entomological Inoculation Rate (EIR) for the malaria vectors was 0.13 ib/m/n. The Average overall EIR for An. gambiae s.l. and An. ziemanni were 0.01 ib/m/n and 0.12 ib/m/n respectively. Only the M form of An. gambiae s.s was found.

CONCLUSION: This study shows that in the absence of the major vector (An. gambiae), other species may play an important role (An. ziemanni) in malaria transmission. However, studies on the behavior and the susceptibility of vectors to insecticides are needed in order to assist the planning and implementation of improved malaria control strategies in this zone.

P816: Insecticide Resistance and Outdoor Transmission: Threats for Malaria Control and Elimination Efforts in Ethiopia

Delenasaw Yewhalaw1, Abebe Asale1, Yeheneh Getachew2, Luc Duchateau1, Niko S peybroeck3

1Department of Biology, College of Natural Sciences, Jimma University, Jimma, Ethiopia; 2Department of Horticulture, College of Agriculture and Veterinary Science, Jimma University, Jimma, Ethiopia; 3Department of Physiology and Biometrics, Faculty of Veterinary Medicine, University of Ghent, Ghent, Belgium; 4Institute of Health and Society (IRSS), Université Catholique de Louvain, Brussels, Belgium

BACKGROUND: There has been scaling up of the coverage of indoor residual spraying and distribution of long-lasting insecticidal nets by many malaria endemic countries in sub-Saharan Africa to reduce malaria morbidity and mortality. However, the growing and wide spread insecticide resistance and outdoor resting and early peak biting time by mosquito population may affect indoor vector control strategy and the envisaged elimination. We here report the results of 4 years insecticide resistance monitoring on populations of the primary malaria vector, Anopheles arabiensis, the underlying resistance mechanisms and its resting behavior and peak biting time from southwestern Ethiopia.

METHODS: The susceptibility levels of populations of An. arabiensis, collected from different localities of south-west Ethiopia was monitored for 4 years (from 2009 to 2012) using WHO susceptibility test kits. Moreover, mechanisms conferring resistance in An. arabiensis were assessed and characterized using allele specific PCR (AL-PCR) and CDC bottle assays using synergists. Further, the resting behaviour and peak biting time of An. arabiensis was assessed from August to December 2012.

RESULTS: Susceptibility test results of populations of An. arabiensis showed that populations of An. arabiensis from the study site showed reduced susceptibility to DDT, permethrin, deltamethrin, lambdacyhalothrin, alpha-cypermethrin, Cyfluthrin, etofenprox and malathion with mortality rates ranged from 1.90% - 66.07%. However, the mosquito populations were susceptible to bendiocarb (mortality 100%). The West Afican kdr allele (L1014F) was detected with an allelic frequency of > 95% but modified ace1-1 was not detected in all tested specimens. CDC bottle assay using synergists revealed the involvement of metabolic resistance in this mosquito population. Moreover, populations of An. arabiensis showed early peak biting activity and both indoor and outdoor and over 50% of the biting activity occurred between 18:00 – 21:00h and was found to be both endophilic and exophilic.

CONCLUSIONS: In conclusion, populations of An. arabiensis showed reduced susceptibility to 8 out of the 12 insecticides recommended
P817: Retrospective study on the efficacy and durability of the use Long-Lasting Insecticidal Nets (LLINs), PermaNet @ 2.0, under field condition use in Benin
Ramziyath Youssouf

BACKGROUND: PermaNet® 2.0, a Long-Lasting Insecticidal Net (LLIN), according to the manufacturers, maintains its efficacy and durability after 20 washings or 3 years. However, it is important to confirm this allegation under field conditions. This study aims to assess the efficacy and durability of PermaNet® 2.0 LLINs under field conditions.

METHODS: The study was conducted in Southern Benin in 4 sites: Ketonou, Missere, Tori and Vossa. Net samples were collected in each locality. Colorimetric test was carried out in laboratory on collected nets samples followed by cone test, to confirm the nets colorimetric test results.

RESULTS: The results showed that most nets have a lower efficacy beyond ten washes (p<0.001). The frequency of washing has been identified as a factor contributing to the declining efficacy of LLINs. Regarding durability, intense washes have a pronounced effect on alteration of the fibers that cause tears or holes in the nets and it also promotes degradation of insecticide incorporated into the net.

CONCLUSION: The present study showed that the efficacy and the durability of LLINs depend on the use of the net under field conditions. It is therefore desirable that the National Malaria control Program (NMCP) that distributes these tools within the communities implement policies for their follow-up. These (NMCP) should also sensitize populations on use conditions for efficacy and durability of LLIN.

P818: Community Case Management of malaria using RDTs and ACT in Saraya district, Senegal: quality of services and lessons learnt
Ndiiaye Youssoupha1, Ndiiaye Jean Louis2, Clotte Badara2, Blanas Demetri3, Bassene Jonas1, Manga Akhenaton1, Ndiah Mansour2, Faye Sylvain1, Bocoum Mamoudou1, Ndiiaye Mouhamed1, Thiop Pape Moussa1, Sene Doudou1, Milligan Paul1, Gaye Omar1, Schellenberg David1

1Ministry of Health and Prevention, Senegal; 2Department of Medical Parasitology, University Cheikh Anta Diop, Dakar, Senegal; 3Department of Sociology, University Cheikh Anta Diop, Dakar, Senegal; 1Cahn School of Medicine, Mount Sinai, New York, USA; 1Cahn Joint Venture Group; 1London School of Hygiene & Tropical Medicine

INTRODUCTION: Community Case Management of malaria (CCMm) has been developed to support malaria control and elimination. It is important to understand the quality of community services provided by Lay Health Workers (LHW) such as Community Health Workers and malaria volunteers. This study assessed LHW’s performance in Saraya district, Senegal.

METHODS: LHWs were observed as they carried out each step of patient management. This included an assessment of technique in performing an RDT, appropriateness of treatment and referral, provision of information on the signs of severe malaria and advice on methods of malaria prevention. The presence of RDTs, ACTs, paracetamol, gloves, thermometers and a stock card were noted. Management of adverse events and documentation of deaths was discussed. Home visits were made for the last three patients in the LHWs’ register and questions asked to patients about the same set of measures that had been observed in the clinic.

RESULTS: In 2010 and 2011, 61 observations on LHWs and 101 home visits were performed. Direct observation reported 54 LHWs as having recorded fever subjectively or with a thermometer before doing an RDT. Nearly all patients were told the correct dosage and duration of malaria treatment. Only 31 LHWs directly observed the patients take their first dose of ACT. Supervisory visits (51) noted availability of RDTs, ACT and paracetamol respectively in 46, 45 and 35 LHW settings. Gloves were found in 22 sites, thermometers in 23 and stock cards in 11. Adverse event notification tools were available in only 8 health points. Referrals were suggested for 90% of patients who were RDT negative, and for 84% of pregnant women, but the proportion of referrals that took place is unknown. LHWs reported 38 deaths during the previous two months. Caregivers reported 60 LHWs having checked for subjective fever prior to doing an RDT. Advice for malaria prevention and recognition of danger signs was rarely given.

CONCLUSION: LHWs showed good compliance with CCMm procedures and RDTs and ACTs were widely available. LHW improvements are needed in the referral system, recording of adverse events and death notifications.

P819: The Influence of in utero Exposure to Plasmodium falciparum Antigens on Susceptibility of Cameroonian Infants to Malaria during the First Year of Life
Samuel Tassi Yung1,2, Rose G.F. Leke1, Diane W. Taylor2
1Department of Tropical Medicine, Medical Microbiology and Pharmacology, John A. Burns School of Medicine, University of Hawaii, USA; 2The Biotechnology Center, University of Yaoundé 1, Cameroon

BACKGROUND: Malaria parasite antigens transferred across the placenta as immune complexes, may stimulate an immune response in the developing fetus. Currently, the impact of this in utero exposure and probable response, on the susceptibility of babies to malaria during infancy is not well understood.

METHODS: We studied a cohort of 361 Cameroonian babies at birth and through their first year of life. In utero malaria-specific antibody response was assessed by detection of immunoglobulin M (IgM) in umbilical cord blood, to a panel of eight malaria antigens, since maternal IgM does not cross the placenta. A newborn was considered to have responded in utero if IgM to at least two malaria antigens or malaria parasite-infected erythrocyte lysate, were detected using a Multiplex Analyte Platform (MAP) assay. Cord blood collected from United States babies was used as negative control. Microscopy and nested Polymerase Chain Reaction (PCR) were used for malaria parasite detection.

RESULTS: Overall, 10.5% of the newborn babies had IgM in their cord blood that recognized a range of blood-stage antigens, including vaccine candidates on the surface of merozoites. The IgM response was significantly higher in babies born to mothers with high placental malaria parasite density (p=0.042), and lower when mothers received malaria chemoprophylactic Sulfadoxine-Pyrimethamine (p<0.001). Infants who were exposed to malaria in utero and produced malaria-specific antibodies before birth had significantly higher rates of P. falciparum infections by microscopy (p=0.01) and PCR (p=0.02) during their first year of life than those without evidence of prenatal exposure and IgM response.

CONCLUSION: These data show that some babies in malaria-endemic regions mount specific antibody responses to malaria antigens in utero but become more susceptible to malaria. In utero immune priming could lead to a modulation of response to malaria antigens during infancy.
P820: A heat stable peroxidase from Vigna sp

Yves M.E.L. Mbassi, Marie S. Evehe, Wilfred Mbacham, John P. Muluh
Laboratory for Public Health Research Biotechnologies, The Biotechnology Center, University of Yaoundé I

BACKGROUND: Peroxidases are used as markers in DNA probes, in enzyme immunoassays or clinical diagnosis, and as catalysts for several industrial applications. In addition, thermostable peroxidases promote the development of new analytical methods and improve immunoenzymatic analytical kits where these enzymes are used as immunoconjugates. Although the most exploited source of peroxidase is Horseradish (Armoracia rusticana), some studies show that peroxidases isolated from plants of tropical regions have a greater potential for such applications, so our study aimed to characterize a thermostable peroxidase isolated from a Vigna sp growing in barren areas of Cameroon.

METHODS: An isoenzyme was purified by acetone precipitation, heat treatment, gel filtration, and ion exchange chromatography. Kinetic parameters (kM, Vmax, Kcat/Km) were determined by the method of Lineweaver-Burk for 6 of the most used substrates in biotechnological applications of peroxidases at optimum pHs determined in this study. Various heat treatments of various durations were carried out: short duration at high temperature, long duration at moderated temperature, long periods at room temperature. The effect of some metal ions on thermostability of the enzyme was also evaluated.

RESULTS: We have purified near homogeneity a thermostable isoperoxidase from Vigna sp radicles. The inactivation kinetics of the purified peroxidase at pH 8 fitted a first-order reaction, and the half-lives were 3.06 weeks, 13.5 hours, 15.5 min and 3.5 min at 50°C, 70°C, 80°C, and 90°C respectively. The calculated activation energy for its thermal inactivation was found to be 221.5 KJ/mol at pH 8. This peroxidase isoenzyme is stable for 4 months at room temperature, loosing only 5% of its initial activity over this period. Its thermal stability is increased 8 times by Ca++ ions. That peroxidase shows great catalytic efficiencies towards the oxidation of diverse substrates usually used in ELISA (Enzyme-Linked ImmunoSorbent Assay) technique. Apparent Km values for O-dianisidine, ABTS, TMB, DAB and OPD were respectively 3.50 mM, 0.12 mM, 1.81 mM, 0.05 mM, 17.22 mM and 2.53 mM; catalytic efficiencies were 5.12×10^6 M⁻¹.min⁻¹, 2.22×10^6 M⁻¹.min⁻¹, 1.59×10⁵ M⁻¹.min⁻¹, 1.82×10⁵ M⁻¹.min⁻¹, 3.17×10⁻¹ M⁻¹.min⁻¹ and 1.79×10⁻¹ M⁻¹.min⁻¹. It has in other hands a very acid optimum pH for the oxidation of ABTS and an optimum temperature of activity above 60°C.

CONCLUSION: Thermal stability of peroxidases is a requirement for long storage capacity and to improve some analytical techniques were these enzymes are used. The unusual catalytic and thermal characteristics of the peroxidase we isolated could make it a potent tool in several biotechnological applications, especially as part of bench top diagnostic kits in Africa that do not require cold chain.

P821: New potential antimalarial hits from Hypericum lanceolatum (Hypericaceae)

Denis Zofou, Hippolyte H.K. Wabo, Moses N. Ngemena, Pierre Tane and Vincent P.K. Titanji
1Biotechnology Unit, Faculty of Science, University of Buea; PO Box 63 Buea, South West Region, Cameroon; 2Laboratory of Natural Products, Faculty of Science, University of Dschang, Cameroon; 3Department of Biochemistry, University of Dschang, Cameroon.

INTRODUCTION: For decades, traditional herbal medicine had constituted a good basis for anti-malarial lead discovery and drug development. Previous investigations showed that over 200 plants species were identified in Cameroon for their use in malaria treatment by traditional healers, and some of these were proven to contain active ingredients with significant anti-plasmodial activity. However, despite the wide use of such plants like H. lanceolatum in Cameroon, their efficacies have no yet been experimentally established.

OBJECTIVE: A bioassay-guided fractionation of the stem bark of H. lanceolatum was conducted in order to assess the in vitro anti-plasmodial activity, and thereby establish the potential of the plant species as source of new malaria drug leads.

METHODS: The antiplasmodial activity was assayed by the lactate dehydrogenase method (pLDH) against the multidrug-resistant W2mef laboratory strain, and a field isolate (SHF4) of Plasmodium falciparum. Cytotoxicity tests were carried out using the LLC-MK2 monkey kidney epithelial cells.

RESULTS: Five compounds were isolated from the most active and least cytotoxic ethylacetate sub-extract: betulinic acid (HLT1), 2,2',5,6'-tetrahydroyxobenzophenone (HLT2), 5-hydroxy-3-methoxyxanthone (HLT3), 3-hydroxy-5-methoxyxanthone (HLT4) and HLT0 (yet to be identified). Three of the tested compounds presented significant anti-plasmodial activities (with 50% inhibitory concentration, IC50 < 5 μM), with 5-hydroxy-3-methoxyxanthone exerting the highest activity, followed by HLT0 and betulinic acid. All the compounds with significant antiplasmodial activity were non-cytotoxic, except for betulinic acid which showed a 50% cytotoxic concentration, CC50 of 25 μg/mL.

CONCLUSION: These findings justify the use of H. lanceolatum stem bark as anti-malarial by traditional healers of Western Cameroon, and could constitute a good basis for further studies towards development of new drug candidates or phytomedicines for malaria.

P823: Randomized non-inferiority trial of dihydroartemisinin-piperaquine compared with sulfadoxine-pyrimethamine plus amodiaquine for Seasonal Malaria Chemoprevention in children in Burkina Faso

Issaka ZONGO

BACKGROUND: WHO recommends that children living in areas of highly seasonal malaria transmission in the Sahel and sub Sahel should receive Seasonal Malaria Chemoprevention with sulfadoxine-pyrimethamine
plus amodiaquine. These drugs retain their antimalarial efficacy in the areas where SMC is appropriate, but alternative regimens may be needed if SMC is used in other areas or if these drugs start to lose their efficacy. DHA-PQ is a potentially suitable alternative, this trial was conducted in an area where SMC with SPAQ is highly effective, to determine whether DHA-PQ is as effective when used for SMC.

**METHODS:** 1500 children aged 3-59 months were randomized to receive SMC with SPAQ or DHA-PQ over three months from August to October. Surveillance was maintained to record incidence of malaria, the primary endpoint was malaria with fever or history of fever with a parasite density of 3000/µL over a period of three months. To monitor incidence in untreated children, a cohort of 250 children outside the main trial were followed up in a similar way over the latter two months of the trial. Parasite DNA was analysed from patients with malaria to determine presence of molecular markers of drug resistance.

**RESULTS:** There were 280 episodes of malaria with parasite density of 3000/µL or more, 119 in the SPAQ group and 161 in the DHA-PQ group. Kaplan-Meier estimates of the proportion of children with a malaria attack was 0.15 and 0.19 respectively, odds ratio 1.36 (95%CI 1.04,1.76). Efficacy of SMC with DHAPQ compared to the control group was 77% (95%CI 67%,84%) and of SPAQ, 83% (74%,89%). The CVIET haplotype of pfcr, the 86Y polymorphism of pfmdr1, and pfdrf59 and dhps437 mutations were more common in malaria cases among children who received SPAQ than in children who received DHAPQ.

**CONCLUSIONS:** SPAQ was more effective than DHAPQ in preventing malaria but both regimens had high efficacy and were safe and well tolerated. DHAPQ is a potential alternative regimen for chemoprevention in children where SPAQ cannot be used.

This trial is registered at www.clinicaltrials.gov, NCT00941785
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