Challenges in malaria research: Progress towards elimination

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ORAL PRESENTATIONS

O1 Malaria surveillance systems: from control to elimination
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The capacity of malaria surveillance systems to provide accurate information on the distribution of and trends in malaria varies widely across the globe. It is influenced by (i) the extent to which patients seek treatment, (ii) whether patients use public sector health facilities, (iii) the proportion of patients that receive a diagnostic test, and (iv) the completeness of recording and reporting systems. When these factors are taken into account, it is estimated that malaria surveillance systems detect less than 10% of all cases globally, though the proportions are higher in the Europe (>90%) and the Americas (50%). The characteristics of surveillance systems vary by geographical region. South-East Asia has the lowest percentage of malaria patients that seek treatment in public health facilities. Confirmatory diagnostic tests (blood slides or RDTs) are used infrequently in Africa, as compared with other regions. Reporting is most complete in the European region.

In April 2012 WHO released operational manuals for malaria surveillance to guide programmes both in the control phase and those in the elimination phase. In the control phase the objective of malaria programmes is to reduce the incidence of and mortality from malaria as rapidly and economically as possible. Many countries with high levels of malaria transmission are low- or lower-middle-income countries, which have low expenditures per person on health care services. This results in weak health systems that are not easily accessed by the population, lower staff to patient ratios, frequent interruptions of medical supplies and limited use of parasitological diagnosis. Such settings pose particular challenges to the development of surveillance systems. Health systems in low-transmission settings are usually stronger than in high-transmission settings, and there may be widespread availability of parasitological diagnosis and appropriate treatment. Malaria may, however, be concentrated in marginalized populations, such as those living in remote border areas, migrant workers and tribal populations, and innovative ways may have to be found to reach these groups.

In the elimination phase cases occur sporadically or in distinct foci and imported cases may comprise a significant proportion of all cases. The aim of malaria programmes is to stop local transmission of malaria, and surveillance is a principal strategy for achieving this. All malaria infections are important and need to be detected, as they may lead to onward transmission (i.e. all persons with parasitaemia are considered a ‘malaria case’, regardless of the presence or absence of clinical symptoms). In practice, this is accomplished in two stages (i) by identifying all areas or foci with local transmission of malaria using reports of malaria cases from public and private sector health facilities. Each malaria case is then investigated to determine whether it was locally acquired or imported and, if so, from where. (ii) if a focus of local transmission is detected, the characteristics of transmission are determined and control and surveillance activities are then intensified in the focus.

O2 The global distribution of Plasmodium vivax and G6PD deficiency prevalence
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Existing understanding of the spatial epidemiology and geographical distribution of Plasmodium vivax is poor. Here we present the first systematic effort to map its endemicity globally. Routine case reporting data were assembled from 17,893 administrative units across the 95 P. vivax endemic countries and combined with biological risk exclusion layers and other medical intelligence data to update the estimated global limits of P. vivax transmission for 2010. Within areas of stable transmission, a second assembly of 9,970 quality-checked and geopositioned P. vivax parasite rate surveys were used with a spatiotemporal Bayesian model-based geostatistical approach to estimate endemicity age-standardised to the 1-99 year age range within every 5x5 km resolution grid square. The model incorporated prevalence data on the refractory Duffy negative genotype to appropriately suppress risk predictions, particularly in Africa. Endemicity was predicted within a relatively narrow range of prevalence throughout the endemic world with the point estimate rarely exceeding 7%. These patterns are described. Radical cure of P. vivax requires treatment of the parasite’s dormant relapsing life stages, for which primaquine is the only drug licenced. This drug may, however, trigger mild to severe haemolysis in patients with a genetically determined deficiency in glucose-6-phosphate dehydrogenase production (G6PDd). We therefore also present the first evidence-based continuous prevalence map of G6PDd globally. Representative community surveys of phenotypic G6PDd prevalence were identified for 1,734 spatially-unique sites globally. These formed the evidence-base for a Bayesian geostatistical model adapted to the G6PD gene’s X-linked inheritance mechanism, which generated a G6PDd allele frequency map across malaria endemic countries. The resulting maps and population estimates reflect potential risk of primaquine-associated harm.

O3 Challenges in surveillance and response
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Surveillance in the context of malaria elimination will needs to shift from measuring reductions in morbidity and mortality to detecting infections (with or without symptoms). The malaria elimination surveillance research and development agenda needs to develop tools and strategies for active and prompt detection of infection. The capacity to assess trends and respond without delay will need to be developed, so that surveillance itself becomes an intervention. Research is needed to develop sensitive field tests that can detect low levels of parasitaemia and/or evidence of recent infection. Examples of recent work on surveillance and response issues in several African countries will be discussed to illustrate approaches in active case
detection and case investigations, cell phone reporting and response, and strategies to access mobile populations.

**04** The importance of the linkage between national and international research funders to support malaria elimination

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Abstract not submitted

**05** Targeting the parasite in the mosquito: rationale and practicality

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From the very earliest days of malaria research and control it has been recognised that attacking the mosquito vectors is one of the most powerful interventions at our disposal. Recognising that these activities are, sensu stricto, attacking the population of parasites as they pass through the vectors, it is now appreciated that attacking the parasite per se within the mosquito vector has equal potential to contribute to malaria control, elimination or indeed eradication.

The rationale behind current anti-parasitic malaria transmission-blocking strategies is profound. Parasite populations in the mosquito host can be 10–100 smaller than those in the human host, offering reduced blocking strategies is profound. Parasite populations in the mosquito host can be 10–100 smaller than those in the human host, offering reduced genetic/molecular potential with which the parasite can combat any intervention. Further, the parasites are, for the first 24 hours, extracellular and directly exposed to any intervention (drug; vaccine or other) delivered in the infectious bloodmeal. Finally, within the vector many of the accessible parasite and mosquito surface molecules have never been exposed to the human immune system, perhaps as a correlate to this they, by comparison with merozoite and sporozoite surface molecules, are neither antigenically variant, nor significantly polymorphic, thus offering stable and geographically widespread targets for intervention.

In response to the new calls for research to underpin possible elimination/eradication campaigns, current efforts are now describing potential and effective new drugs and vaccine strategies targeting the parasites in the mosquito bloodmeal. Recent studies examining the potency of transmission blocking vaccine candidates, and the identification of new and transmission blocking drugs will be reviewed. Questions must be asked as to what relevance the outputs of current laboratory based transmission blocking experiment have to the key population-based parameters that must be measured in the field. As an example:- If an intervention causes a 50% reduction in oocyst number per se within the mosquito vector has equal potential to contribute to malaria control, elimination or indeed eradication.

**06** Finding hotspots: the role of active surveillance methods in malaria control and elimination

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It is evident that malaria infection and transmission display fine scale clustering over all transmission settings. Such clusters, or hotspots, could be a group of households, or even a single household, whose occupants suffer from an abnormally high exposure to infectious mosquitoes and are a source of infection to households outside the cluster. Whilst it has been suggested that targeting interventions at hotspots is likely to be a cost-effective method to reduce transmission, the challenge remains to develop methods for their identification. Active Case Detection (ACD), whereby defined populations are screened and treated where necessary, may offer one solution. There are, however, a number of factors that need to be considered before ACD is implemented, including its timing and frequency, whether it should be conducted pro- or re-actively, transmission setting and diagnostic method used. This presentation will examine and discuss the potential use and effectiveness of ACD in relation to the spatial epidemiology of malaria using examples over different transmission settings.

**07** Rapid mapping of seasonal malaria transmission risk for strategic elimination planning in Swaziland

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Background As successful malaria control programs move towards elimination, they must identify residual transmission foci, focus on both asymptomatic and symptomatic infections, and manage importation risk. High spatial and temporal resolution maps of malaria risk can support all of these activities, but new approaches are required to provide accurate case-based risk maps for very low prevalence countries like Swaziland, where fewer than 500 cases were reported in 2011.

Materials and methods Household locations and travel histories of confirmed malaria patients were recorded through routine surveillance by the Swaziland National Malaria Control Programme for the higher transmission months of Jan to Apr 2011 and the lower transmission months of May to Dec. Household locations with locally-acquired infections were compared against a random set of background points with respect to variables related to environment, population density, vector control, and distance to the households of imported cases. Comparisons were made separately for the high and low transmission seasons. The regression tree classification approach Random Forest was used to generate maps predicting the probability of a locally-acquired case at 100 m resolution across Swaziland during the high and low transmission seasons.

Results Results indicated that case households during the high transmission season tended to be located at lower elevations, closer to stream channels, in more sparsely populated areas, with higher rainfall and lower temperature than random background points (all p<0.01). No significant difference was evident with distance to the nearest imported case household. Similar differences were evident during the low transmission season, but environmental variables like distance to stream channels and water bodies were no longer significantly different, while low season case households were located significantly nearer to those of imported cases (p=0.02). Maps from the fit models suggested better predictive ability during the high season.

Conclusions The rapid, high-resolution mapping approaches described here appear useful for helping elimination programs understand the epidemiology of a disappearing disease. Generating case-based risk maps at high spatial and temporal resolution will allow control programs to direct interventions in response to evidence-based measures of risk and ensure that the impact of limited resources is maximized to achieve and maintain malaria elimination.

**08** A comprehensive risk map for malaria in Kinshasa, Democratic Republic of Congo

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Malaria Journal 2012, 11(Suppl 1):C08

Background The Democratic Republic of Congo (DRC) is the second most malarious country in the world. However, there is a paucity of epidemiological data on the risk pattern of malaria.

Methods In 2009 (dry season) and 2011 (end of the rainy season), two-stage cluster sampling malaria surveys were conducted in the
capital city Kinshasa with the twofold aim of (1) assessing malaria parasite prevalence, anemia and associated malaria risk factors, and (2) producing a malaria risk map using a geographic information system (GIS).

**Results** A total of 6410 children aged 6-59 months (3058 in 2009 and 3352 in 2011) were tested for both malaria (using rapid diagnostic tests) and anemia (by Hemoccu™). Nine health zones (HZ) were sampled in 2009 with an average prevalence for malaria and anemia of 6.6% (95% CI 5.8-7.5) and 66.0% (64.5-67.4) respectively, while in the 25 HZs in 2011 the prevalence was 17.0% (15.7-18.3) and the anemia rate was 62.6-65.9%. Overall, the rate for both surveys was 11.9% (11.2-12.8) for malaria and 65.1% (63.9-66.7) for anemia. To compare comparability of the results between surveys, two HZs from 2009 were resampled in 2011. Prevalence for malaria in 2009 and 2011 was: Ngiri Ngiri 1.0% versus 0.8% and Selembao: 14.1% versus 26.8%. Prevalence for anemia was: Ngiri Ngiri 62.5% versus 55.4% and Selembao: 67.1% versus 61.4%. In a multivariate analysis of the 2011 data, significant protective factors for malaria risk were: educational level of the respondent (OR = 0.12, 95% CI: 0.03–0.56) and sleeping under an ITN (OR = 0.52, 95% CI: 0.43–0.63). All key parameters were mapped to the level of the HZs (n=35). Malaria parasitemia, anemia and fever prevalence were found to be much lower in the city center than in the peri-urban suburbs, where transmission rates remain high. ITN usage around 1000 per 1000 people.

**Conclusions** For the first time a comprehensive picture of the epidemiology of malaria has been prepared for Kinshasa, a mega-city in a highly endemic zone. This provides a solid baseline information for planning future malaria control interventions.

Low level malaria transmission continued on Santiago and, in 2003, reappeared on the island of Boa Vista. The annual parasite incidence for the whole archipelago has remained below 0.3 per 1000 population, exceeding 0.5 per 1000 population on Santiago Island only once, in 2000. Over the last 20 years, activities have been restricted to passive case finding and investigation with case-based surveillance on Santiago, and to early detection of imported cases elsewhere. Unfortunately, in 2006, an unexpected 8 malaria deaths occurred. Formulated in 2007, the National Health Policy sets out the strategy for fully eliminate malaria by 2020. The Government developed a National Strategic Plan 2009-2013 with an integrated approach and the following main strategies: drug policy change to artemisinin-based combination therapy (ACT) for *P. falciparum*; case detection among all febrile patients with positive travel history; full reporting of microscopically confirmed cases; case and foci investigation; vector control – including focal larval control where appropriate, and IRS. National funding for malaria has been increased; in 2011, a 5-year Global Fund grant was secured to support the programme transition to elimination.

**Conclusions** In Cape Verde, malaria transmission has already been interrupted twice within the last 50 years, confirming that elimination is technically feasible; the challenge is now sustainability. The importance of preventing malaria reintroduction should be fully taken into account if elimination is to be achieved.

**O10 Progress towards elimination: a case study of Sri Lanka**

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This abstract is submitted as part of the panel session on case studies for elimination by the WHO Global Malaria Programme and the UCSF Global Health Group.

**Background** As malaria transmission declines and malaria programs shift their focus from malaria control to elimination, it is vital to have documentation of the strategies that countries have used and are currently applying as they seek to eliminate malaria. Our case study of Sri Lanka, which has a long history of malaria control, including a period of near elimination and resurgence in the 1960s, aims to capture the key factors behind the country’s decline in malaria over the last decade.

**Materials and methods** This case study employed qualitative and quantitative methods, using data triangulation to compare and contrast trends. A review of available literature was conducted, and district and national data were collected on incidence, surveillance and vector control. Trends were observed across years and districts, in particular comparing conflict and non-conflict districts. Thirty-three key informant interviews were conducted. Expenditures in two districts for two years were compiled to identify changes in expenditure.

**Results** Malaria control in Sri Lanka began in the early 1900s [1]. Indoor residual spraying (IRS) was introduced in 1945 and, in combination with surveillance, led to a decline in cases to 17 in 1963 [1]. Only four years later, however, two outbreaks of *P. vivax* in 1967 led to a major epidemic (1967-68) [2]. Factors contributing to the epidemic were the reduction of DDT, emerging vector resistance to DDT, complacency of malaria control officers, lack of funding and population movement [2,3,4] As a result Sri Lanka again scaled up IRS mobile units but the damage had been done. In the last decade, Sri Lanka has made great strides in reducing its malaria burden. Malaria incidence in Sri Lanka has declined by 99.9% since 1999. During this time, there were major increases in the proportion of malaria infections due to *Plasmodium vivax*, and those occurring in adult males. New vector control strategies were introduced, such as spatial insecticide rotation and long-lasting insecticide-treated nets. A strong passive case detection system is the foundation for diagnosis, while active case detection grew from identifying 1.1% of all infections in 2000 to 13.1% in 2007. Vector control and surveillance measures were maintained in conflict areas. For example, coverage of...
indoor residual spraying of risk populations in conflict districts was 45.9% in 2005 (10.9% in non-conflict districts). One of two districts in the study reported a 48% decline in malaria programme expenditure per person at risk from 2004 to 2009, and a decline in prevention costs and an increase in surveillance costs.

**Conclusions** Malaria is now at low levels in Sri Lanka – 124 indigenous cases were found in 2011. Evidence-driven policy and an ability to adapt to new challenges contributed to this decline.

**References**


**O11 Achieving malaria elimination and certification in Turkmenistan**

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This abstract is submitted as part of the panel session on case studies for elimination by the WHO Global Malaria Programme and the UCSF Global Health Group.

**Background** This case study presents and evaluates the strategies and policies applied for containment of re-emerging malaria outbreaks in Turkmenistan since the 1990s, and the process followed for achieving malaria elimination. Evidence-based lessons for countries that are considering or embarking upon elimination are distilled. The case study is a part of a series of malaria elimination case studies conducted by the WHO Global Malaria Programme and the University of California, San Francisco, Global Health Group. Key partners in the case study work were the National Malaria Control Programme, Ministry of Health, and WHO Regional and Country Offices.

**Materials and methods** A comprehensive search was made of English and Russian language materials related to malaria in Turkmenistan, as well as selected materials in Turkmen language. Published and grey literature; National Malaria Programme data and other official data from the Ministry of Health; information from direct observations; and WHO archives were consulted. Epidemiological, programmatic, social and economical determinants were extracted and analysed to evaluate (1) the malaria epidemiological situation; (2) social and economical factors that influence malaria situation; and (3) programme operations.

**Results** Turkmenistan achieved malaria elimination by the 1960s, after which only sporadic imported and introduced cases of Plasmodium vivax were reported. In the 1980s and 1990s the malaria threat increased due to rising receptivity in some areas (owing to major water projects such as construction of the Karakum Canal, expanded irrigation, and increased rice production), as well as to increasing vulnerability in districts bordering Afghanistan related to growing population movement. Malaria importation from Afghanistan increased, followed by an increase of autochthonous cases. Outbreaks of vivax malaria were registered in Mary province on the border with Afghanistan in 1998-99 (in a military training camp) and in 2002-03 (among petroleum workers). The main interventions to contain the outbreaks were: intensive case detection (daily house-to-house visits; mass blood surveys) with subsequent treatment of those found to be positive; epidemiological investigation of all cases and foci; radical treatment of patients; IRS; and larviciding. The population in active transmission foci received seasonal chemoprophylaxis with chloroquine and after-season radical treatment with 14 days of primaquine.

Following the outbreaks, malaria elimination was achieved by: (1) strong political commitment and sustained national funding; (2) introduction of a National Strategy and Plan of Action for Malaria Elimination; (3) case-based surveillance of confirmed cases through quality-assured laboratories; (4) integrated vector control in foci; and (5) cross-border collaboration with Afghanistan. The last autochthonous cases were registered in 2004. In 2010, the country was certified by WHO as free of malaria.

**Conclusions** The Turkmenistan case study is an example of the correct application of evidence-based malaria elimination strategies and policies to overcome a reintroduction of malaria transmission and subsequently achieve malaria elimination. It illustrates the need for continued political commitment, programmatic efforts and substantial funding needed to design and implement a malaria elimination programme. The Turkmenistan experience highlights the importance of a clear plan for prevention of malaria reintroduction following elimination, and the need for continued financial resources to execute these critical activities.

**O12 Eliminating malaria and preventing its reintroduction: the Mauritius case study**

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This abstract is submitted as part of the panel session on case studies for elimination by the WHO Global Malaria Programme and the UCSF Global Health Group.

**Background** Sustaining elimination of malaria in areas with high receptivity and vulnerability will require effective strategies to prevent reestablishment of local transmission, yet there is a dearth of evidence about what such approaches should involve. Mauritius offers a uniquely informative history, with elimination of local transmission in 1969, re-emergence in 1975, and second elimination in 1998.

**Materials and methods** To provide evidence for future elimination programs, Mauritius’s elimination and prevention of reintroduction (POR) programs were analyzed through a comprehensive review of literature and government documents, supplemented by program observation and interviews with policy makers and program personnel.

The Por of the country’s most costly intervention, a passenger screening program, was assessed quantitatively using simulation modeling.

**Results** Following the introduction of malaria in Mauritius in the mid-1800s, P. vivax and P. falciparum malaria were hyperendemic until the government launched an aggressive campaign to interrupt transmission and eliminate the parasite through indoor residual spraying (IRS) in 1948. Between 1948 and 1963, incidence rates declined from 105 cases per 1,000 population at risk to 0.04 at an estimated cost of $5.75 per capita per year (pcpy) between 1948 and 1949 and $2.99 pcpy between 1960 and 1961. Anopheles funestus was eliminated during this time, leaving An. gambiae as the main vector. Local P. vivax transmission was re-established in 1975 after large cyclones created new breeding sites and para-sitaemic workers from endemic countries arrived to rebuild the damaged infrastructure. Lax interventions (e.g. surveillance and vector control) during the first POR program may have also contributed to this resurgence, as well as increased importation risk.

Mauritius launched a second elimination campaign from 1982 to 1988 through implementation of a combination of focal interventions, widespread larviciding, and an extensive case response system at a cost of $4.43 pcpy. The country currently spends $2.06 pcpy on its POR program that includes robust surveillance, routine vector control (larviciding island-wide and IRS at the ports of entry), free chemoprophylaxis to travelers, and prompt and effective diagnosis,
treatment, and response. Thirty-five percent of POR costs are for a passenger screening program through which passengers arriving from malaria endemic countries, report having been in an endemic country in the last six months, or who are febrile upon or soon after arrival are tested at the ports of entry or are contacted by surveillance officers at their residence. Between 2005 and 2008, an average of 42,612 blood smears collected through passenger screening were examined for malaria parasites detecting an average of 10 positive cases each year. Modeling suggests that the estimated 14% of imported malaria infections identified by this program reduces the annual risk of local transmission by approximately 25%.

Conclusion The Mauritius experience demonstrates that it is possible to eliminate malaria and prevent its reintroduction in a country with relatively high receptivity and moderate vulnerability but that continuous vigilance and some control to reduce and maintain low vector density is critical. Strong leadership and substantial predictable funding are critical to consistently prevent resurgence in Mauritius and must be sustained.

O13 Health systems stewards and health systems researchers: a critical partnership for malaria elimination
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The Global Malaria Action Plan places scaled-up effective malaria control as a necessary prerequisite for malaria elimination. Effective and sustained malaria control depends on vastly strengthened health systems in malaria endemic countries. However definitions of health system strengthening, and the means to achieve it are still lacking. Emerging approaches of system-wide thinking that applies systems thinking tools in implementation science can open new possibilities to accelerate system strengthening with a particular focus on the systems effectiveness of malaria surveillance and control interventions. This presentation will discuss how tipping point revolutions in health systems could be harnessed to strengthen health systems to become elimination ready. These include social network analysis to better manage stakeholder partnerships that bring malaria control and implementation research closer together; new concepts in governance of health systems to improve both system design and systems management; new approaches to diagnose system effectiveness bottlenecks and target system-level interventions; and modern mHealth strategies to improve procurement and supply chains as well as surveillance as an intervention in real time. For rapid progress across these dimensions, closer and better managed partnership among health system managers, health systems researchers and implementation scientists is essential.

O14 Communities, policy-makers and scientists: a critical partnership for malaria elimination
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Partnership between all stakeholders involved in malaria efforts has been demonstrated to be a pillar of successful malaria control, a precursor for malaria elimination. Over the last decade, collaboration between donors, governments, academia, industry (private sector), civil society and to reasonable extent communities has borne fruits that were only a dream in the recent past. Manuals about the prerequisites for countries moving from control to elimination abound. They point out the need for country programs to embrace a paradigm shift that entails not only thinking differently but also managing differently. Key to the move from control to elimination for a country is the sustained “free from local transmission” status for three years at least, thus having malaria off the list of a country’s key health problems, driven by a strong political commitment to follow through overall development efforts including the Millennium Development Goals, development of strong health systems and enactment of adequate policies for vigilance/surveillance together with enhanced legislation to support some of the policies. In addition, a cooperating epidemiological condition that allows positive parasitological rates of below established thresholds is critical. This paper while acknowledging the importance of all partners in the malaria control equation, posits that the partnership between communities, policy-makers and scientists be strengthened. Communities that embrace and believe that malaria can be controlled are better able to be engaged in surveillance and vigilance, and are also better able to demand for services and follow through with utilization of necessary tools during the pre-elimination to elimination phase. This paper further argues that scientists who are the ones charged with providing evidence upon which policies and strategies are based have to ensure their evidence is unpacked to enable policy-makers make rational decisions – based on science and not political rhetoric. Countries that have conditions that are conducive to moving to elimination should be supported and encouraged to do so. On the other hand, countries in which conditions for elimination are fraught with challenges including long or year-round transmission (high endemicity), low coverage of effective malaria control interventions and lack of adequate funding among others should be advised against taking the elimination route prematurely.

Examples of countries that have registered progress in Africa through deliberate strengthening of partnerships as described above will be provided. In addition, the paper will provide proposals about channels that can be used to reach policy-makers, who must of necessity not only steward their countries’ control to elimination agenda, but also provide strong leadership for inevitable cross-border collaboration.

O15 Supply and demand for antimalarial drugs and diagnostic testing in the era of subsidies: multi-country findings
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Abstract not submitted

O16 Overcoming the affordability barrier for effective and high quality life saving malaria medicines in the private sector in rural Uganda: The Consortium for ACT Private Sector Subsidy (CAPSS) pilot study
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Malaria Journal 2012, 11(Suppl 1):O16

Background Artemisinin-based combination therapies (ACTs), the treatment of choice for non-complicated falciparum malaria, are unaffordable and inaccessible in the private sector, yet the private sector is the first port of call for malaria treatment across most of rural Africa. Between August 2007 and May 2010, the Uganda Ministry of Health and the Medicines for Malaria Venture conducted the Consortium for ACT Private Sector Subsidy (CAPSS) pilot study to test whether access to effective malaria treatment could be improved through the provision of highly subsidized ACTs in the private sector.

Methods Four intervention districts (Pallisa, Budaka, Kamuli and Kaliro) were purposefully selected to receive branded subsidized medicines- “ACT with a leaf”, while the fifth district (Soroti) acted as the control.
Baseline and evaluation outlet exit surveys and retail audits were conducted at all licensed private drug outlets in the intervention and control districts. A survey-adjusted, multivariate logistic regression model was used to analyse the intervention’s impact on: ACT uptake; access to ACTs within 24-hours of symptom-onset; and displacement of sub-optimal antimalarials.

**Results** At baseline, the market share of ACTs was <1%. However, at evaluation, “ACT with a leaf” had a market share of 69% in the interventions districts. Access to ACTs within 24 hours of symptom onset rose from 0.8% at baseline to 26.2% (95% CI: 23.2-29.2%) at evaluation in the intervention districts. In the control district it modestly rose from 1.8% to 5.6% (95% CI: 4.0-7.3%). The odds of accessing ACTs within 24 hours in the intervention compared to the control districts was 0.46 (95% CI: 0.08-2.68, p=0.4), at baseline and significantly increased to 6.11 (95% CI: 4.32-8.62, p<0.0001) at evaluation. Children less than 5 years-old had “ACT with a leaf” purchased for them more often than those aged above 5 years. There was no evidence of price gouging.

**Conclusions** Our data demonstrate that a supply-side antimalarial subsidy coupled with an intensive communications campaign significantly increased the uptake of ACTs in the private sector in Uganda.

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**O17**

The combined effect of determinants on coverage of intermittent preventive treatment of malaria during pregnancy in the Kilombero Valley, Tanzania

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**Malaria Journal 2012, 11(Suppl 1):O17**

**Background** Intermittent preventive treatment during pregnancy (IPTp) at routine antenatal care (ANC) clinics is an important and efficacious intervention to reduce adverse health outcomes of malaria infections during pregnancy. However, coverage for the recommended two IPTp doses is still far below the 80% target in Tanzania. This paper investigates the combined impact of pregnant women’s timing of ANC attendance, health workers’ IPTp delivery and different delivery schedules of national IPTp guidelines on IPTp coverage.

**Materials and methods** Data on pregnant women’s ANC attendance and health workers’ IPTp delivery were collected from ANC card records during structured exit interviews with ANC attendees and through semi-structured interviews with health workers in south-eastern Tanzania. Women’s timing of ANC visits and health worker’s timing of IPTp delivery were analyzed in relation to the different national IPTp schedules and the outcome on IPTp coverage was modelled.

**Results** Among all women eligible for IPTp, 79% received a first dose of IPTp and 27% were given a second dose. Although pregnant women initiated ANC attendance late, their timing was in line with the national guidelines recommending IPTp delivery between 20-24 weeks and 28-32 weeks of gestation. Only 15% of the women delayed to the extent of being too late to be eligible for a first dose of IPTp. Less than 1% of women started ANC attendance after 32 weeks of gestation. During the second IPTp delivery period health workers delivered IPTp to significantly less women than during the first one (55% vs. 73%) contributing to low second dose coverage. Simplified IPTp guidelines for front-line health workers as recommended by WHO could lead to a 20 percentage point increase in IPTp coverage.

**Conclusions** This study suggests that facility and policy factors are greater barriers to IPTp coverage than women’s timing of ANC attendance. To maximize the benefit of the IPTp intervention, revision of existing guidelines is needed. Training on simplified IPTp messages should be consolidated as part of the extended antenatal care training to change health workers’ delivery practices and increase IPTp coverage. Pregnant women’s knowledge about IPTp and the risks of malaria during pregnancy should be enhanced as well as their ability and power to demand IPTp and other ANC services.
Background
Throughout Africa, the private sector plays an important role in malaria treatment complementing formal health services. However, this sector is faced by a number of challenges including poor dispensing practices by unqualified staff. The Accredited Drug Dispensing Outlet (ADDO) program was introduced in Tanzania in 2002 to improve the quality of retail services and especially of dispensing practices. The study adapted the often contested mystery shopping methodology and trained local community members to assess practices of ADDO dispensers. The study then compared the assessed dispensers’ practices before and after ADDO interventions.

Methods
Mystery shoppers were identified in the villages with the assistance of Health Demographic Surveillance System field staff. A total of 865 visits were made to general shops and drug shops between 2004 and 2009. Three case scenarios were developed to assess the quality of treatment; a) child aged 2-4 months, with fever/hot body for one day and problems with drinking/breastfeeding, b) child aged 2-4 years, with recurring fever/hot body for 3 days problems with drinking, eating, diarrhea and tiredness/not playing as usual and c) adult, with recurring fever/hot body for 2 days, headache, dizziness and loss of appetite.

Results
Study findings indicate improvements in dispensers’ knowledge and practices in management of fever, especially after the roll out of ADDO program in the study area. A 30 percent increase was noted after ADDO interventions on four assessed indicators developed based on the national malaria control guideline on malaria case management. On the other hand advice on the use of Insecticide Treated Nets as a measure to prevent malaria was not consistent over years even after ADDO interventions. Children aged two to four years and adults were more likely to be provided with anti-malarials than children between two to four months. Despite challenges posed against the methodology, findings reveals how useful the mystery shopping technique can be for community assessments of ADDO interventions in retail outlets.

Conclusion
Study findings signify the importance of ADDO interventions in improving malaria case management in drug retail outlets. If ADDOs are closely monitored and strengthened to provide appropriate malaria treatment and the program is rolled throughout the country, a reduction in malaria morbidity and mortality is possible in the country. Innovative community based participatory research approaches and more systematic mystery shopping techniques would allow for comparative community-based assessments of ADDO interventions across regions.

O21
Chemotherapeutics for vivax malaria
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Plasmodium vivax imposes significant burdens of morbidity and mortality across the malaria endemic world. Treatment of this infection requires a blood schizontocide against the acute attack and hypnozoitocide against relapses. Chloroquine combined with primaquine has been the therapy of choice for radical cure of vivax malaria since the 1950s. Primaquine, however, was never optimized or adapted for use in endemic zones and its toxicity in prevalent G6PD-deficient patients (typically 5-20%) sharply limits its effectiveness. Resistance to chloroquine has emerged in Southeast Asia and now threatens the Indian sub-continent where most P. vivax occurs. The research community faces the steep challenge of developing new radical cure strategies. This presentation explores those challenges and the means to meet them, principally optimizing primaquine as a partner to new ACTs for maximum efficacy and more practical dosing and safety.

O22
Abstract not submitted for online publication
**O23**

**Dissecting T cell or antibody immunodominance in a complex host-pathogen system**

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**Background** Vaccines are considered the most cost-effective public health measure for the prevention of infectious diseases, with many demonstrated successes. However the Plasmodium parasite, causative agent of malaria, has eluded decades of efforts aimed at developing an effective intervention. Contributing to this is the complexity of the parasite life cycle and our poor understanding of the mechanisms and antigenic targets of host immunity to the parasite.

**Materials** To enhance our understanding of the host-parasite relationship and facilitate rationale vaccine design, we have generated independent proteome-wide datasets of antibody responses and T cell responses to *P. falciparum*. Parasite antigens have been prioritized on the basis of the frequency and magnitude of immune responses in individuals either experimentally infected or naturally exposed to malaria. We are integrating these complex datasets to develop metrics of immunological, structural and genetic parameters associated with antigen immunodominance. A range of computational tools and comparative genomic analyses have been applied to extract information on putative structural and functional features associated with both immunodominance and antigenicity. These analyses have also taken into account information on gene and protein features, transcript and protein expression, protein localization, post-translational modifications, and sequence conservation between different *P. falciparum* strains and *Plasmodium* species.

**Results** Our data demonstrate that the most antigenic parasite-encoded molecules are not randomly distributed throughout the proteome since a large number of potential antigens are not recognized. Importantly, we establish that the antigens sets that are highly reactive for T cells are distinct from those that are highly reactive for antibodies. Additionally, while antigens recognized preferentially by antibodies are highly polymorphic, the most T cell reactive antigens are highly conserved amongst *P. falciparum* strains and different *Plasmodium* species. This observed lack of epitope polymorphism contrasts with dogma in the field that important antigens or epitopes are polymorphic as a consequence of parasite evasion of host immune responses.

Further analyses are underway to determine the localization and the expression kinetics of immune targets and to identify any structural and functional characteristics associated with the relative immunogenicity of *P. falciparum* antigens or epitopes.

**Conclusions** This study represents, to the best of our knowledge, the first attempt to quantitatively and qualitatively define the parameters of immunodominance in humans in response to a complex pathogen. These data will facilitate the rational design of vaccines against malaria and provide the foundation for similar studies of other pathogens that threaten public health.

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**O24**

**Plasmodium vivax in Papua New Guinea: high diversity and gene flow among endemic populations signal roadblocks for elimination**

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**Malaria Journal 2012, 11(Suppl 1):O24**

The highest prevalence of *Plasmodium vivax* is observed in the lowlands of Papua New Guinea (PNG), the only country in the Western Pacific Region that experienced an increase in malaria cases over the last decade. Contrastingly, prevalence is lower in the Solomon Islands, a country aiming to eliminate malaria, where the number of confirmed cases decreased by approximately 50% from 2000 to 2010. Population structure can inform interventions against malaria. Genetic diversity, gene flow and linkage disequilibrium (LD) between loci are thought to influence the emergence and spread of drug resistance and may affect efficiency of future vaccines. In endemic areas where transmission has been reduced to low levels, genotyping could play an important role in tracking outbreaks and to identify the origin of imported malaria cases.

In areas of a *P. vivax* endemicity lower than in PNG considerable genetic differentiation between populations was found, suggesting limited gene flow. To understand the population genetic structure of *P. vivax* in the South Pacific, we have used 14 molecular markers to genotype 295 *P. vivax* samples from four sites in PNG and from the Solomon Islands. Diversity was very high, with expected heterozygosity values ranging from 0.62 to 0.98 for the different markers. As a result, the effective population size was also found to be high. Among the four PNG sites, a near absence of population structure was observed (*Fst*<0.015). When comparing PNG and the Solomon Islands, population structure was found to be weak (*Fst*=0.03 to 0.044). *P. vivax* populations in the South Pacific are much less structured than populations from areas of lower endemicity in Latin America and Asia. In PNG, the presence of a large *P. vivax* reservoir seem to overcome the geographical barriers to transmission. Intensified, sustained control programs, which are coordinated among endemic countries and also target asymptomatic carriers, seem to be required to eliminate *P. vivax* in the South Pacific.

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**O25**

**Interaction between iron/folic acid and malaria**

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This abstract is submitted as part of the round-table discussion sponsored by Sight and Life.

A recent trial reinforced earlier concerns that iron supplementation can increase malaria rates. The World Health Organization subsequently restricted its recommendations in malaria-endemic areas from universal supplementation to targeted supplementation of iron-deficient children, but continues to advocate universal supplementation in pregnancy. Resurgent interest in iron has led to further studies to assess its safety, particularly in pregnant women; to identify markers for rapid, low-cost screening for deficiency; and to develop safe but efficacious iron interventions.

The hepcidin-axis recently emerged as a newly discovered arm of the innate immune system. Hepcidin is now known to regulate iron absorption and metabolism, but also to mediate impaired recycling and absorption of iron in infections. Current evidence suggests that plasma hepcidin concentration may predict haematological and infectious absorption and metabolism, but also to mediate impaired recycling and absorption of iron in infections. Current evidence suggests that plasma hepcidin concentration may predict haematological and infectious interventions. In endemic areas where transmission has been reduced to low levels, genotyping could play an important role in tracking outbreaks and to identify the origin of imported malaria cases.

**O26**

**Elimination challenges in the Pacific: vivax and submicroscopic parasitemia**

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**Malaria Journal 2012, 11(Suppl 1):O26**

Major progress has been made in reducing the burden of malaria in the Solomon Islands and Vanuatu. This has been achieved by improving health systems, increasing deployment of ITN, improving diagnostics, and by deployment of more effective antimalarials, including ACT. These
advances have highlighted the challenges in achieving elimination of malaria in these countries, and may provide useful lessons for other settings where control activities are less advanced. Specific challenges include the significant prevalence of submicroscopic parasitemia that can only be detected by molecular methods such as PCR. This poses significant challenges to active case detection and to programmatic needs to monitor progress in elimination. As well, the significant prevalence of asymptomatic submicroscopic parasitemia in an epidemiological setting where herd immunity is waning raises questions regarding the nature of anti-disease immunity in such settings. Further, the relative prevalence of P. vivax infection is increasing compared to P. falciparum. A specific challenges this poses is the need to address the issue of latent liver stage infection with hypnozoites in a setting where the prevalence of clinically significant G6PD deficiency exceeds 10%. In this presentation these challenges all be discussed along with research underway to address them.

O27 Integrating diagnostics for elimination
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There are increasing reports of declining malaria prevalence in a number of African countries. This presents a new set of challenges for accurate identification and enumeration of the parasite burden. This is not solely related to generating estimates of malaria with sufficient precision (which may be poor for entomological measures for example) but to the realisation that these measures constitute an important component of surveillance. Pro-and reactive surveillance will be required to maintain the gains that have been made by control programmes by better targeting of resources to areas of sustained transmission which are likely constitute the reservoirs for infection. Surveillance is also an integral component of elimination campaigns. To facilitate surveillance and monitoring, new tools for the detection of infection and exposure to infection will be needed. Molecular amplification assays such as PCR or LAMP will allow identification of sub-microscopic infections that are likely to be the reservoir of infection at low transmission. Serological measures detect antibodies to malaria parasites that reflect exposure to infection and can be performed on large sample numbers. It is likely that these methodologies will need to be used both in conjunction to capture as many likely infections as possible but also separately as the scale and sensitivity of screening change. This presentation will provide further rationale for integrating these approaches, show current examples of their utility in low endemicity settings and discuss options for subsequent development of this approach.

O28 Mass screening tools for glucose-6-phosphate dehydrogenase deficiency: validation of the WST8/1-methoxy-PM5 enzymatic assay in a highly malaria-endemic area in Uganda
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Background Glucose-6-phosphate dehydrogenase (G6PD) deficiency is believed to confer protection against malaria and its distribution and prevalence are geographically correlated with malaria endemicity. This enzymopathy has been identified as the cause of haemolysis following administration of the antimarial drug primaquine. Screening for G6PD deficiency prior to administration of primaquine together with artemisinin combination therapy for treatment or mass-drug administration is being considered for malaria elimination. Current conventional methods for G6PD screening have limitations for field use.

Methods The WST8/1-methoxy-PM5 method, recently adapted to assay G6PD activity in a 96-well format using dried bloodspots, was validated using a current gold standard enzymatic assay (R&D Diagnostics Ltd®). A study was conducted to identify prevalence of G6PD deficiency in Tororo, a highly malaria-endemic region in Uganda. The performance of the test under various temperature, light, and storage conditions was evaluated.

Results The WST8/1-methoxy-PM5 assay was found to have 72% sensitivity and 98% specificity when compared to the commercial enzymatic assay. Its calculated AUC was 0.904 suggesting good agreement. Most of the cases misclassified had borderline values of G6PD activity either between mild and normal activity values, or between moderate and severe deficiency values. Other misclassifications were related to outlier haemoglobin values. Although severe G6PD deficiency was not found in the area, the test enabled identification of low G6PD activity. The assay was found to be highly robust in terms of light sensitivity, performance under temperature variations, and storage conditions for bloodspots, assay mixes and tested samples.

Conclusions The assay was comparable to the currently used standard enzymatic test, yet offered advantages in terms of cost, storage, portability, and use in resource-limited settings. As with other G6PD tests, outlier haemoglobin levels (e.g., as a result of recent haemolytic crises) may confound G6PD level estimation.

O29 Utilising malariometric data in Real Time: a strategy to roll back malaria and sustain local elimination
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Malaria Journal 2012, 11(Suppl 1):O29

Current scale up strategies for malaria control are based on massive input of resources as well as expertise from major donors from a variety of sources but long term sustainability is insecure. Objectives are not always based on public health principles, and outcomes are measured on an immediate scale of demographic or logistic estimates rather than epidemiological indices. If the objective is to reduce transmission as well as to reduce mortality and disease in endemic communities, new strategies sustained through local infrastructure and expertise must be developed. There is a need to focus on empowering local infrastructure to collect data from rural areas, process them in real time, analyze for outbreak conditions and mobilize the local health system to intervene strategically in place and time. In Zambia, the rural health service is doing this on a pilot scale.

In endemic areas where transmission is less than holoendemic (or stable), transmission is seasonal with peaks during the rainy season and troughs when the weather is cold or hot and dry. During the peak malaria season malaria is widespread, but during the low transmission season malaria can be quite restricted and focal with a high proportion of asymptomatic infections. If a major vector control intervention is implemented, then transmission becomes reduced both during the peak but also during the dry season. This has been shown clearly in the Macha district of Zambia where data on malaria case incidence rates has been collected weekly since August 2008. Staff at 14 rural health centres (RHC) report weekly by SMS texting to the research institute at Macha the number of cases diagnosed positive by rapid diagnostic test (RDT) during the previous week. During the low transmission season, it becomes clear just where residual foci occur and what appear to be salient risk factors for low season transmission. It is also clear how and when the case positivity rate spreads after the advent of the rains. Using GIS it is clear where the foci persist and where the parasite reservoir would be vulnerable to attack. These foci were identified by following cases diagnosed in RHC during the low transmission period in 2010 to their homestead, and by examining other residents of the homestead by RDT and PCR for asymptomatic cases. There were significantly more malaria infections detected at these homesteads than in homes selected randomly. We propose to target such foci in real time using
**O30**

### Molecular diagnosis for screening and elimination of malaria: performance of the first commercially-available malaria LAMP test

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### Background

The ability to screen for asymptomatic malaria infection at a field level is increasingly recognized as a key strategy in malaria elimination campaigns. However, molecular methods necessary to detect very low parasite density infections, such as PCR, are restricted to reference-level laboratories and require considerable training to perform. To be effective, such techniques must be close enough to the positive cases to enable rapid treatment. Loop-mediated isothermal DNA amplification (LAMP) is highly sensitive and specific, faster than PCR, requires minimal processing and instrumentation, and allows result detection with the naked eye.

### Materials and methods

FIND has been working with the Hospital for Tropical Diseases in London and Eiken Chemical Company (Japan) in the development of a simplified LAMP assay for the diagnosis of malaria. An optimized test targeting different sequences in the mitochondrial DNA was developed for the detection of parasitaemias below 1 parasite/μl of blood in less than 40 minutes. Prototypes of this test have been compared to PCR with samples from febrile patients in two clinical trials, one in London (travelers) and other in an endemic setting in Uganda.

### Results

Both clinical trials have demonstrated that LAMP is equivalent to nested PCR in sensitivity and specificity with faster time-to-results. In London with 705 samples, sensitivity and specificity of the LAMP *P. falciparum* primers were 98.4% and 98.1% respectively, and for the LAMP Pan primers, 97.0% and 99.2% respectively. In Uganda, 272 samples were tested with the LAMP *P. falciparum* primers and sensitivity and specificity were 93.3% and 85% respectively. This performance of the LAMP assay for malaria was achieved using two simple DNA extraction methods that take only 15 minutes per sample. The study in Uganda also demonstrated that technicians without molecular training could perform the test after a short training period in a simple laboratory space with basic equipment.

### Conclusions

This LAMP test has potential applications both as a reference standard for other diagnostics, for primary diagnosis of returned travelers in non-endemic countries, and as a tool for population screening in malaria elimination campaigns. A high-throughput assay suited to large-scale screening studies is on development.

### Funding

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**O31**

### New drugs for the control and elimination of malaria: a snapshot of the pipeline

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**Malaria Journal** 2012, 11(Suppl 1):O31

Medicines are having a terrific impact on the lives of malaria patients. Great progress has been made with understanding the safety and efficacy of fixed dose artemisinin combination therapies, with five medicines either prequalified by WHO or soon to be prequalified, allowing treatment of children for as little as 25 cents. Artesunate injections are now being established as standard of care for severe malaria, offering significant improvement in healthcare, at a low cost from prequalified suppliers. Medicines can also be used to protect vulnerable populations. Seasonal malaria chemoprophylaxis can protect children for less than 60 cents per year; chemoprophylaxis in pregnancy has significant potential for the lives of the mothers and babies. However, we are facing an infectious enemy, which can and does develop resistance. New medicines are now in phase II clinical trials which have the potential to overcome any emerging resistance, and offer the hope for a single dose cure. We have programs to develop new safer molecules to block transmission of the parasite and to prevent relapses, and these are starting to define potential clinical candidates, after four years of investment. Finally, the need for chemoprotection means that we need new medicines with high potency and long duration. To facilitate these needs have released a ‘malaria box’: a set of 400 physical compounds, and also all the data on over 20'000 hits. Over the next few years, then innovative new partnerships with industry and academia based on this open access to data will help us define a new era in antimalarial drug development.
residual drug concentrations. Unfortunately, it is impossible to observe this emergence because parasites (assuming 10⁵ emerge) are below the microscopic limit of detection (assumed to be 10⁸) and so we are forced to rely on ‘apparent’ WoS i.e. the time at which they have grown to patency. We use the validated mechanistic pharmacokinetic-pharmacodynamic (PK/PD) model described in Winter & Hastings [1] to simulate WoS for increasingly resistant infections and to ascertain how accurately field observations estimate the ‘true’ WoS of selection. Observed estimates of the WoS generally provide a good match to the ‘true’ WoS when resistance levels are moderate to high (i.e. increases in the half-maximal killing rate or IC50 of 10-fold or more). However, field estimates may overestimate it when resistance levels are low or when parasites are able to successfully cause reinfections in the first few days following treatment. The latter is usually accompanied by high levels of resistance and hence treatment failures that should provide warnings that this effect may occur.

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References


O34

A new P. falciparum gametocyte drug screening assay based on pLDH detection

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Plasmodium gametocytes (GCT) have recently been proposed as a crucial target for the development of new antimalarials in order to achieve malaria elimination and eventually eradication. At present, however, a widely accepted and routinely used screening method for potential gametocytocidal drugs does not exist. The aim of our work was to adapt the parasite lactate dehydrogenase (pLDH), already standardised for drug screening on asexual stages, to measure gametocyte drug sensitivity. In clinics the GCT-pLDH, which is present during all the five stages of gametocyte development, can be measured with good sensitivity through OptiMAL, an immunochromatographic diagnostic test. The pLDH assay is fast, simple, not expensive and does not require complex equipment or special waste disposal. It can be applied to field isolates since transgenesis is not needed.

Gametocytogenesis of two different strains of P. falciparum, 3D7 and NF54, was induced in vitro using a standardized protocol, asexual parasites were removed by N-acetylglucosamine treatment, and GCT were seeded in 96well plates. A linear correlation between the percentage of gametocytoma, microscopically counted by Giemsa staining, and the optical density, measured spectroscopically by pLDH assay, was demonstrated. A good signal to noise ratio was obtained with the pLDH assay, and the Z' factor was calculated as indicator of the robustness of the method. Our data also indicate that GCT have a pLDH activity higher than asexual parasites. GCT were treated for 48-72h with primaquine, the gold standard against mature gametocytes in vivo, which was used to validate most of the GCT screening methods in literature; dihydroartemisinin, active on young GCT; and methylene blue, an old antimalarial recently characterised also for its anti-GCT activity. Finally, epoxomicin was tested since its strong gametocytocidal effect has been recently reported. Dose-response curves were obtained with all the four drugs. However, some discrepancies were observed between the Giemsa staining and the pLDH detection at high concentrations of the drugs, suggesting that morphological abnormalities, detected microscopically, precede the decay of pLDH activity in drug-treated GCT. In order to better understand these observations, we prolonged the treatment for further 72h. This extra-incubation period allowed us to calculate, from the pLDH assay, the IC50 (as the 50% inhibition compared to control untreated GCT) of the tested compounds, which were comparable to those obtained by Giemsa staining. These results demonstrate the feasibility of pLDH assay to measure GCT content in culture. Although more specific probes for GCT viability need to be standardized for measuring stage-specific drug activity, pLDH can be used as the first, fast and cheap screening method to find potential gametocytocidal drugs.

O35

Abstract not submitted for online publication
The continued geographic expansion of Malaria Journal
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Artemisinin resistance in Plasmodium falciparum has emerged in South East Asia. Evidence of resistance in Western Cambodia was documented over six years ago, and last year clear evidence of resistance was reported on the Thailand-Myanmar border. As artemisinin derivatives are the cornerstone of antimalarial treatment both for uncomplicated and severe malaria, their loss will increase morbidity and mortality, and could derail current containment and elimination efforts. In the treatment of severe falciparum malaria artesunate reduced mortality by approximately one third compared with quinine. This resulted from greater parasitocidal activity against circulating ring stage parasites, the very property that is lost in artemisinin-resistance. If we have to return to quinine for the treatment of severe malaria, mortality will rise again. In the treatment of uncomplicated malaria artemisinin resistance results in slower parasite clearance and consequently slower therapeutic responses. Times to recovery are slower, treatment failure rates higher, and transmissibility of the resistant strain greater. The reduced antiparasitic effect means that the partner drug now has to eliminate a greater proportion of the infecting parasite biomass, and so there is a greater probability of selecting for partner drug resistance. The future of artemisinin resistance is uncertain; the mechanism underlying resistance has yet to be elucidated. With present levels of resistance artemisinins are still efficacious, albeit much more slowly than before. Whether higher levels of resistance can and will occur is uncertain. Nevertheless, given that previous pandemic spread of antimalarial resistance from South-East Asia killed millions, containing this threat is of the highest global health priority.

The global challenge of antimalarial drug resistance
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The last decade has witnessed dramatic progress in global malaria control and elimination efforts, in large part because of the scale-up of vector control interventions and improved access to effective antimalarials, especially artemisinin-based combination therapies (ACTs). Malaria cases have fallen by >50% in 43 countries since 2001. Since 2000, malaria mortality rates have declined by >25% globally, and >33% in the WHO African Region. These reductions are dependent on the efficacy of ACTs. In 2010, more than 180 million ACT treatment courses were procured for the public sector alone. Resistance to artemisinins was first confirmed on the Cambodia-Thailand border in late 2006, and reported in 2008. The first containment project – the Artemisinin Resistance Containment and Elimination (ARCE) project – was funded by BMGF and coordinated by WHO-GMP; it ran from late 2008-2011. The project did not eliminate malaria from the affected area; however, active case detection, strengthened community case management and better vector control did result in a sustained and dramatic reduction in Plasmodium falciparum burden. To mobilize global and local stakeholders for containment and ultimately elimination of artemisinin resistance, WHO, together with RBM, launched the Global Plan for Artemisinin Resistance Containment (GPARC) in January 2011. The GPARC defines priorities to contain or eliminate artemisinin resistance where it already exists, or to prevent it where it has not yet appeared. While strong country-level activities are the central building blocks in the response, artemisinin resistance does not respect national boundaries. Resistance has generally been identified in areas with high numbers of migrants and close to national borders. Consequently, national responses to the threat of resistance are not sufficient; strong regional coordination is crucial. Despite the emergence of artemisinin resistance in the Greater Mekong subregion, ACTs remain the most effective treatment for uncomplicated falciparum malaria; most patients with delayed response are cured if the partner drug remains effective. However, mefloquine resistance is widespread in Thailand and Cambodia, and piperaquine resistance may have emerged in Western Cambodia, limiting the number of treatment options for uncomplicated malaria. In addition to the existing foc, there is growing evidence of resistance to artemisinin, as defined by delayed parasite clearance times in south-eastern Myanmar and southern and central Viet Nam. It is not known if these new foci represent spread or de novo emergence of artemisinin resistance.

In response to new data, containment efforts have been started in western Thailand, south-eastern Myanmar and Viet Nam. These new programmes will draw on the lessons learned during the containment project in Cambodia and Thailand. In addition, routine monitoring must be strengthened for the early detection of artemisinin resistance, ensure that recommended first-line ACTs are effective, and that timely changes in treatment policies can be made. Many aspects of artemisinin resistance, including the identification of molecular markers and better understanding of resistance mechanisms and antimalarial treatment failure are still not well understood. Consequently, there is an urgent need for further research to refine our knowledge of artemisinin resistance, including the identification of molecular markers and better understanding of resistance mechanisms and antimalarial treatment failure. These efforts require full funding to ensure that artemisinin resistance does not spread and compromise global success in malaria control and elimination.

The power of pooled analysis: WWARN community reaches a critical mass of data
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Early warning of emerging resistance is needed to identify new foci, predict their spread and inform prompt containment action to counter and slow the resistance to antimalarials. The Worldwide Antimalarial Resistance Network (WWARN) works to provide tools to this end. WWARN has developed systems that transform heterogeneous clinical, pharmacology, molecular and in vitro data from all parts of the world into standardized information that can be analysed and directly compared. Bigger data sets give greater statistical power. The repository currently has 75,000 individual patients’ data (3 million records) - 50% of the published ACT clinical data - and represents the largest compilation of malaria data now available. ACTs are still highly efficacious in most parts of the world, with the important exception of some specific locations in South East Asia. Therefore, the number of reported clinical failures reported is still small. It is therefore critical to identify and deploy now, efficient methods to track the earliest signs of reduced efficacy. This will require interrogation of very large sets of data to test risk factors associated with failures and work with the community to validate informative early markers. The current WWARN repository also allows questions of public health relevance to be answered. For example, one can test the consequences of variance in dosing strategies of key ACTs and their effects on clinical efficacy, and early parasitological responses. Outputs from the data analysis also highlight gaps in this retrospective data collection and allow planning for filling those gaps. Prospectively, WWARN is developing research tools and services to support diverse malaria research communities to improve the comparability and quality of data being collected, and to increase the power of pooled analysis. Presented on behalf of the WWARN community.

Safety and efficacy of artemether-lumefantrine against uncomplicated Plasmodium falciparum malaria during pregnancy: a systematic review
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Background Malaria during pregnancy, especially Plasmodium falciparum malaria, is linked to increased morbidity and mortality,
which must be reduced by preventive measures and effective case management [1-3]. The World Health Organization (WHO) recommends artemisinin-based combination therapy (ACT) to treat uncomplicated *P. falciparum* malaria during the second and third trimesters of pregnancy, and quinine plus clindamycin during the first trimester [4]. However, the national policies of many African countries currently recommend quinine throughout pregnancy. Therefore, the objective is to provide a summary of available data on the safety and efficacy of artesunate-lumefantrine (AL) in pregnancy.

**Materials and methods** A systematic English-language research identified 16 publications from 1989 to October 2011 with reports of artesunate or AL exposure in pregnancy, including randomized clinical trials, observational studies, and systematic reviews.

**Results** Overall, there were 1,103 reports of AL use in pregnant women: 890 second/third trimester exposures; 212 first trimester exposures; and 1 case where the trimester of exposure was not reported. In the second and third trimesters, AL was not associated with increased adverse pregnancy outcomes compared with quinine or sulfadoxine-pyrimethamine, showed improved tolerability relative to quinine, and its efficacy was non-inferior to quinine. Although, few reports suggest that the pharmacokinetics of anti-malarial drugs may change in pregnancy, the majority of studies reported high cure rates and adequate tolerability. As there are fewer reports of AL safety in the first trimester, additional data are required to assess the potential to use AL in the first trimester.

**Conclusions** These findings reinforce the WHO recommendation to treat uncomplicated *P. falciparum* malaria with quinine plus clindamycin in early pregnancy and ACT in later pregnancy.

**References**


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**O40**

Abstract not submitted for online publication

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**O41**

Thinking outside the ‘health’ box: interventions for malaria control

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In the last decade malaria has declined sharply in many countries in the tropics, due largely to the massive expansion of control programmes. However, the future may not be as bright as drug and insecticide resistance rises and aid budgets constrict. Since it is well recognised that malaria declined in many parts of the world due to socio-economic improvements, here I consider whether development today could be an effective weapon against malaria.
**O42**  
Characterization of the mating systems of *Anopheles gambiae* in malaria control perspective  
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The current work is focussed on ways in which the manipulation of mosquito mating behaviour has the potential to contribute to integrated programme for the control of malaria vectors. One line of investigation explores means of reducing vector populations by significantly reducing mating with lure-and-kill and mating disruption measures. The second line is looking at variation in mating success between and within swarms of *Anopheles gambiae* and its underlying factors with an ultimate goal of designing mosquito rearing scheme that produce competitive mating males.

A complete map of swarm distribution in Vallée du Kou was constructed and swarms were physically described. Overall swarms were tightly linked to specific man-made markers within the village and a significant difference in swarm numbers and size was observed between households. The pattern distribution of swarms across space was provide semi-field conditions and provide a more direct way of exposing the makers to sunlight, the contrast pattern and the openness of the marker to air circulation. Exploration of the energetic budget in relation to swarming and mating showed that sugars and glycogen are the main energetic sources that fuel males mating activities. The distribution of wing size of mated males was focused around a central value suggesting that intermediate size of males is advantageous in *An. gambiae* mating system.

A better knowledge of key parameters that account for male mating success will be of significance to control strategies based on the release of genetically modified or sterilised males. Similarly, understanding the ecological parameters that are correlated with the presence or absence of swarms would be valuable for the implementation of mating disruption strategies.

**O43**  
Does mosquito mortality in WHO insecticide susceptibility tests relate to mosquito mortality in LLIN experimental hut studies?  
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Malaria Journal 2012, 11(Suppl 1):O43

Background With the rise of insecticide resistance, there are concerns that insecticide treated nets (LLINs) will become less effective against malaria transmission. The WHO insecticide susceptibility (tube/cylinder) test is a standard for estimating mosquito susceptibility to insecticide. The test measures the proportion of mosquitoes that die when exposed to an insecticide with a certain concentration. Whereas, with fully susceptible mosquito populations, mortality is 100% with 0.05% deltamethrin, but with resistant populations, mortality can be below 15%. However, little is known how susceptibility results in these tests relate to the effectiveness of LLINs in the field. Experimental huts put in relation to results provide a more direct way of estimating the effect of susceptibility of mosquitoes to interventions than WHO susceptibility tests. This work assesses the degree to which mortality in WHO susceptibility tests correlates to mortality in experimental hut studies with LLINs.

Materials and methods For seven wild mosquito populations, published experimental hut results (1-5) were available for the *An. gambiae* and *Culex quinquefasciatus* cage. Twenty times washed, and unwashed nets, for which the insecticide content was also measured. Nets were either artificially holed (hut assays for six populations), or intact (hut assays for one population). For six of these populations, mortality in WHO susceptibility tests were also published. These data were used to estimate deterrence (reduction in hut entry), the proportion of mosquitoes attacking of those entered, and pre- and post prandial mortality, as a function of LLIN insecticide concentration and as a function of holed area. These variables were also used to define personal protection (reduction in bites received), and corrected mortality (mosquito mortality), as a function of insecticide concentration and holed area. Using these parameterizations, the corrected mortality in experimental huts for all populations was calculated for a standard net with a fixed deltamethrin concentration of 17.44 mg m^-2 and 96 cm^-2 holed area.

Results The Pearson correlation coefficient for the correlation between corrected mortality values and mortality in WHO susceptibility tests was strong and positive at r=0.92. However, for personal protection, the correlation was much weaker (r=0.54).

Conclusions Our analysis concludes that the WHO susceptibility tests give a good indication of the killing efficacy of LLINs in semi-field conditions. However, the efficacy of LLINs in the field is not only determined by direct mortality, but also by personal protection and deterrence, for which correlations are much weaker.


**O44**  
Engineering mosquito population for vector control  
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Malaria Journal 2012, 11(Suppl 1):O44

Background and results The development of genetically engineered malaria-resistant mosquitoes has shown, as a proof-of-principle, the possibility of targeting the mosquito’s ability to serve as a disease vector (1,2). The translation of these achievements into control measures relies on the availability of an effective gene drive technology to spread a genetic modification from laboratory mosquitoes to field populations. We have suggested that homing endonuclease genes (HEGs), a class of simple selfish genetic elements, could be exploited to develop vector control strategies aimed at spreading in a target population either novel genes that impair the mosquitoes ability to function as vector for malaria or genetic modifications that disrupt their reproductive capability (3, 4, 5). To assess the ability of HEG based constructs to spread a genetic modification into target mosquito populations we have generated transgenic mosquitoes carrying a synthetic genetic element containing the l-SceI homing endonuclease selectively activated in male during spermatogenesis. We show that the l-SceI element is able to rapidly invade receptive *A. gambiae* cage populations, validating mathematical models for the transmission dynamics of HEGs. Molecular analysis confirms that the expression of l-SceI in the male germline induces high rates of both cleavage of receptive chromosomes and gene conversion, which results in the gain of the l-SceI gene, and underlies the observed genetic drive. Furthermore we also show that different HEGs can be engineered to reprogram their sequence specificity to selectively target *A. gambiae* sequence.
These findings provide a new perspective for the implementation of genetic control measures by demonstrating a mechanism by which linked genes could be spread through vector populations. Genes that interfere with A. gambiae ability to transmit Plasmodium falciparum malaria without unbalancing key mosquito physiological processes are yet to be found, however candidates genes that impair mosquito reproductive capability are potentially available. In previous reports we showed that the homing endonuclease I-PpoI recognizing a unique site within the Anopheles gambiae 28S ribosomal genes could be used to selectively target X chromosome carrying spermatozoa. Our data demonstrated that in heterozygous males, the expression of I-PpoI in the testes induced a strong bias toward Y chromosome–carrying spermatozoa. However these male mosquitoes also induced complete early dominant embryo lethality in crosses with wild-type females. Irrespective of the inheritance of the I-PpoI the spermatozoa carried a substantial amount of I-PpoI protein that attacked the maternally inherited chromosome X of the embryo. Here we show that transgenic male mosquitoes expressing a destabilized form of I-PpoI during the process of spermatogenesis generated vital male only progeny thereby decoupling the sex distortion and the embryo lethality phenotype resulting from targeting the X chromosome. Our results show how, using sequence-specific genetic drive elements like HEGs, the step from the genetic engineering of individuals to the genetic engineering of populations can be taken.

Acknowledgement

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References


O45 The SolarMal Project: innovative mosquito trapping technology for malaria control

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The use of insecticides against mosquitoes, and drugs to treat infection, continue to form the mainstays of malaria control programmes, but the long term success and sustainability of these approaches is threatened by the development of insecticide and drug resistance. New complementary approaches to control must be explored. The development by Okumu and others [1] of a blend of synthetic chemical attractants which was capable of attracting more Anopheles gambiae s.s. than a human, provided the key breakthrough towards creation of a mass trapping system which could be used for malaria control. By luring Anopheles mosquitoes to traps in numbers that are high enough to suppress population size and reduce biting intensity, a decline in malaria transmission could be realized. Here we describe our plans for the development and testing of odour-baited traps for malaria control in Western Kenya.

The SolarMal project aims to demonstrate proof of principle for the elimination of malaria from Rusinga Island, Western Kenya, using a nationwide adopted strategy of LLINs and case management, augmented by mass trapping of mosquito vectors. The use of novel technology and scientific development underpins all areas of the project; from the optimisation of chemical baits to attract mosquitoes, to the design of a new mosquito trap and the installation of solar panel systems to provide power to run the traps. Electronic tablets are used to record health and demographic surveillance data.

The mosquito traps operate according to a counterflow mechanism previously shown to be highly effective in collecting anopheles mosquitoes [2] and are designed to collect mosquitoes outdoors prior to house entry. Odour baits placed within the traps mimic human odourants [3].

In a unique variation on the stepped wedge intervention strategy, which we refer to as the hierarchical design, intervention implementation begins at one randomly selected household and expands radially until a cluster of houses with the intervention is created. The intervention implementation then commences in a second geographically distinct location, then a third, fourth, fifth etc, continuing until the whole island is covered.

Outcome measures of malaria parasite prevalence and incidence, as well as estimates of malaria transmission intensity, will be used to assess the impact of the intervention. We expect the results to demonstrate that the use of odour baited traps is an effective, novel means of integrated malaria control.

References


O46 Dramatic changes in malaria after the free distribution of mosquito nets in Papua New Guinea

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Background Papua New Guinea (PNG) is a South Pacific island nation with a complex malaria epidemiology. Four malaria species are transmitted by a variety of anopheline vectors filling the diverse ecological niches. Attempts to eliminate malaria in PNG in the 1950s-70s failed largely due to operational difficulties related to the unique implementation environment. Since 2004, the national malaria control program has been supported by two consecutive grants from the Global Fund resulting in the first country-wide free distribution of insecticide treated mosquito nets.

Methods Two cross-sectional household surveys carried out in 2008/09 and 2010/11 in randomly selected villages across PNG investigated changes in malaria control intervention coverage and population
prevalence of malaria infection. Malaria surveillance in sentinel sites documented trends in the incidence of clinical cases and the prevalence of malaria infection among fever cases in health facilities. Prevalence of Plasmodium spp. was assessed by rapid diagnostic test (RDT) and light microscopy.

Results Country-wide household ownership of long-lasting insecticide treated nets (LLIN) reached 65% (n=1958) in 2009 and over 80% (n=1986) in 2011; usage in the target group of children under five years amounted to 40% (n=1599) and over 55% (n=1768) in the respective years. Data from sentinel surveillance sites suggest that prior to the first LLIN distribution (2005-2009) both ownership and usage of LLIN were below 10%. No other malaria control interventions were introduced on a large scale during the mentioned period. Simultaneously, Plasmodium spp. prevalence in the general population decreased from 14% (n=6442) in 2009 to below 7% in 2011 (n=7978). While the decrease was significant for P. falciparum, P. vivax parasite rates remained virtually unchanged resulting in a shift from P. falciparum to P. vivax dominance in all regions. A significant decrease was also noted in malaria cases in sentinel health facilities where the proportion of fever cases with a positive RDT dropped from 56% pre-distribution (n=1330) to 18% post-distribution (n=681); however, the P. falciparum to P. vivax shift was less dramatic in clinical cases. Simultaneous entomological studies found a decrease in entomological inoculation rates but also short-term changes in biting behaviour.

Conclusions The dramatic effect of the Global Fund supported LLIN distribution on malaria in PNG poses new challenges to the national malaria control program. Implications for surveillance, prevention and treatment choices are discussed in consideration of experiences from comparable settings.

O47 Malaria vaccines for eradication
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In achieving the goal of malaria eradication, vaccines could play a crucial role. Current attempts to develop malaria vaccines are primarily focused on Plasmodium falciparum and are directed towards reducing morbidity and mortality. This is the case of the RTS,S vaccine candidate, the most developed at the moment and currently undergoing the final analysis of a multicentric, international Phase III clinical trial. Continued support for these efforts is essential, but if malaria vaccines are to be used as part of a repertoire of tools for malaria eradication, they will need to have an impact on malaria transmission, contributing to lower the reproductive rate to less than one.

The consultative group on vaccines of the Malaria Eradication Research Agenda (malERA) initiative identified key features of vaccines especially suited for malaria eradication. The group introduced the concept of “Vaccines that Interrupt Malaria Transmission” (VIMTs), broadening the classical definition of Transmission Blocking Vaccines (TBVs) that target sexual and mosquito stages of the parasite, in order to also include any other parasite life stages that may interrupt transmission of malaria. This would include pre-erythrocytic and asexual stage vaccines to reduce prevalence and densities of sexual forms of the parasite, as well as vaccines against mosquito gut antigens impeding parasite development in the vector.

Moreover, malaria eradication would necessarily need to target not only P. falciparum but also P. vivax, for which therapeutic vaccines against hypnozoites or preventive vaccines with effect against multiple parasite stages could have enormous impact.

Development of VIMTs require the advancement of basic understanding of interactions of the malaria parasite with humans and vectors, as well as the development of tools to measuring infectivity at the individual level and assessing transmission within a given population. VIMTs may have primarily an effect at the population level and could be crucial not only for eradication, but also in areas where malaria control is still the main issue to be addressed. In order to accelerate the actual implementation of such vaccines, it is also important to address regulatory issues and to develop appropriate delivery platforms.

O48 Adaptive clinical trials of three PFSPZ products for development of a whole sporozoite vaccine that prevents Plasmodium falciparum infection, disease and transmission
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Malaria Journal 2012, 11(Suppl 1):O48

An ideal, single stage vaccine useful for elimination of Plasmodium falciparum infection at the pre-erythrocytic stage of the parasite life cycle, thereby preventing all P.f.-caused disease and transmission from humans to mosquitoes. The only approach to immunization shown to consistently induce greater than 90% protection against infection and protection sustained for at least 10-28 months has been immunization by mosquito bite with whole Pf sporozoites (SPZ) of two types. The first type, radiation-attenuated PFSPZ, invade hepatocytes and expresses new proteins, but cannot replicate. The second type fully develop in hepatocytes, producing tens of thousands of merozoites that invade erythrocytes, but are unable to fully develop within erythrocytes because they are killed by an anti-malarial drug. This approach called chemoprophylaxis with sporozoites (CP5) harnesses the infectious agent’s inherent replicative properties to amplify production of Pf-specific immunogens spanning multiple developmental stages, and then eliminates the infectious agent with an anti-infective drug before the onset of disease. Sanaria was founded to develop PFSPZ vaccines. The first vaccine developed and tested was the PFSPZ Vaccine. The PFSPZ Vaccine is comprised of aseptic, purified, radiation attenuated, cryopreserved PFSPZ. It was shown to be safe and well-tolerated when administered ID or SC to 80 volunteers in the U.S., but sub-optimally immunogenic. It is now being tested in the U.S. and soon in Tanzania when administered by IV injection, since it induced high levels of PFSPZ-specific CD8+ T cells in the livers of immunized non-human primates when administered IV. A second product, PFSPZ Challenge, is comprised of non-irradiated, fully infectious PFSPZ. PFSPZ Challenge has been shown to infect 100% of volunteers after ID or IM administration by needle and syringe. It has been or will be tested for optimization of administration by the ID, IM, and IV routes in 2012 or early 2013 in the Netherlands, UK, Tanzania, U.S., Germany, Spain, and Kenya. A third product, PFSPZ-CVac, is comprised of PFSPZ Challenge administered to volunteers receiving chloroquine chemoprophylaxis. It will be assessed in 2012-2013 in the Netherlands, Mali, Germany and Tanzania. Assessment of these three products in synergistic, interactive and adaptive clinical trials will facilitate progress toward optimizing administration and dosage regimen of all three whole PFSPZ products, as well as those developed in the future from genetically altered parasites, thereby facilitating licensure of one or more PFSPZ-based vaccines. Progress and plans for development will be discussed.

O49 A novel Plasmodium vivax vaccine based on recombinant chimpanzee adenovirus ChAd63 and MVA expressing TRAP
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Malaria Journal 2012, 11(Suppl 1):O49

Background P. vivax is the most geographically widespread human malaria and is considered to be the most prevalent form in some regions of Latin America, Central and South-East Asia, accounting for up to 390 million clinical infections every year and an estimated 2.6 billion people being at risk of infection with P. vivax [1, 2]. An effective vaccine against this protozoan would have a major global impact on the disease burden [3]. Modified Vaccinia Ankara (MVA) and the chimpanzee adenovirus ChAd63 are two clinically relevant viral vectors that have been shown to induce strong and protective antibody and T-cell responses against P. falciparum TRAP, both in pre-clinical studies and clinical trials [4-7].

Materials and methods We developed recombinant ChAd63 and MVA vectors expressing P. vivax TRAP (PvTRAP), which were used to assess T-cell and antibody responses upon sequential immunisation (prime-boost) of mice. Vaccine efficacy was assessed through challenge with a newly developed transgenic P. berghei expressing PvTRAP.
Results High antibody titres and frequencies of PvTRAP-specific T cells were induced in all tested inbred and outbred mouse strains. The newly developed parasite showed similar fitness to wild type P. berghei and was successfully used to infect and assess protection in vaccinated mice. The Ad-MVA prime-boost regimen induced good protective levels regardless of the mouse strain.

Conclusions The strong immunogenicity and protective efficacy elicited by the recombinant ChAd63 and MVA viruses expressing PvTRAP indicate that this vaccine approach has a good potential to be tested in clinical trials in the near future.

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References

O51
Plant-produced transmission blocking Plasmodium falciparum Pfsp25 subunit and VLP based vaccine candidates
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Malaria is a serious mosquito-borne disease caused by a protozoan parasite. Vaccines can target different stages of the pathogen’s life cycle. Transmission blocking vaccines target mosquito stages of the parasite life cycle, and will support eradication programs to ease the disease burden at the population level. Pfsp25 is a sexual stage protein of Plasmodium falciparum using highly polymorphic molecular markers in conjunction with high resolution typing. Pfsp25 is easily detected despite a background of ongoing infections. Simultaneously, blood samples from trial participants become available for RNA based detection and quantification of gametocytes by qRT-PCR. Vaccine effects on gametocyte prevalence can be detected by targeting gametocyte-specific transcripts.

Materials and methods We have evaluated RNA sampling techniques for malaria field surveys. Collecting samples directly into RNAprotect solution gave best results. Gametocytes were detected by qRT-PCR using marker Pfsp25.

In our cohort studies asexual parasites were genotyped using marker msp2 and fragment sizing by capillary electrophoresis [1,2]. In the vaccine trial (Phase 2b vaccine trial of Combination B conducted in Papua New Guinea [3] a PCR-RFLP methodology was used. We have developed the statistical approaches to determining the actual number of P. falciparum clones acquired per time per individual host, corrected for imperfect detectability [4].

Results Molecular parameters describing the P. falciparum infection dynamics were estimated based on high precision genotyping data from cohort studies or from a clinical trial with repeated follow up bleeds at intervals between 2 weeks and 2 months. The full time-series of presence and absence of clones in consecutive samples from one individual forms the basis from which FOI, duration of infection, and clone detectability were estimated.

FOI in vaccinated children from the Combination B vaccine trial was significantly reduced in vaccine recipients only for parasites carrying a 3D7-type msp2 allele corresponding to 3D7 MSP2 component of infecting combination B.

Conclusions We demonstrated proof of concept of this approach in a vaccine trial of the Combination B malaria vaccine. We propose to consider the FOI as an outcome measurement in vaccine trials and to collect and preserve in the field setting in parallel blood samples useful for RNA extractions for monitoring vaccine effects on transmission stages.

References

O52
Eliminating malaria in a sub-Saharan Africa: debate it or just do it?
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Malaria Journal 2012, 11(Suppl 1):O52

At the outset of the RBM movement in late 1998 with the declared goal of halving the global malaria burden, there was much talk that was eventually (by ~2005) followed by resources and country-led action in scale up of malaria control, notably in sub-Saharan African countries, that has achieved the anticipated burden reduction in
Malaria elimination and eradication is back on the global health agenda. The Roll Back Malaria / World Health Organization Global Malaria Action Plan sets out an ambitious plan for improved control, leading to regional elimination and includes as the ultimate goal the eradication of malaria. The ambitious goal of malaria eradication can however not be achieved with the tools and approaches currently available. Research and development of new tools must accompany and complement the Global Action Plan. We critically review and discuss the progress so far made to pursue the R&D agenda and about the key R&D issues where renewed attention and investments are required [1]. The following areas will be discussed on the basis of innovations made and practical applications already launched and validated.

- The strengthened focus on *P. vivax*: *In vitro* culture and study of the biology of hypnozoites
- Drugs to be used for mass drug administration to clear infections and provide prophylaxis to prevent new infections
- Vaccines that aim at interrupting transmission
- New vector control approaches for (i) outdoor biting / resting mosquitoes and (ii) achieving permanent reductions of vectorial capacity in areas where transmission is predominantly due to *A. gambiae*
- New approaches for fast and accurate assessment of transmission at community level and strengthened diagnostic, monitoring, and surveillance tools/approaches that are linked and embedded in the health and social health systems
- New approaches in mathematical modelling to inform Target Product Profiles of tools, and predict expected outcomes of intervention strategies for elimination
- Tools to scientifically assess and determine health system readiness for moving from control to elimination.

The review will conclude by pinpointing the challenges that are still ahead of us in the development of the science for elimination and application of its results to effectively complement the Global Action Plan.

References

Update from the Asia Pacific Malaria Elimination Network (APMEN)
Maxine Whittaker

Background Community engagement and participation has played a critical role in successful disease control and elimination campaigns in many countries. Despite this, its benefits for malaria control and elimination are yet to be fully realised, and research in this area has been identified by MalERA as a priority. The Pacific Malaria Initiative – a partnership between Vanuatu, Solomon Islands, AusAID, WHO, SPC and its in-country partners has been supporting operational and applied research activities to understand effective ways to engage the communities in their programmes. The Asia Pacific Malaria Elimination Network has had a focus of work in community engagement, and draws upon lessons from the country programmes within the region. This paper reports upon some of the challenges faced in elimination, and the tools, approaches and insights gained in the Asia Pacific Region at the implementation level of elimination of malaria.

Results The paper will present strategies developed and/or trialled in countries in the Asia Pacific Region to develop sustainable engagement by communities in the targeted locations to maintain and support malaria control activities and be engaged in the identification of malaria cases, and protection of borders. As defined in the national malaria elimination strategy, these include: Community participation to reduce transmission and reservoir of infection (including IRS, source reduction, LLINs); Community based treatment support for people who are using malaria treatment (*vivax* or *falciparum*) (early recognition of fever, active case detection, directly observed treatment and adherence, community based distribution support, test before treatment behaviour); Develop and strengthen community self monitoring of community level surveillance.

Conclusions The operational realities of moving towards malaria elimination demonstrate the need to address community participation and engagement. Approaches that have been successful in other elimination and eradication activities may form one set of strategies to trial. Additionally new tools such as GIS can support the sustaining of engagement in these efforts.

How protected are populations if transmission relapses? Insights from mathematical modeling and simulation

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Background Malaria control measures have been successful in reducing malaria mortality and morbidity in sub-Saharan Africa in the last decade. In particular, in Príncipe Island, São Tomé e Príncipe, after 5 years of control measures, *Plasmodium falciparum* incidence has decreased 99% and prevalence measured by slide-positivity rate was below 1% in 2009 [1]. However, this method lacks sensitivity for detection of asymptomatic and sub-patent parasite carriage that has implications on transmission [2]. Furthermore, control measures have the adverse effect of promoting decrease of immunity against the parasite, and a relapse in transmission might therefore have more severe consequences on infected individuals [3].

Materials and methods Mathematical models are developed to assess age and time trends on malaria infection and immunity against conserved and variant surface antigens of *P. falciparum*. The effect of interventions and transmission relapse on the dynamics of infection and immunity to markers of transmission intensity, such as the merozoite surface protein-3 (MSP-3) and of protection against disease, like *P. falciparum* erythrocyte membrane protein 1 (PFEMP1) at the population level are studied. To calibrate the model, sero-epidemiological data collected in 2005 and 2008 in Príncipe island with parasitemia determined by polymerase chain reaction (PCR) are used. Results In 2005, with two years of control measures in Principe Island, *P. falciparum* PCR parasitemia was above 25% and, assuming endemic equilibrium, the basic reproduction number was still above 1. Patterns of seropositivity to conserved and anti-PFEMP1 antibodies reflect cumulative exposure with age. Simulations of interventions
that reduce the transmission coefficient mirror well the progression of infection and anti-MSP-3 immunity in the population in 2008 and suggest that prevalence of infection decays faster than immunity. However, when transmission relapses occur, the onset of immunity is slower than the increase of infected individuals, posing problems for severity of new infections. The simulated number of variants against PFEmp1 decayed steeply with interventions and the effect on particular variants associated with severe disease could give some clues on future malaria morbidity.

Conclusions Based on data from a settlement that has been subject to control measures we explore conditions under which the decay on the diversity of the immune repertoire can increase morbidity if transmission relapses and we aim to propose and inspire strategies to undermine the impact of this issue.

References


O56
Tools for malaria elimination in the Kingdom of Saudi Arabia
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Malaria Journal 2012, 11(Suppl 1):O56

In 1998, the Kingdom of Saudi Arabia (KSA) suffered its worst malaria epidemic. A total of 36,139 locally transmitted cases were recorded and incidence reached as high as 44/1000 in malarious regions, in Southern KSA. Since then, malaria control has been scaled up significantly, with ITNs, larviciding and improved case management. These activities had a significant impact, in 2011 only 29 locally transmitted cases were recorded in the country, reducing incidence to <0.01/1000, well below the WHO recommended rate of 5/1000 that a country should consider before elimination. KSA is now one of the 32 countries that are facing the challenge of malaria elimination.

In order eliminate malaria KSA needs to enhance its surveillance programme, ensuring timely and accurate collection of all malaria operational data. This will be achieved by the integration of passive case detection with reactive and active case detection to rid the country of these last few cases and to prevent malaria from re-establishing itself from imported cases. This data can be used to map, and target, areas of transmission and track any outbreaks. A successful elimination campaign will require the incorporation of other relevant data, for better targeting and faster response to disease detection.

KSA will achieve this by Arabisation of the Malaria Decision Support System, originally developed in collaboration with malaria programmes in southern Africa [1]. This system integrates a number of tools covering case surveillance, intervention planning and monitoring, entomological monitoring and survey development. It also incorporates the ability to generate maps and standard reports at the ‘click of a button’. One of the key challenges to elimination is detected every case and to respond to that case. The MDSS builds on experiences from Africa [2,3] where simple tools allow for the detection of reactive cases, reactive follow up and any action carried out within 48 hours. These tools also allow for the mapping of malaria clusters for better targeting of resources and detection of disease outbreaks, something that will be critical to KSA in avoiding resurgence from imported cases.

Here we demonstrate how the MDSS can be used to track malaria from moderate endemicity to an elimination phase and demonstrate how the system could then be used to assist in elimination of malaria and its maintenance.

References


Abstract withdrawn

Abstract withdrawn

Wild sage (Salvia Officinalis) as a potential anti-malarial drug

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Background During the intra-erythrocytic stage, Plasmodium parasites degrade hemoglobin resulting in Ferriprotoporphyrin IX (FX) accumulation; toxic to the parasite [1]. β-Hematin, a synthetic polymer made from Ferriprotoporphyrin-IX is structurally, chemically and spectroscopically identical to purified Hemozoin is used in in-vitro studies [1]. Resistance to Chloroquine, highlights the need for new drugs. Earlier attempts showed the effect of Pyrimidine derivatives in in-vitro inhibition of β-Hematin [2], and Cisplatin complexes [3]. We concentrate on finding new molecules from natural products; (Salvia officinalis).

Materials and methods Plant materials, collected from areas around Jerusalem, were dried at room temperature; leaves and stems separately grounded. Extraction was by soaking 5g of dried plant parts in 40 ml of 35% ethanol or ultrapure-water; left standing for about 24-hours. Extracts were then filtered using MN615-Φ 90 nm filter paper, rotary evaporated at 50°C then lyophilized to constant weight. Stock solutions were prepared in water.

Semi-quantitative method The procedure was according to [4], ultrapure-water for negative control, chloroquine or Amodiaquine for positive control. The final precipitate of β-hematin dissolved in 200μl of 0.1M NaOH to give alkaline hematin for spectroscopic quantification at 405-nm.

Results The efficiency of sage leaf extracts compared to controls is shown below. Each absorption value is the average of five experiments. The mechanism of inhibition is probably through formation of a complex between active compounds in these extracts and ferriheme; this complex prevents the formation of β-Hematin.

<table>
<thead>
<tr>
<th>Test</th>
<th>Absorbance</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Control (H2O)</td>
<td>2.26</td>
<td>2.14</td>
</tr>
<tr>
<td>Negative control 35% Ethanol</td>
<td>2.22</td>
<td>2.01</td>
</tr>
<tr>
<td>Chloroquine 0.1mg/ml</td>
<td>0.045</td>
<td>0.047</td>
</tr>
<tr>
<td>Amodiaquine 0.1mg/ml</td>
<td>0.058</td>
<td>0.055</td>
</tr>
<tr>
<td>Chloroquine in 35% ethanol 0.1mg/ml</td>
<td>0.055</td>
<td>0.062</td>
</tr>
<tr>
<td>Stock-leaf extract in 35% ethanol 1 mg/ml</td>
<td>0.060</td>
<td>0.057</td>
</tr>
<tr>
<td>Stock-leaf extract in 35% ethanol 0.5 mg/ml</td>
<td>0.044</td>
<td>0.074</td>
</tr>
<tr>
<td>Stock-leaf extract in water 1 mg/ml</td>
<td>0.099</td>
<td>0.080</td>
</tr>
<tr>
<td>Stock-leaf extract in water 0.5 mg/ml</td>
<td>0.378</td>
<td>0.162</td>
</tr>
</tbody>
</table>

Conclusion The extract is a natural product and has been used in folk medicine without reported toxicity. Although the results for the extracts are lower than for the positive controls we must take into account the fact that the extracts are crude, we are already working on isolating the ingredients; results will be published in the near future.

References:
Results
Results indicated that in laboratory, 100% L1 larvae died within 24 hours post-infection and 100% of both L2 and L3 larvae died within 7 days post-infection, regardless of nematode concentration. In field, Anopheles larval density 5 days post-application decreased from 35 larvae per liter to 4 larvae, and from 17 larvae to 1, respectively in site 1 and 2. During a whole rainy season in 2011, monthly nematodes spraying resulted in suppression of larval An. gambiae in treated sites.

Conclusions
The present study indicated that the Mermithid nematode R. iyengari is effective for malaria vector control in Benin, West Africa. R. iyengari mass production using local materials is easy. Integrating this nematode into An. gambiae management system is therefore possible.

Acknowledgements
This work has been supported by both Universities of Abomey-Calavi and California, Riverside. The participation of unpaid volunteers for nematodes spraying is highly appreciated.

References

P6
Socio-economic inequity in accessing malaria control interventions in Nigeria: analysis of changes between 2003 and 2008
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Background
Malaria is the major health problem in Nigeria, accounting for 60% of outpatient consultations and 30% of hospital admission [1]. The Federal Ministry of Health (FMoH) of Nigeria has adopted cost-effective malaria control interventions as tools for achieving ambitious objective of halving malaria burden by 2015 as contained in the National Malaria Strategic Plan (NMSP). The interventions include prompt and effective case management, Insecticide Treated Net (ITN) and Intermittent Preventive Treatment (IPT) while parasitological diagnosis is an adjunct to effective case management. The coverage for these interventions is very low as only 8% of households own ITN, 1.1% of under five children with malaria have access to Artemisinin-based Combination Therapy (ACT) within 24 hours of onset of fever and only 6.5% of pregnant women have access to 2 doses of IPT [2].

Methods
We used concentration index (C) to measure changes in socioeconomic inequity in access to malaria control measures using Nigeria Demographic and Health Surveys 2003 and 2008.

Results
There was increase in access to all the malaria control measures studied between 2003 and 2008: ownership of any bed nets (11.8% to 16.9%), under-5 children who slept under treated bed net (1.4% to 5.3%), under-5 children with fever who received non-Artemisinin-Combination Therapies (ACT) (9.6% to 27.5%) and pregnant women who received intermittent preventive treatment (1.1% to 7.8%). In 2008 there is concentration of treated net use among the rich (pro-rich inequality) which was more pronounced in the North West and South east regions and least pronounced in the South South. The pattern of inequalities of use treated bed nets were similar those observed in ownership of treated net. There is pro-rich inequality in the prompt and effective treatment of malaria using non-ACT, ACT combination and use of intermittent preventive treatment by pregnant women. In most cases the inequalities were more pronounced in the northern regions.

Conclusion
Though access to most malaria control interventions increased across all wealth quintile between 2003 and 2008, there are significant differences in access to some of these interventions that favour the better-off of society as a whole and some geopolitical regions.

Acknowledgements
The data used in this study were made available through MEASURE DHS Archive. The data were originally collected by the ICF Macro, Calverton USA.

References
by Ifediba and Vanderberg to obtain viable, mature gametocytes en masse. We designed an assay to determine the activity of antimalarial drugs based on the intracellular ATP content of purified stage IV-V gametocytes after 48h of drug exposure in 96/384-well microplates. Measurement of drug activity on asexual stages and cytotoxicity on HepG2 cells were also obtained to estimate the specificity of the active drugs. The methodology is fully described at Lelièvre et al [1].

**Results** The assay was validated by comparing traditional microscopy examination with the ATP bioluminescence assay using a set of 6 anti-plasmodial drugs. We obtained comparable IC₅₀ values with both methods. After validation, 16 clinically relevant antimalarial drugs presenting different mechanism of action were tested. Only epoxomicin (0.42nM) and methyl blue (0.49μM) showed IC₅₀ values in the range of nanomolar.

Epoxomicin is an inhibitor of proteasome activity, exerting a toxic effect on the parasite, but as this function is also essential in mammalian cells its cytotoxicity is of concern. Primaquine has been reported to destroy the inner structure of *Plasmodium* mitochondria. It is assumed that primaquine activity depends on the formation of metabolites, more active than the parent compound. This α-aminooxyquinoline has long been known to reduce the prevalence of circulating gametocytes in the peripheral bloodstream of patients. Due to the absence of the active metabolite involved in its mechanism of action, primaquine remains inactive in vitro (20.9μM).

Methylene blue (MB) was identified as a specific inhibitor of *P. falciparum* gametocytes and HepG2 cell line (6.52μM), founding a good activity but quite high cytotoxicity.

**Conclusions** The work described represents another significant step towards determination of activity of new molecules on mature gametocytes with an automated assay suitable for medium/high-throughput screening. Considering that the biology of the sexual stages is very different from asexual forms, screening of compound libraries would allow us to discover novel anti-malarial drugs to target gametocyte-specific metabolic pathways.

**References**


**P8**

**Assessment of desiccants and their instructions for use in rapid diagnostic tests**

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Malaria Journal 2012, 11(Suppl 1):P8

**Background** Malaria rapid diagnostic tests (RDTs) are protected from humidity-caused degradation by the inclusion of a desiccant in the device packaging. The present study assesses malaria RDT products for the availability, type and design of desiccants and their information supplied in the instructions for use (IFU).

**Materials and methods** A panel of malaria RDTs was assessed for the desiccant type supplied in the device packaging and for the information mentioned in the IFU. The criteria used during this assessment were based on recommendations of the World Health Organization, the European Community, the European Chemicals Agency and own observations.

Desiccant sachets were defined as self-indicating (all beads coated with a humidity indicator that changes color upon saturation), partial-indicating (part of the beads coated) and non-indicating (none of the beads coated).

In addition, silica gel sachets containing a humidity indicator were individually assessed for humidity saturation indicated by color change and, in case of partial-indicating silica gels, for the presence or absence of indicating beads.

**Results** Fifty malaria RDT products from 25 manufacturers were assessed, of which 14 (28%) products were listed by the “Global Fund Quality Assurance Policy” and 31 (62%) were CE-marked. All but one of the products contained a desiccant, which was generally silica gel (47/50, 94%) supplied as a sachet enclosed in the device packaging. Thirty (40%) RDT products (one with no desiccant and 19 with non-indicating desiccant) did not meet the WHO guidelines recommending to add a self-indicating desiccant to the RDT device packaging. However, where self- or partial-indicating silica gel was added (n = 22 and 8 respectively), the toxic cobalt dichloride was always used as humidity indicator.

Less than half (14/30, 47%) of the IFUs of RDT products with indicating desiccants mentioned to check the humidity saturation before using the test. Moreover none of the IFUs included information on properties, safety hazards and disposal of the desiccant.

For the criteria assessed here, no difference was observed for Global Fund-listed and CE marked RDT products compared to those which were not.

A total of 15,577 silica sachets from 16 malaria RDT products were visually inspected immediately after opening the device packaging. Color change indicating humidity saturation was observed for 8/16 RDT products, at a median incidence of 0.8% (range 0.05% - 4.6%) of sachets inspected.

In all RDTs with partial-indicating silica gel, sachets without any color indicating bead were found (median proportion 13.5% (0.6% - 17.8% per product) and an additional light source was needed to be able to assess the color of the humidity indicator.

**Conclusions** The design of desiccants currently provided in RDTs shows several shortcomings. Improvements can be made regarding desiccant type (self-indicating desiccant which is easy to inspect), desiccant safety (phasing out of cobalt dichloride) and information supplied in the IFU.

**P9**

**Hemozoin impairs cell cycle progression and promotes chemokine release in human microvascular endothelial cells**

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**Background** Cerebral malaria (CM) is a fatal complication of *Plasmodium falciparum* infection caused by the cytoadherence of infected erythrocytes to brain endothelial cells followed by micro-circulatory obstruction, blood-brain barrier (BBB) damage, ring hemorrhages, inflammatory response and neurological sequelae. The combination of both parasite and host factors are involved in the pathogenesis of CM. In particular, malarial pigment, hemozoin (HZ) was shown to interfere with monocytes and endothelial cell functions. Recently our group demonstrated that HZ enhanced total gelatinolytic activity in endothelial cells by inducing ex novo matrix metalloproteinases-9 (MMP-9) and promoting proMMP-9 protein expression (Prato et al., 2011).

**Materials and methods** In the present study human dermal microvascular endothelial cells (HMEC-1) were treated with native HZ isolated from *P.falciparum* cultures. Cell morphology was evaluated by optical microscopy, chemokine (CXCL-8; CCL-5) production by ELISA, proliferation/viability by MTT assay and trypan blue count, apoptosis and cell cycle by FACS analysis, using Annexin V/ Propidium Iodide (PI) and only PI, respectively.

**Results** Modifications of cell morphology were observed in HZ-treated cells, which showed elongated form instead of the classical polygonal shape. Moreover, HZ stimulated the production of the chemokines...
Materials and methods
management of malaria. As a first step to gather evidence on the 
Plasmodium malariae
Mayuge District. A group of children (N = 163) within the SIMI cohort 
collected onto Whatmann 3MM filter paper. Treatment decisions in the 
two-three days after samples were taken. In addition, blood spots were 
point, a blood smear archive was made and microscopy was performed 
RDT-positive on Day 17 were also followed up on Day 24. At each time-
real-time PCR diagnosis of 
Genomic DNA was extracted from blood spots using chelex and 
for
on Day 17 were treated with oral quinine. All children were tested 
Day 7 were retreated with AL and those who were malaria positive 
AL at baseline. Children who were malaria positive by OptiMAL RDT on 
Background
including many mixed infections of 
consistently high prevalence of malaria was found in young children 
treatment for uncomplicated falciparum malaria. During a longitudinal 
study (SIMI project) investigating the dynamics of intestinal 
malaria was still responsible for 10% of the total disease burden. Mothers, 
guardians and caregivers of children play a vital role in the prevention, 
early detection and management of malaria. The general and daily 
priorities of caregivers living in a malarial area are not well understood, 
particularly as they have to balance competing social, economic and 
health constraints. A better understanding of household behaviour 
with respect to health education is imperative for the reduction of 
malaria incidence and the success of malaria control strategies. 
The investigation compared the relative importance assigned by 
female caregivers in communities under a successful vertically-
managed malaria control programme to malaria awareness on the one 
hand and to social and economic concerns on the other.
Materials and methods
In Uganda artemether-lumefantrine (AL) is the first-line treatment for uncomplicated falciparum malaria. During a longitudinal 
study (SIMI project) investigating the dynamics of intestinal 
chistosomiasis and malaria in Ugandan lakeshore communities, a 
consistently high prevalence of malaria was found in young children 
(including many mixed infections of Plasmodium falciparum with 
P. malariae and/or P. ovale sspp.), despite use of AL for home-based 
management of malaria. As a first step to gather evidence on the 
effectiveness of AL in this setting, a community-based observational 
study was initiated in an area of intense malaria transmission. 
Results
Forty children (26.3%) were microscopy-positive for malaria 
on Day 7 and 33 (21.3%) on Day 17. After genotyping, 33 (21.9%) and 
17 (11.7%) children were shown to have recrudescence infections on 
Days 7 and 17, respectively. Of the 28 children who had received two 
consecutive AL treatments, 11 were microscopy positive on Day 17. 
Multi-species Plasmodium infections were common, with 41.1% of 
children positive for P. falciparum/P. malariae, 9.2% positive for P. 
falciparum/P. ovale sspp. and 8.0% for all three species at baseline. By 
real-time PCR 39.9% of those children infected with falciparum malaria 
at baseline were P. falciparum positive at Day 17 and 9.2% of those who 
were infected with P. malariae at baseline were P. malariae positive 
at Day 17. On Day 24, after two or three consecutive anti-malarial 
treatments, 10 children were infected with P. falciparum and two with 
P. malariae.

Conclusions
Our results suggest that AL may not be as effective as 
previously thought for treatment of malaria at a community-based 
level in Uganda and that further more formalised efficacy studies of 
this drug in high transmission settings are required, particularly in areas 
with mixed-species malaria infections are common and where mass 
administration of anthelminthic drugs is being carried out.

Background
Control of malaria remains one of the world’s chief current 
public health challenges, particularly in sub-Saharan Africa [1] where 
malaria is still responsible for 10% of the total disease burden. Mothers, 
guardians and caregivers of children play a vital role in the prevention, 
early detection and management of malaria. The general and daily 

Acknowledgements
We thank Mr P Kruger and R Mudzielwana (Malaria 
Control Programme, Limpopo Province) for assistance in approaching 
the communities. The authors’ immense gratitude goes to the village 
women caregivers who voluntarily participated in the study.
References

P13
Malaria ecotypes: their usefulness for stratification in current malaria control and modeling
Allan Schapira1, Konstantina Bouvrika1,2
Swiss Tropical and Public Health Institute, Socinstrasse 57, P.O. Box CH-4002 Basel Switzerland; 1University of Basel, Petersplatz 1, CH-4003 Basel, Switzerland Malaria Journal 2012, 11(Suppl 1):P13

To deal with the variability of malaria, control programmes need to stratify their malaria problem into a number of smaller units on the basis of the epidemiology of malaria or on determinants, such as ecology. Relying on published research and grey literature we reviewed earlier classifications of malaria based on ecology. 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. Such classification provides a framework for planning, when it is recognized that the implications of any ecotype depend on the biogeographical region, sometimes sub-region, and that knowledge on physiography must be supplemented by information on natural, anthropic and health system processes. Only two ecotypes can be delimited with some accuracy and have relatively constant implications for control within certain biogeographic regions: forest environments within the Indomalay and the Neo-tropic and urban malaria, which has different implications in Africa and in the Indian sub-continent.

P14
The paradox of the effectiveness of IRS insecticides (including DDT) and its impacts on human health – what can we fix if it isn’t broken?
Hindrik Bouwman1, Henrik Kylin1, Maria (Riana) Bornman1
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The effectiveness of DDT and other insecticides when properly used as indoor residual spray (IRS) to combat malaria is not in question [1]. However, the high body burden of DDT of those protected is very high [2], and the human health consequences due to IRS insecticides of those protected are of great concern [1,2,3]. What may be questioned though are the effectiveness, health impacts, social consequences, and sustainability of some IRS alternatives. Many promising ‘silver bullets’ (using anything but IRS) to beat malaria over the last number of decades have come and gone. Yet, the one proven method, IRS, gets less recognition or attention. IRS interrupts transmission where most infections occur – the home. It is also at home where those most likely to suffer malaria – babies, children and pregnant mothers – are to be found. The negative part of the IRS message though, remains the inevitable co-exposure of the very same susceptible groups to IRS chemicals. Protection by IRS comes at a cost, creating a paradox – the inevitable co-exposure of the very same susceptible groups to IRS chemicals used [1,3]. Policy formulation, negotiating fora, and the development of research priorities via consensus (some possibly burdened with other agendas) seem not to be good platforms to deal with intractable paradoxes. IRS with chemicals seems out of Vogue and often relegated in favour of the enticing promises of high-tech or new methods.

IRS as a method has remained almost unchanged since de Meillon pioneered it in South Africa in 1936 [4]. Combining basic biological knowledge about reproductive behaviour of the female vector mosquito with residual toxic chemicals within and close to residential areas where most infections occur, is effective at preventing transmission, but bad at preventing chemical exposure and uptake of the chemicals by residents. We believe that a vast scope of options to improve on IRS remain to be explored that, while maintaining effective transmission prevention will also significantly reduce human exposure to IRS chemicals. Options for further exploration include inter alia: better application, more selective areas of indoor application, mosquito irritability and repellency, better formulations, and new chemicals [1].

Maintaining a proven top-down IRS strategy supported by an effective hospital and clinic system requires a minor inconvenience but no other behavioural changes by the inhabitants [5], ecological engineering, biological interventions or modifications, or vaccinations. The mostly non-intrusive IRS allows inhabitants and communities the freedom for social interactions and economic betterment unhindered by the inconvenience of most some other current forms of preventing malaria. For the foreseeable future, IRS with adequate supporting health infrastructure will remain a mainstay of malaria prevention, will most likely have a role in malaria elimination in any endemic area, and/or will remain the fall-back method in case of failure of alternatives. In the mean time, we can and should re-evaluate what works (IRS), and make it work better.

References

P15
Stakeholder development of the Malaria Decision Analysis Support Tool (MDAST)
Zachary Brown1, Randall Kramer1,2, Clifford Mutera1,2, Dohyeong Kim3, Marie Lynn Miranda4, Birkinesh Amenneshewa2, Adriane Lesser5, Christopher JPaul1,2
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Background Although exceptional progress has been made towards controlling and eventually eliminating malaria from sub-Saharan Africa, recent efforts have sometimes faltered. Reasons for this include the development of resistance in parasites and vectors to current control strategies, volatile funding streams, and funding allocations which sometimes do not efficiently achieve the goals of project managers, policy makers, or citizens. The project described here implements an approach to evidence-based policy for malaria control using a decision analysis framework proposed by Kramer et al. [1]. The project consists of the stakeholder-driven implementation of that framework through the development of a Malaria Decision Analysis Support Tool (MDAST) in Kenya, Tanzania, and Uganda. Results from the project to
date point towards large anticipated value from stakeholder-driven implementation of a tool such as MDAST at the policy, programmatic, and technical levels.

**Materials and methods** MDAST is an evidence-based framework to assess health, social, economic, and environmental outcomes that can result from alternative malaria control strategies. It was developed through an iterative process consisting of the following activities:

- **Recruit stakeholders who are experts and decision makers in malaria control policy across governmental and academic sectors in Kenya, Tanzania, and Uganda.** Elicit influence diagrams from stakeholder workshops about factors determining outcomes of different interventions.
- **Review field and modeling research on the short- and long-run effectiveness of dominant malaria control interventions.**
- **Develop a rapidly deployable, open-access, and customizable software tool using the Analytica® Decision Analysis Platform.** The software combines stakeholders’ influence diagrams and our review of scientific research.
- **Demonstrate and elicit stakeholder feedback on the tool through hands-on workshops.**
- **Refine the software to reflect stakeholder feedback on scientific content and ease-of-use/interpretability.**

**Results** For the analysis of risks that (a) were identified by stakeholders as important in determining effectiveness of different policies, and (b) have not previously been combined in a practical, flexible tool. These features include the dynamic selection of insecticide resistance in the mosquito population, as well as options of different long lasting insecticidal net (LLIN) distribution mechanisms (mass distribution or voucher-subsidized).

Through anonymous written surveys during the workshops, participants indicated high levels of enthusiasm for using the tool, and provided essential feedback on how it can be improved (e.g. additional IRS insecticides and the capacity to rotate them, and better representation of larvicing), and identified barriers to implementation (e.g. context-specific data for calibrating MDAST to reflect local conditions).

**Conclusions** The MDAST project demonstrates the need for compact systems to exchange evidence between scientific, policy, and program management communities for analyzing the potential outcomes of alternative policy decisions. MDAST works to address this need in participating countries. Continued engagement with stakeholders, and with scientists producing the primary research on which this tool relies, is necessary to complete implementation of MDAST and develop extensions of similar tools to additional locations and situations.

References


**P16**

**HIV-positive Nigerian adults harbour significantly higher serum lumefantrine levels than HIV negative individuals seven days after treatment for *Plasmodium falciparum* infection**

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**Background** Management of co-infection with malaria and HIV is a major challenge to public health and yet potential drug-drug interactions between antimalarial and antiviral regimens have not been adequately investigated in people with both infections. Both of the constituent components of artemether-lumefantrin (AL) the first-line regimen for malaria treatment in Nigeria, and nevirapine, a major component of highly active antiretroviral therapy, are drugs metabolised by the cytochrome P450 3A4 isozyme system, known to be induced by nevirapine. We examined potential interactions between lumefantrine and nevirapine in 68 HIV-positive and 99 HIV-negative adults, all of whom were diagnosed with asymptomatic *Plasmodium falciparum* infections by microscopy. Post hoc PCR analysis confirmed the presence of *P. falciparum* in only a minority of participants.

**Materials and methods** 68 out of 80 attendees at the HIV clinics tested were identified as positive for *P. falciparum* and returned for day 7 follow-up (85%). None of these individuals reported concurrent symptoms suggestive of clinical malaria. 126 additional volunteers agreed to have a rapid HIV test performed, of which 99 were found to be both negative for HIV and infected with *P. falciparum*. None of these individuals were symptomatic. All 167 participants were treated with a full adult course of AL, and followed up on days 3, 7 and 28 for repeat blood sampling. We recorded and examined the distribution of lumefantrine concentration at day 7 in all study participants.

**Results** Using the PCR data as a more reliable test for parasite carriage, we found weak evidence that HIV positive people were more likely to be parasitaemic at day 0 (OR 2.05, 95% C.I. 0.917 - 4.60; P = 0.054), which may reflect slightly higher parasite densities in this group. HIV-positive subjects were not significantly more likely to be PCR positive for *P. falciparum* at day 3 and/or day 28 after AL treatment than were HIV negative individuals (OR 1.75, 95% C.I. 0.776 - 3.95; P = 0.141). HIV status, and thus nevirapine use, was found to have a significant effect on the concentration of lumefantrine 7 days after treatment (Wilcoxon ranksum test z = -3.270, P=0.0011), with a median concentration in the HIV negative group of 2.75μM (IQR 1.03 – 4.31), and in the HIV positive group of 3.35μM (IQR 2.07 – 5.37). There was a significant association between HIV status and lumefantrine concentration at 7 days post AL treatment (z = -2.830, P=0.0046). Day 7 capillary blood levels of lumefantrine were significantly higher in nevirapine-treated HIV positive participants than in 99 HIV negative controls (P=0.0011). Higher day 7 levels of lumefantrine were not associated with lower risk of persistent PCR-detectable parasitaemia at day 3 post-treatment.

**Conclusion** Nevirapine increases peripheral lumefantrine levels in AL-treated adult African malaria patients. Preliminary data suggest that higher lumefantrine concentrations do not provide any parasitological benefit to nevirapine-treated HIV patients.

**P17**

**Abstract withdrawn**

**P18**

**Measuring the blockade of malaria transmission: analyzing the results of mosquito feeding assays**

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**Background** Mosquito Feeding Assays are currently the only method used to assess the effectiveness of malaria transmission blocking interventions (TBIs) currently under development. Infectious gametocytes are fed to mosquitoes which are then dissected after a fixed time interval to determine how efficiently oocysts have developed on the midgut. Feeding assays include The Standard Membrane Feeding Assay (SMFA), the Direct Membrane Feeding Assay and the Direct Feeding Assay, which use different methods of parasite presentation but all assess mosquito infectivity in the same way. Operation and analysis of these assays varies between laboratories: field scientists often measure TBI efficacy as reduction in the prevalence of infected mosquitoes whilst laboratory scientists are more likely to quote efficacy as a change in the number of oocysts within the mosquito. These metrics give outputs that differ widely, resulting in need for greater understanding of how these feeding assay SMFA inform TBI assessment.

**Materials and methods** Data from 538 different SMFAs (conducted on *Plasmodium falciparum* and *P. berghei*, in either *Anopheles gambiae* or *A. stephensi*) is used to illustrate why generalized linear mixed models should be used to analyze mosquito feeding assays data.
Results The relationship between oocyst prevalence and intensity is complex, yet predictable. We demonstrate that the distribution of oocysts between mosquitoes is highly over-dispersed, making efficacy estimates based on reductions in intensity highly uncertain. Analysis of 30 feeding assays carried out on the same TBI confirms that the observed reduction in prevalence depends upon the parasite exposure (as measured by oocyst intensity in the control group), with assays which have lower exposure appearing more effective. By contrast, if efficacy is estimated as a reduction in oocyst intensity, then this candidate demonstrates constant efficacy, irrespective of the exposure level.

Conclusions To report transmission-blockade efficacy accurately, the results of membrane feeding assays should give both the prevalence and intensity of oocysts in both the control and intervention group. Candidates should be assessed against a range of parasite exposures to allow laboratory results to be extrapolated to different field situations. Currently, many studies assessing TBIs are underpowered and uncertainties in efficacy estimates rarely reported. Statistical techniques that account for oocyst over-dispersion can reduce the number of mosquitoes that need to be dissected and allow TBI candidates from different laboratories to be accurately compared.

P19 Modeling the cost-effectiveness of mass screening and treatment for reducing Plasmodium falciparum malaria burden

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Background Past experience and modeling suggest that, in most cases, mass treatment strategies are not likely to succeed in interrupting Plasmodium falciparum malaria transmission. However, this does not preclude their use to reduce disease burden. Mass screening and treatment (MSAT) is preferred to mass drug administration (MDA), as the latter involves massive over-use of drugs. This paper reports simulations of the incremental cost-effectiveness (ICER) of well-conducted MSAT campaigns as a strategy for Pfalciparum malaria disease burden reduction in settings with varying receptivity (ability of the combined vector population in a setting to transmit disease) and access to case management.

Materials and methods MSAT incremental cost-effectiveness ratios (ICERs) were estimated in different sub-Saharan African settings using simulation models of the dynamics of malaria and a literature-based MSAT cost estimate. Imported infections were simulated at a rate of 2 per 1,000 population per annum. These estimates were compared to the ICERs of scaling up case management or insecticide treated net (ITN) coverage in each baseline health system, in the absence of MSAT.

Results MSAT averted the most episodes, and resulted in the lowest ICERs, in settings with a moderate level of disease burden. At a low pre-intervention entomological inoculation rate (EIR) of 2 infectious bites per adult per annum (ib/pa), MSAT was never more cost-effective than scaling up ITNs or case management coverage. However at pre-intervention EIRs of 20 and 50 ib/pa and ITN coverage levels of 40 or 60%, respectively, the ICER of MSAT was similar to that of scaling up ITN coverage further.

Conclusions In all the transmission settings considered, achieving a minimal level of ITN coverage is a “best buy.” At low transmission, MSAT probably is not worth considering. Instead, MSAT may be suitable at medium to high levels of transmission and at moderate ITN coverage. If undertaken as a burden-reducing intervention, MSAT should be continued indefinitely and should complement, not replace, case management and vector control interventions.

P20 Estimating malaria transmission in Sarangani Province, the Philippines using serological markers of infection

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Background The Philippines is among the 39 countries aiming for malaria elimination. The major challenge is finding residual transmission foci in difficult to access villages of Southern Mindanao, Philippines. The sensitivity of microscopy to detect asymptomatic infections declines with decreasing malaria prevalence. The aim of this project is to use antibody markers of P. falciparum and P. vivax infection to locate residual transmission foci and to determine effects of control measures in Sarangani Province, the Philippines.

Methods Filter paper blood spots from 907 participants (aged 1-86 yr) were collected in a cross-sectional survey from nine villages in Sarangani Province, Philippines between June-August 2010. Sera extracted blood spots were tested for presence of antibodies to Pfalciparum and P. vivax apical membrane antigen 1 (AMA1) and merozoite surface protein 1 (MSP1x) using indirect ELISA [1]. The mixture model was used to define the cut off value for presence or absence of antibody to the antigen tested. The dichotomized sera were fitted into reversible prevalence catalytic models using maximum likelihood estimation to generate age-specific seroprevalence curve [2]. Statistical analyses were done using the STATA® v12 software (Stata Corp., Texas).

Results The age-specific seroprevalence curves for PfAMA1 and Pf-MSP1x showed that the force of P. falciparum infection (λ) per year in Sarangani Province was 0.005 (95% CI: 0.003-0.009) and 0.03 (95% CI: 0.02-0.05), respectively. This result was supported by records of annual parasite incidence (API per 1,000) in Sarangani that decreased from 3.49 in 2005 to 0.57 in 2009. There was strong evidence (P<0.001) that the difference in exposure to P. falciparum varies between the nine villages. The age-specific seroprevalence curve to Pv-AMA1 showed that in 2010 the force of P. vivax infection (λ) per year was 0.02 (95% CI: 0.01-0.04), which reflected a decline in transmission as observed in seroprevalence curves to PF-AMA1 and PF-MSP1x. The force of infection using Pv-MSP1x was not calculated because its seroprevalence curve showed a horizontal line (probability positive of 0.1). This suggested that either P. vivax epidemic occurred in the province where individuals exposed developed antibodies to P. vivax at one point in time as observed in Vanuatu [3]; or a relapse of P. vivax infections due to activation of liver hypnozoites. There was strong evidence (P<0.001) that P. vivax transmission differed between the villages surveyed.

Conclusion The results show that P. falciparum and P. vivax transmission continue at very low levels in Sarangani Province, the Philippines despite strengthened control efforts. It is recommended that elimination efforts are intensified in villages where antibody prevalence to P. falciparum and P. vivax infections is higher relative to the nine villages surveyed.

References

P21 DDT exposure levels and semen quality of young men from a malaria area in South Africa

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Background The organochlorine pesticide DDT (1,1,1-trichloro-2,2-bis(chlorodiphenyl)ethane) has been used for malaria vector control in Limpopo Province, South Africa, since 1945. It is one of the...
Persistent Organic Pollutants (POPs) that are noted for their toxicity, persistence and bio-accumulative characteristics. Evidence of health effects in laboratory animals and wildlife are found in the scientific literature and include altered hormone activity [1]. DDT is still being used in many parts of the developing world for malaria vector control. Indoor spraying programmes in the Limpopo province include houses that are not painted on the inside. Exposure to DDT can be direct (spraying of houses) or indirect (through the food chain). DDT has got estrogic properties and its degradation product, p,p’-DDE, is an anti-androgens [2]. In response to mounting concerns about the endocrine disrupting influence of environmental chemicals on human health, this epidemiological study was initiated in a malaria area where DDT is still used. The aim of the study was to investigate the DDT/DDE exposure levels, effects on seminal parameters and possible adverse effects on human sperm genetic integrity in a non-occupationally exposed population of young health men, living in a malaria area.

**Materials and methods** This cross-sectional study involved 209 young males recruited in an endemic malaria area (Limpopo Province, South Africa) where DDT is sprayed annually. DDT and DDE levels were measured in plasma. Semen analyses were done according to the WHO (1999) criteria. The flow cytometric sperm chromatin structure assay (SCSA) and Anilin Blue (AB) methods were used to assess sperm DNA/chromatin integrity [3].

**Results** The lipid adjusted p,p’-DDT (mean±SD) concentration was 109.2±106.6 μg/g lipid whereas the p,p’-DDE concentration was 246.2±218.5 μg/g lipid. Several sperm motion parameters including the percentage of motile sperm were impaired with higher DDT and DDE concentrations (r=-0.27; p=0.001 and r=-0.20; p=0.001 respectively). Sperm motility and morphology were also negatively correlated with sperm DNA damage (-0.19; p=0.008 and -0.22; p=0.002). The results point to a weak association between DDT/DDE plasma concentration and the incidence of sperm with chromatin defects.

**Conclusions** The results suggest that non-occupational environmental DDT/DDE exposure have negative effects on seminal parameters and might impact on the sperm chromatin integrity and DNA damage of young South Africans. In response, the *University of Pretoria Centre for Sustainable Malaria Control* was established to make a sustainable contribution towards the creation of a malaria-free Africa. South Africa is working towards malaria elimination and is facing many challenges regarding effective, safe and sustainable vector control methods.

**References**


**P22**

**A novel method for large-scale culture of *Plasmodium falciparum* asexual blood stage and gametocytes in a Wave Bioreactor cell culture system**

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The clinical manifestations of *Plasmodium falciparum* malaria occur when parasites are multiplying within erythrocytes. During this stage, some parasites develop into sexual forms (male and female gametocytes), that are transmitted to the mosquito during their feeding on blood. The development of new drugs or vaccines targeting both asexual and sexual blood stage parasites is important to strengthen existing tools for fighting against malaria, and this is an essential component of the overall strategy of malaria eradication. However, there are still many unsolved questions concerning the cellular development and cell biology of *P. falciparum* asexual parasites, as well as for the onset and the development of the gametocyte stage. This is partly because of the inability to obtain good workable quantities of parasite material. Routine bulk-growing of cultures in most laboratories involves establishing multiple culture flasks, which is very time consuming and labour intensive.

To obtain large quantities of parasite material with high consistency, we recently reported the use of the Wave Bioreactor system for the culture of asexual blood stage of *P. falciparum* in suspension [1]. Controlled wave-induced motion within the bioreactors provides low-shear, stable hydrodynamic conditions and very efficient gas transfer that is ideal for parasite cultivation. We used the Wave Bioreactor™ 20/50 EHT which is composed of a single-use plastic Cellbag, the cultivation chamber, that sits on a heated and rocking platform. We established important parameters for maintaining *P. falciparum* cultures, such as rocking motion, temperature and gas flow. Moreover, by monitoring parasite growth along with glucose consumption, pH dynamic and lactic acid production we characterized the development of the parasites within the bioreactor. We showed that malaria parasites grow far better in these rocking cultures than in static flask cultures in terms of preserving parasite cell synchrony and reducing the number of multiple infected erythrocytes. Finally, we established a simple and straightforward protocol for bulk cultures of up to 1L of culture of *P. falciparum* within five days.

To take advantage of the benefits of *P. falciparum* culture in suspension, we have now developed a simple protocol for gametocyte production and isolation from 1L culture in the wave bioreactor. This protocol includes 2 phases; the growth of the asexual parasites to stress conditions to induce gametocytes, followed by the maturation of gametocytes. In our experiments, approximately 8-10% of the asexual parasites commit to gametocytes. We reproducibly obtained mature gametocytes within 10 to 13 days (males in 10-12 days and females in 11 to 13 days). Batches of mature gametocytes can be collected from the same wave bag culture over several days. Finally, flagellate tests confirm that gametocytes produced in the wave bioreactor are viable and, therefore, likely infectious for mosquitoes.

The use of the Wave Bioreactor is a breakthrough method that will allow large scale production of *Plasmodium falciparum* asexual blood stage and gametocytes for antigen or organelle isolation, high throughput screening of compound libraries, for infection of mosquitoes and for whole cell blood-stage malaria vaccine development under GMP compliant procedures.

**References**

standardized ex vivo methodology that can be applied during the early phases of the drug development process. Cross-resistance is evaluated through a panel of specific multi-drug resistant strains designed to cover all genetically validated resistance mechanisms known to occur in the field. Second, the genetic ability of P. falciparum to evolve a genetically encoded resistance mechanism is quantified by measuring the minimal inoculum for resistance (MIR), that is the minimal number of parasite from which a resistant mutant is likely to be selected ex vivo by a constant low level of drug pressure. Further, the generation of resistant parasites possibly facilitates the understanding of the compound mode-of-action and permits the identification of resistance markers, which are essential for resistance monitoring during the clinical development and post-marketing surveillance phases. Altogether, these and other parameters, such as resistant parasite fitness and gametocyte production, define a comprehensive profile, which allows the identification of overt risks and the active prioritization of the most robust antimalarials in a cost-effective manner.

References

P24
Recognition of Plasmodium falciparum gametocyte surface antigens by plasma antibodies in asymptomatic Ghanaian school children
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Malaria Journal 2012, Volume 11 Suppl 1:24

Background Malaria transmission-reducing interventions are key components of malaria control and elimination [1]. However, little is known about the immune responses directed at circulating Plasmodium falciparum gametocytes in humans, knowledge of which would be useful in the development of anti-gametocyte vaccines, which would have the capability to reduce malaria transmission from humans to mosquitoes. In a study in the Gambia, mature gametocyte-infected erythrocytes of P. falciparum were found to carry antigens (gametocyte surface antigens, GSA) that were recognised by malaria patient’s plasma antibodies. These anti-GSA antibodies, taken at a single timepoint, were weakly associated with lower duration of gametocyte carriage in these treated patients [2,3]. We then sought to determine longitudinal patterns in GSA antibody prevalence and its relationship to possible immune suppression of gametocyte carriage in vivo.

Materials and methods Flow cytometry of cultured gametocyte-infected erythrocytes from 3D7 and from two recently adapted gametocyte-producing lines was used to detect and measure plasma antibodies recognising the erythrocyte surface. Plasma was obtained from asymptomatic P.falciparum-positive children attending school in a rainforest region in Ghana. These children were treated with dihydroartemisinin piperquine, and followed up weekly for 1 month.

Results and conclusions By microscopy, 8.9% (15/168) of the children enrolled carried gametocytes and a further 20% of them developed gametocytes during subsequent follow-up. (NASBA is also now being carried out to identify sub-microscopic gametocyte carriers.) Preliminary results from 113 samples tested in flow cytometry show that more than 50% of those in the sub-group of children with gametocytes at enrolment carry antibodies to GSA, and we expect this proportion to increase as gametocytes are developed during the follow-up. Further longitudinal flow cytometry, and NASBA analyses will enable us to understand the dynamics between immune responses to gametocytes and gametocyte carriage following treatment of asymptomatic malaria.

References

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falsepositive rapid diagnostic tests for malaria and deletion of the pfhrp2 and pfhrp3 genes should continue in South America.

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References

P28
Antimalarial treatment by health care providers in Port Harcourt, Nigeria
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Background In Nigeria, malaria accounts for 60% of outpatient visits, 30% hospitalization, and is estimated to be responsible for about 11% of overall maternal mortality, 25% of infant mortality, and 30% of under-five mortality [10]. The disease is particularly virulent among pregnant women and the under-five years of age, due to their low levels of immunity. It impedes economic growth and keeps households in poverty. Lack of access to diagnostic testing before treatment is one of the weaknesses in the management of malaria in Nigeria [10]. This study examines the treatment practice for malaria among health care providers (HCPs) in Port Harcourt.

Materials and methods This was a cross-sectional study among HCPs, and data collection was by use of pre-validated questionnaires and in-depth interviews. The data was analyzed using SPSS Version 17.

Results A total of 273 HCPs (doctors, nurses, pharmacists, community health workers (CHWs) and private medicine vendors (PMVs)) were randomly selected from health care facilities in Port Harcourt. Of the HCPs, 100% of doctors & pharmacists; 89.6% nurses; 33.3% PMVs; and 25% CHWs are aware of the World Health Organizations (WHO) treatment guidelines. The ACTs (69.2%) and sulphadoxine /pyrimethamine (7.7%) were the most prescribed drugs for uncomplicated malaria in children. Other drugs prescribed were: Chloroquine, Quinine, and Artesunate (group 1), 5.1%; and Pyrimethamine and Paracetamol (group 2), 2.6%. For severe malaria in children, Quinine (46.2%), the ACTs (20.5%) and intravenous arteether (12.8%) were mostly used. The other drugs prescribed were: Chloroquine, Quinine, and Artesunate (group 1), 5.1%; and Pyrimethamine and Paracetamol (group 2), 2.6%. For severe malaria in adults, the ACTs (66.7%) and sulphadoxine /pyrimethamine (17.9%) were most prescribed in addition to the other drugs in groups 1 and 2. For severe malaria in adults, Quinine (46.2%), the ACTs (20.5%) and intravenous arteether (12.8%) were mostly prescribed. For pregnant women, sulphadoxine /pyrimethamine (76.9%), the ACTs (10.3%), Artesunate (7.7%) and Quinine (5.1%) were mostly prescribed. Regarding adherence to WHO treatment guidelines, only 44.3% of HCPs [doctors, 52.1%; Nurses, 23.1%; Pharmacists, 11.6%; PMVs, 7.4% and CHWs, 6.0%] used diagnostic testing before treatment. Proximity to a good laboratory, laboratory costs, and availability of diagnostic tools are major factors that influence HCPs’ decisions in carrying out proper diagnosis before treatment.
Conclusion This study shows that, while the ACTs are widely used for the treatment of malaria in Nigeria, a larger proportion of the treatment is not based on diagnostic evaluation. Many HCPs recognize that diagnostic testing should precede treatment, but do not have the required facilities for it.

References

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P30
Cysteine-Rich Protective Antigen (CyRPA) as promising blood-stage candidate protein for inclusion in a malaria subunit vaccine

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Background The development of an effective malaria vaccine is recognized as one of the most promising approaches that would provide a cost-effective intervention for addition to the currently available malaria control measures. Since the fully annotated P. falciparum genome has become available in 2002, reverse vaccinology represents a new opportunity to identify novel malaria vaccine candidate antigens. Screening of predicted P. falciparum open reading frames for proteins that could elicit parasite-inhibitory antibodies has led to the identification of the Cysteine-Rich Protective Antigen (CyRPA) as promising blood-stage candidate protein for inclusion in a malaria subunit vaccine.

Materials and methods On the basis of available genome-wide transcriptomic and proteomic information generated since 2002, we have selected uncharacterized ORFs for evaluation of their potential as vaccine candidate antigens. To generate tools for the characterization of candidate antigens, we have developed a cell-based approach for monoclonal antibody production: (I) generation of stably transfected mammalian cells, expressing high levels of target antigen on their surface in a native conformation; (II) immunisation of mice with transfected cells; (III) hybridoma cell generation by screening with the transfectants [1]. Stage-specific expression of CyRPA in schizonts and free merozoites was shown by Western blot analysis and confirmed by indirect immunofluorescence staining of synchronized blood-stage parasites. Generated anti-CyRPA mAbs showed parasite growth inhibitory activity due to inhibition of merozoite invasion. The in vivo growth inhibition was assessed by passive immunisation experiments in P. falciparum infected NOD-scid IL2Rγnull mice engrafted with human erythrocytes [2]. To demonstrate that growth inhibitory anti-CyRPA Abs could be induced by active immunization, CyRPA was recombinantly expressed as secreted protein in mammalian cells and directly purified from culture supernatant. Vaccine-induced polyclonal anti-rec_CyRPA Abs showed that the antigen is highly immunogenic in mice. Monoclonal antibodies against rec_CyRPA have been raised and are currently being characterized.

Results and conclusions Our data on localization, stage-specific expression pattern, and functional assays suggest a role of CyRPA in erythrocyte invasion by the merozoite. Importantly, CyRPA elicits Abs that inhibit merozoite invasion in vitro and in vivo. Thus, CyRPA represents a promising malaria blood-stage vaccine candidate antigen. It fulfills three key criteria applied to select asexual blood-stage antigens as vaccine candidates: (I) the protein is conserved; (II) Abs against the antigen inhibit parasite growth in vitro and (III) are protective in animal models. We expect that characterization of further parasite proteins with this strategy will identify additional vaccine candidate antigens from the extracellular stages of P. falciparum. This will extend the panel of vaccine antigens for incorporation into an effective multivalent, multi-stage malaria subunit vaccine.

References

P31
Malaria control in potable water and in biodiversity rich habitats: Need and opportunities for biological control agents

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Background During recent years, the contribution of man-made aquatic/semiaquatic habitats for breeding of malaria vector Anopheles culicifacies has increased, challenging the efforts of eliminating indigenous malaria from Sri Lanka. Some serve as important aquatic habitats with rich biodiversity while others are constructed to extract potable water and for agriculture in water sparse dry zone. These restrictions call for alternative control measures. A study explored the presence of malaria larvae and possible use of an indigenous fish species as a part of an on-going attempt for alternative malaria control methods.

Materials and methods Three types of potential mosquito breeding habitats; abandoned clay pits and quarry pits from wet (rain fall
Conclusions

Coleopterans were the dominant food items present in all time periods at 1230 hours (p<0.001). Results suggested that insect parts and fullness during 1630 hours whilst females had the peak gut fullness interaction explained the gut fullness with males having a peak gut parts and insect larvae) and class Maxillopoda (Copepoda). A time sex class Insecta (Coleoptera, Hymenoptera and other unidentifi ed insect analysis, are being monitored whist the sensitivity of being experimentally introduced to above habitats and larval densities were calculated. Simultaneously, Aplocheilus parvus, a common surface dwelling predatory indigenous fish species available in both lotic and lentic systems in all zones of Sri Lanka , was collected every 2 hours for 24 hours to determine its preference for mosquito larvae.

Results

The results indicated that 62% of clay pits and 76% of quarry pits were positive for Anopheline larvae while 31% and 37% of agricultural wells contained Anopheline larvae in Rathmale and Wagollakada respectively. Malaria vector An. culicifacies was present in 3% of clay pits at a density of 0.001 larvae/dip and 24% of quarry pits at the density of 0.027 larvae/dip. Among agricultural wells, 11% was positive for An. culicifacies in Rathmale at 0.097 larvae/dip whereas 11.63% wells in Wagollakada had a density of 0.106 larvae/dip. Moreover, potential malaria vectors An. varuna, An. vagus and An. jameisi were also recorded from all three types of habitats. Agricultural wells in Rathmale were also the habitat for Culicines at 0.899larvae/dip including vector of Japanese Encephalitis Culex tritaeniorhynchus. According to the diet composition analysis, A. parvus diet mainly consisted of adult or larval stages of class Insecta (Coleoptera, Hymenoptera and other unidentified insect parts and insect larvae) and class Maxillopoda (Copepoda). A time sex interaction explained the gut fullness with males having a peak gut fullness during 1630 hours whilst females had the peak gut fullness at 1230 hours (p<0.001). Results suggested that insect parts and Coleopterans were the dominant food items present in all time periods (p<0.001).

Conclusions

The need for alternative malaria control measures is highlighted. Currently A. parvus and guppy (Poecilia reticulata) are being experimentally introduced to above habitats and larval densities are being monitored whilst the sensitivity of A. parvus to commonly used agricultural chemicals are being tested.

Acknowledgements

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P33

Methods for costing malaria service delivery using secondary data

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Malaria is one of the major public health problems for low income countries, a major global health priority, one that carries a dramatic economic impact. Funding and consequently coverage of both preventive interventions and of case management for malaria control have been rising and both international donors and governments of malaria endemic countries need the tools and evidence to assess which are the best and most efficient strategies. To aid with these decisions we developed an open access malaria costing database that effectively summarizes an extensive body of literature on costing and effectiveness of malaria preventive interventions and case management. The database comprises 150 publications spanning from 1985 through 2012 from a total of 42 countries. We collected costs, detailed site and intervention data on insecticide residual spraying campaigns, insecticide treated net distribution and re-treatment campaigns, delivery of intermittent preventive treatment, and treatment of uncomplicated and severe malaria. Unit costs and other intervention data were coded into standardized categories and expressed in common 2008 international dollars allowing us to compare economic outlays across sites and interventions. We discuss findings from these descriptive analyses and illustrate how these data can be used to extrapolate costs to other countries.

Acknowledgements

The database structure and methodology builds largely on the work of White et al [1]. The project benefitted from constructive input by Thomas Smith, Joshua Yukich, and Nicolas Marie. Anika Quillitzsch assisted with literature searches and data entry.

References


P34

Abstract not submitted for online publication
A Bayesian model for estimating within-host *P. falciparum* haplotype frequencies

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The necessity for effective surveillance of antimalarial resistance is all the more reinforced by the recent emergence of resistance to artemisinin derivatives. However, the accurate detection of resistant haplotypes from patient samples that are infected with multiple parasite clones is nontrivial. When the multiplicity of infection exceeds one, the allele sequences of the constituent clones at genotyped loci are convoluted. Nevertheless, statistical methods can be used to reconstruct the allele sequences, infer distinct haplotypes and ascertain their frequencies from prevalence data. We have developed a Bayesian model for estimating haplotype frequencies. The model estimates haplotype frequencies based on prevalence data collected for one or more molecular markers known to be associated with antimalarial resistance. Prior knowledge of the MOI is not required. The model uses a Metropolis-Hastings Monte Carlo Markov chain algorithm to explore the different possible haplotype compositions that are compatible with the sample observed, and calculates the likelihood of the data given the current estimate of the haplotype frequencies. For each haplotype the model returns a distribution of frequency estimates from which the mean and its credible interval are derived. For each sample the model returns a distribution, over the possible haplotype compositions with which it is compatible. The model is validated using simulated data sets for which the true haplotype estimates are known. We present results of the application of our model to estimate haplotype frequencies for a set of historic data from Africa in which the prevalence of mutations associated with sulphadoxine-pyrimethamine resistance were obtained.
P37 A systematic review of published antimarial clinical trials: parasite clearance of artemisinin-containing regimes in the treatment of uncomplicated malaria:

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Background Parasitaemia on day 3 has been proposed as useful alert of potential artemisinin resistance, however, the normal variation of parasite clearance observed in ACT clinical trials is poorly documented. We reviewed the trends in parasite clearance following treatment with an artemisinin antimalarial regimen

Methods A PubMed literature search identified all studies of uncomplicated falciparum malaria published between January 2000 and December 2011. Studies were individually reviewed to identify clinical efficacy studies. Data from clinical studies using an artemisinin derivative were extracted and entered into a Microsoft Office Access database for analysis

Results In total 65,078 patients were enrolled in 213 clinical trials of artemisinin-containing regimes with 413 treatment arms containing either an artemisinin derivative alone (n=26) or in combination with a partner drug (n=387). The proportion of patients remaining parasitaemic at 24, 48 and 72 hours was documented in 115 (28%), 167 (40%) and 153 (37%) treatment arms, respectively. Excluding resistance studies in Cambodia, the median proportion of patients still parasitaemic was 53.8% (range 3-95, IQR=30.5-69.2) at 24 hours, 6% (range 0-65.9, IQR=2-11.5) at 48 hours and 0% (range 0-12.6, IQR=0-2) at 72 hours. Comparing studies from 2000-2005 and 2006-2011, the median proportion of patients remaining parasitaemic at 72 hours decreased in Africa (1.6% vs. 0, p=0.0004), but increased in Asia (0.8% vs. 1.2%, p<0.0001). Overall in 95% of these studies the proportion of patients with peripheral parasitaemia was <6% at 72 hours.

Conclusion These results highlight a normal range of parasite clearance times that will underpin a surveillance system based on day 3 positivity and the impact of heterogeneity in study design, host and parasite factors. Greater understanding of factors influencing parasite clearance will come from an analysis of data from individual patient records.

P38 Longitudinal study assessing the return of chloroquine susceptibility of Plasmodium falciparum isolates from travellers returning from West Africa

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Background From the 1940s up to the 1990s, chloroquine (CQ) was the main malaria therapy worldwide. Following the CQ resistance burden in Africa, most African countries have discontinued CQ during the past 2 decades, and now promote artemisinin-based combination therapy (ACT), as the first-line treatment for uncomplicated malaria. The policy changed in West Africa during the last decade (2002 in Cameroon; 2003 in Senegal and Cote d’Ivoire; 2004 in Mali). The aim of this study is to describe the evolution of CQ resistance in West Africa, through travellers returning from this region.

Methods The study was conducted by the Malaria National Reference Centre, France. The database collated in vitro response of reference and clinical isolates for CQ and the pfcrtK76 molecular marker for CQ susceptible Pf malaria from travellers returning from Cameroon, Senegal, Cote d’Ivoire and Mali. As a proxy of drug pressure, CQ intake for children under five years of age with fever was extracted from the Demographic Health Surveys (DHS) and Multiple Indicator Cluster Surveys (MICS) for the study period (1-2). Logistic regression models were used to detect trends in the susceptible isolates proportions.

Results From 2000 to 2011, around 700 isolates were genotyped for each country. The frequency of the pfcrt76 wild-type significantly increased for Cameroon (CM) (from 10% to 41%, Slope=0.09, p<0.10), Cote d’Ivoire (CI) (from 37% to 63%, Slope = 0.14, p<0.10), and Senegal (SN) (from 22% to 53%, Slope=0.17, p<0.10). The geometric mean of the 50% growth inhibition (IC50) of CQ decreased from 181nM (95% confidence interval, 87-374) (25% CQ sensitive) to 51nM (37-71) (63% CQ sensitive) in CM, from 75nM (43-130) (41% CQ sensitive) to 29nM (22-39) (84% CQ sensitive) in CI and from 86nM (51-145) (41% CQ sensitive) to 39nM (26-60) (75% CQ sensitive) in SN. Analyses performed from 2004 to 2011, when most of West African countries have officially discontinued CQ, confirmed previous results and also show a significant increase of the prevalence of pfcrt76 wild-type genotype for Mali (ML) (Slope = 0.07, p=0.02). Meanwhile, CQ use among children with fever significantly decreased during this period.

Conclusions An increase of CQ susceptibility following official withdrawal is observed in travellers returning from Cameroon, Cote d’Ivoire, Mali and Senegal. The length of time between policy changes and their subsequent implementation, as well as the cross resistance between antimalarial drugs, may affect the time for a significant recovery of CQ sensitivity. This information should be compared to country level CQ efficacy data.

Acknowledgements Data for DHS was obtained from Macro, Inc, www.measuredhs.org. Data for MICS was obtained from UNICEF, www.childinfo.org. This study was supported in part by a grant for doctoral studies to M. Gharbi from the Doctoral Network of the Ecole des Hautes Etudes en Santé Publique, Rennes, France. This abstract is being presented on behalf of the French National Reference Center for Imported Malaria study group.

References

1. MeasureDHS, ICF Macro, [http://www.measuredhs.com].
pharmacodynamic hallmark of AD is rapid parasite clearance, the clinical phenotype of slow clearance characterises resistance. This indicator remains critically important to monitor the extent of the problem in the absence of molecular marker(s) associated with artemisinin resistance and lack of sensitivity of current in vitro tests. Frequent parasite counts are needed to define clearance rate but it is uncertain what sampling frequency is required to ensure reliable estimates.

**Materials and methods** WWARN established a study group project to assess this question. Twelve studies with 4552 patients with frequent parasite counts, from Cambodia, Thailand, Laos, Bangladesh, Mali, Tanzania and Kenya were included in the analysis. Patients were treated with artesunate alone or in combination with a partner drug. The WWARN Parasite Clearance Estimator [1,2] was used to produce standardized estimates of parasite half-life (HL). Parasitaemia-time profiles with 6-hourly parasite counts available in the first 48 hours (h) were used to examine the effect of different sampling strategies on HL estimates - four measurement schedules were investigated at: (a) 0.6, 1.2, 2.4 or (b) 0.6, 1.8, 2.4 or (c) 0.12, 1.8, 24 or (d) 0.12, 24 and then every 12h. Bootstrapping was used to estimate the sampling distribution of HLs for subsets of the profiles with different distributions of HLs. A simulation study was performed to investigate optimal schemes. Parasite counts were generated from an overdispersed Poisson distribution based on the variability observed in the study data and assuming a first order elimination process.

**Results** The median (range) of estimated HLs was 3.1h (0.6–17.4). Estimates varied significantly between study location and year (p<0.001), with median HLs ranging from 1.9-6.3 h and the coefficient of variation ranging from 26-52% between studies. Nearly 50% (2251/4552) of the profiles had 6-hourly counts. In these profiles the median (range) for the difference between the original HL estimate and that from the 4 schemes were -0.02 (-3.4 to 3.8), -0.06 (-3.3 to 3.5), -0.09 (-3.6 to 3.4), -0.15 (-5.0 to 3.6) h, respectively. The overestimation of the HL by the restricted schemes was greater for profiles with short reference HL. Bootstrapping showed that the median HL was overestimated by the 4 schemes in the majority of bootstrap samples. The schemes overestimated the proportion (%) of profiles with a HL >3h, on average by 6, 7, 9, 12% in bootstrap samples with slow clearing parasites (50% of HL longer than 3h) and 39, 44, 54, 72% in bootstrap samples with fast clearing parasites (20% of HL longer than 3h), relative to the scheme with 6 hourly measurements. A number of alternative sampling designs derived from the simulation study will be presented and discussed.

**Conclusion** Our data indicate that the estimation of HL is dependent on sampling times for fast clearing parasias. 12 hourly counting is satisfactory in patients with slow clearance but the estimation of short HLs requires more sophisticated sampling schemes. Suggested schemes will need to be tested in a clinical study.

**References**


**P40**

The power of pooled analysis to inform optimal dosing strategies for Artemisinin Combination Therapies (ACTs)

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**Background** Antimalarial efficacy is dependent on administration of a curative dose determined by the pharmacokinetic and dynamic profile of the drug and the age and weight of the patient. Optimal dosing strategies are frequently compromised by pragmatic constraints resulting in patients receiving a wide range of mg/kg dose. WWARN has established a scientific research project aiming at pooling all relevant patient level data from ACTs studies conducted in malaria endemic areas. This study aimed at investigating the consequences of variance in dosing strategies of key ACTs and their effect on clinical efficacy.

**Methods** A systematic review generated a list of all antimarial clinical studies since 1960. Studies relevant to specific pooled analyses were identified and principal investigators approached to contribute individual patient data, which were then compiled into a standard format according to a transparent Data Management and Statistical Analysis Plan [1]. Data were collated for artemether-lumefantrine (AL), artesunate-amodiaquine (AS+AQ) and dihydroartemisinin-piperaquine (DHA+PQP) and analysed separately according to a priori analytical plan to identify key risk factors for treatment efficacy, recrudescence and new infection by day 28. Univariate and multivariate risk factors were identified using Cox’s regression model with frailty shared across the studies to adjust for the differences between studies. Optimal mg/kg dosage of partner drugs which best predicted the PCR adjusted recrudescence were explored using logrank statistics for pre-defined weight/age categories.

**Results** The WWARN repository currently contains over 75,000 individual patient records, 54% of which were treated with ACTs. In the current pooled analysis there are 10,913 patients treated with AL, 6,073 with AS+AQ and 4,739 with DHA+PQP; constituting 45%, 40% and 35% of the entire published data for these three drugs respectively. For AL, significant multivariate risk factors for recrudescence were baseline parasitaemia (log-scale): [AHR: 1.11, 95% CI: 1.00-1.23] and low weight category of 5-14 kg: [AHR: 2.08, 95% CI: 1.06-4.11]. However, the mg/kg dosage of lumefantrine was found not to be associated with the recrudescence failures [P=0.83] in the final model. Patient treated with non-fixed combination of AS+AQ were at 2.8 fold (95% CI: 1.53-5.29) greater risk of recrudescent failure compared to those treated with fixed dose combination (FDC). For the non-fixed combination of AS+AQ, logged baseline parasitaemia [AHR: 1.24, 95% CI: 1.07-1.45], low age category [age 1-5 years (AHR: 3.23, 95% CI: 0.90-11.94)] and the mg/kg amodiaquine dose [AHR: 0.96, 95% CI: 0.93-1.01] were the major risk factors for recrudescence in the final multivariate model. No risk factors were significantly associated with failure with the FDC. For DHA+PQP, patients in age group <12 years [AHR: 4.13, 95% CI: 1.06-15.94] and patients receiving an overall piperacilone dose of <48 mg/kg [AHR: 1.69, 95% CI: 0.95-3.02] were at greater risk of recrudescence.

**Conclusions** Pooled analyses of diverse clinical studies are feasible using standard algorithms and semi-automated processing and can provide new insights on risk factors of failure. Large standardised datasets provide substantial power to explore the impact of different dosing strategies and derive optimal treatment protocols.

**Acknowledgements** We are grateful to all those investigators who kindly shared their data to the WWARN Dose Impact study groups.

**References**


**P41**

Merozoite surface protein 3.3C-specific antibodies block the intraerythrocytic development of Plasmodium falciparum and induce parasite apoptosis

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_Malaria Journal 2012, 11(Suppl 1):P41_

**Background** Antibodies mediate naturally acquired immunity to the asexual blood stages of human malaria. Merozoite surface antigen-specific antibodies inhibit the *in vitro* growth and development of the parasite *P. falciparum*, although the functional mechanisms of this inhibition are not fully understood. In this study, we investigated the functional mechanisms of *in vitro* growth inhibition by antibodies to merozoite surface protein 3.3C.

**Materials and methods** Antibodies were raised by immunization with a recombinant antigen derived from the C-terminal region of merozoite surface protein 3.3 (MSP3.3C). *P. falciparum* blood stage parasites were cultured in the presence of *α*-apical membrane antigen 1 specific, α-MSP3.3C specific or naïve rabbit IgG. Parasite DNA content and morphology, antibody localisation and the presence of apoptotic

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markers were monitored over the parasite life cycle by microscopy, flow cytometry and indirect immunofluorescence antibody tests. **Results** MSP3.3C-specific Immunoglobulin G (IgG) displayed unusual and highly potent anti-parasite properties in growth inhibition assays. This activity appears to be caused by inhibition of the intraerythrocytic development of the parasite and not by inhibition of merozoite invasion. Notably, we have shown that antibodies to MSP3.3C can access the intraerythrocytic parasite post merozoite invasion and effectively block further development of the parasite within the host erythrocyte. Our data indicate that specific IgG to MSP3.3C can prevent the export of MSP3.3 through the parasitophorous vacuole membrane into the erythrocyte cytoplasm. In addition, anti-MSP3.3C antibodies induce several characteristic features of programmed cell death within the parasite. **Conclusions** The mode of action of MSP3.3C-specific antibodies is to gain access to the intraerythrocytic parasite post invasion and arrest parasite development. This research further our understanding of the functional mechanisms underpinning in vitro growth inhibition by merozoite surface antigen-specific antibodies and highlights the potential of MSP3.3 as a blood stage vaccine candidate.

**P42** Field implementation using chlorophyll derivatives with sunlight for malaria, filaria and dengue fever vectors control in infested Africa swamps

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In this work, we present the successful field implementation of using Photodynamic modality to control Malaria, Filaria and Dengue Fever vectors in infested epidemic swamps in Uganda, Ethiopia and Sudan. As the Photodynamic technique has become a major approach for the control of human parasites and noxious insects. Field investigations were carried out based on laboratory and semi-field results. In these trials, chlorophyll derivatives were added to the infested swamps to be taken by the mosquito larvae and the accumulated photoactive compound (photosensitizer) inside the larvae body induces upon sunlight exposure an oxidation stress, that results in organism death. As example in Kasangati and Namamve cities of Wakiso a district in Uganda, chlorophyll derivatives, as sunlight active photosensitizers was applied to cover 250 000 square meter of infected swamps and sand pits (4 gm/m2). The infected cities were mapped for this field application to cover 250 000 square meter of infected swamps and sand pits (4 gm/m2). The obtained results reveal that SAFE is highly effective as it ensures up to 100% mortality of mosquito larvae. The effectiveness of SAFE accumulation in the larval bodies was qualitatively and quantitatively investigated using the Confocal Laser Scanning Microscopy (CLSM) technique. The active ingredient of SAFE (chlorophyll derivative) exhibit several advantages: they are low cost, natural products extracted from green plants and endorsed by the Food and Drug Administration (FDA) as food additives. In addition, they are used in very low concentrations (μM), can be easily applied in the field by being dissolved in an aquatic environment and most importantly they are highly effective.

**P43** Sunlight active formulated extract (SAFE): an Egyptian invention for malaria vector control

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This invention introduces an innovative modality for malaria vector control that combines both effectiveness and efficiency with the highest levels of human safety and environment friendliness.

The Novality of this (PCT) patent lies in developing a new ecologically safe modality using a natural plant extract (chlorophyll derivatives) and mosquito larvae attractant as sunlight active photo-larvicide for control of Anopheles gambiae, Culex pipiens and Aedes aegypti. In this context, the accumulated photoactive compound (photosensitizer) in the larval body induces, upon sunlight exposure, an oxidation stress that results in organism death. The obtained results reveal that SAFE is highly effective as it ensures up to 100% mortality of mosquito larvae. The effectiveness of SAFE accumulation in the larval bodies was qualitatively and quantitatively investigated using the Confocal Laser Scanning Microscopy (CLSM) technique. The active ingredient of SAFE (chlorophyll derivative) exhibit several advantages: they are low cost, natural products extracted from green plants and endorsed by the Food and Drug Administration (FDA) as food additives. In addition, they are used in very low concentrations (μM), can be easily applied in the field by being dissolved in an aquatic environment and most importantly they are highly effective.

**P44** Cultural logics: a key issue in a Kaizen approach for malaria elimination

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**Background** Patients’ adherence to malaria treatment is a key issue in malaria control and elimination. But adherence remains problematic even when drug supply is good, putting human behaviour at the forefront of interest. The current approach to behaviour change strongly relies on communication interventions [1]. Such information and communication for behaviour change addresses people’s explicit and individual knowledge, on the premise that deficient or incorrect medical knowledge can be corrected and change behaviour. It hardly considers people’s implicit and collective knowledge or cultural logics that underlie concepts and practices.

In this presentation, we propose a different approach. To use a metaphor, instead of observing, counting and measuring mushrooms, we study the mushroom’s mycelium – the invisible web of threads beneath the soil that acts in symbiosis with its environment and pushes the mushrooms, the only visible part of the entire organism, across the soil. To know about the mushrooms (actions), one needs to understand the mycelium in its environment (the ‘web of meanings’, in Geertz’ [2]) cultural logics.

**Cultural logics in malaria studies** Based on our work from Peru and Tanzania, we show how such cultural logics can explain certain behaviours. Concretely, we analyse the cultural logics behind malaria treatment adherence and the cultural construction of side effects in the Peruvian Amazon based on the hot-cold theory that permeates popular models of health and illness all over Latin America. We revise the cultural logic of witchcraft behind treatment-seeking behaviour and behind perception of symptoms in Tanzania. We look at the logic of illness progression to explain the observed sequence of treatment actions and we hypothesise about the logic of purity and danger for explaining apparently irrational behaviour for severe malaria, again in Tanzania.

**Kaizen approach for elimination** At first sight, the study of cultural logics might seem to provide interesting adds-on in understanding behaviour whose results can be included in the information and communication strategies for behaviour change. A closer look, however, paints a profoundly different picture. Cultural logics, i.e. the mycelium of knowledge, are implicit in people’s narrations, and they require anthropological skills and theories to identify them and make them explicit. The methodological approach is based on grounded theory and mixed methods. Grounded theory is an iterative process that entails inductive coding from the data. The analysis is done by “weaving in theoretical ideas and concepts without permitting them to drive or constrain the study’s emergent findings” [3]. The emphasis lies
on ethnographic, qualitative (QUAL) in combination with a quantitative strand (quan) to test the relevance of identified cultural logics for practices. The collective nature of cultural logics requires a particular approach for implementation. Information and communication that targets individuals for changing behaviour is unlikely to show an effect other than the mere accumulation of knowledge. Understanding cultural logics provides the clues for problem recognition at the collective level. But these logics moreover provide the tools for solving the problems. Like in the Kaizen approach [4], an intervention that takes cultural logics into account needs to be process- and people-oriented. Kaizen methodology includes making changes and monitoring results, then adjusting. Through continuous and incremental improvement, in dialogue with communities, the mycelium can slowly be reshaped and better mushrooms can grow.

References

P45 Therapeutic efficacy of chloroquine and primaquine for Plasmodium vivax malaria treatment in southeast Iran

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Background Plasmodium vivax is the main cause of malaria infection in Asian, Central and South American countries [1]. It accounts for more than 90% annually of the reported malaria cases in Iran [2]. Plasmodium vivax resistant to chloroquine has emerged in some regions of Asia and resistance or tolerance to primaquine has been demonstrated in several countries [3,4]. The aim of this study was to determine the therapeutic efficacy of chloroquine and primaquine for Plasmodium vivax malaria treatment in southeast Iran.

Material and methods A total of randomly selected 270 patients with confirmed P. vivax infection participated in 28-day in vivo study that extended for 2 years for detecting relapse infection. Chloroquine and primaquine were administrated during 3 days and 8 weeks respectively in 2010. The thick and thin film blood smears were screened for malaria parasites by microscopy. The nested PCR was applied using the Plasmodium 18 subunit ribosomal ribonucleic (Ssr RNA) genes for detecting mixed infections and diagnosis of parasites in the samples with low parasite on days monitoring the drug resistance.

Results Fever resolved on the first day in all subjects. Microscopy findings showed that P. vivax was cleared in 15%, 50%, 95%, and 100% of patients on days 1, 2, 3 and 4, respectively. All 270 subjects showed ~120 Bp band in the nested PCR which was indicative of P. vivax malaria on the zero days. Six patients (2.2%) had specific P. vivax band in nested PCR on day 5. No recurrence was observed on days 7, 14 and 28 in thick blood smear and nested PCR. Mean (±standard deviation) parasite clearance time was 2.41 (±0.8) days. Two patients had P. vivax malaria clinical and parasitological infection following 8 and 12 months after primary P. vivax malaria infection.

Conclusions The findings of this study showed susceptibility of P. vivax to chloroquine in Southeast Iran. This finding is compatible with results of neighboring countries Pakistan and Afghanistan. Nested PCR was a suitable assay to determine exact malaria parasite clearance time in our study. The further investigation is being conducted in two reinfection cases by PCR - Single strand conformational polymorphism method to differentiate between relapse and new P. vivax infection.

References

P46 Exploration of larvicidal activity of Vernononia anthelmintica (L.) wild seed crude extracts in different solvents against malaria (Anopheles stephensi) and dengue (Aedes aegypti) vectors

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Malaria Journal 2012, 11(Suppl 1):P46

Background A large part of the population in the world is affected by one or more vector-borne diseases. The most effective way to prevent such diseases is to control the vectors [1]. Plant based insecticides are one of the best alternatives for the hazardous chemicals [2]. Leaves and fruits of Vernononia anthelmintica have been reported to have larvicidal properties against malaria vector [3]. In this study the larvicidal activity of the seeds of V. anthelmintica has been investigated for the first time.

Materials and methods In this study, larvicidal activity of crude ethanol, hexane, acetone chloroform and methanol extracts of the seeds of V. anthelmintica were tested against late III/early IV stages larvae of malaria (Anopheles stephensi (Liston)) and dengue (Aedes aegypti (Linnaeus)) vectors [4, 5].

Results All tested extracts showed strong larvicidal activity against the both vectors. The most effective extract against malaria vector was ethanol followed by chloroform and methanol extracts (LC50 1.95, 3.535 and 3.974 ppm; LC90 10.49, 18.325 and 15.979 ppm). Whereas in case of dengue vector chloroform was most effective (LC50 2.76 and LC90 14.01) followed by methanol and ethanol extracts LC50 3.395 and 3.461 ppm (LC90 12.95 and 12.804 ppm) (Table 1 and 2).

Conclusion This is the first report of cent percent mortality against the vectors of malaria and dengue using minimal doses of the seed extracts of V. anthelmintica. Further work for the isolation and characterization of larvicidal compounds is in progress.

Acknowledgements The authors are grateful to the Indian Council of Medical Research (ICMR), New Delhi, India for providing financial assistance in the form of research project for the present investigation. Authors are also thankful to University of Delhi as well as Centre of Medical Entomology & Vector Management, National Centre for Disease Control, Delhi, India for providing experimental support. Alina Hellert is grateful and deeply indebted to Prof. Veena Agrawal for the opportunity to do this research work in her laboratory and to all the members of her research group for their support and assistance.

References
Table 1 (abstract P46). Larvicidal activity of different extracts of *V. anthelmintica* seeds against Late III/Early VI instar larvae of *A. stephensi*. No mortality was observed in the controls.

<table>
<thead>
<tr>
<th>Solvent used for extraction</th>
<th>Concentration of crude extract (ppm)</th>
<th>Mortality (%)</th>
<th>LC\textsubscript{50} (ppm) (LCL - UCL)</th>
<th>LC\textsubscript{90} (ppm) (LCL-UCL)</th>
<th>( \chi^2 )</th>
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<tbody>
<tr>
<td>Ethanol</td>
<td>50</td>
<td>100</td>
<td>1.945 (1.488 – 2.392)</td>
<td>10.492 (8.479 – 13.837)</td>
<td>5.220</td>
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<td>25</td>
<td>99</td>
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<td>12.5</td>
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<td>Hexane</td>
<td>50</td>
<td>72</td>
<td>22.452 (18.692 – 27.831)</td>
<td>147.764 (100.792 – 248.774)</td>
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<tr>
<td>Acetone</td>
<td>50</td>
<td>83</td>
<td>9.124 (7.606 – 10.957)</td>
<td>74.713 (53.036 – 118.306)</td>
<td>2.231</td>
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LC\textsubscript{50} Lethal concentration that kills 50% of exposed larvae; LC\textsubscript{90} Lethal concentration that kills 90% of exposed larvae; LCL Lower confidence limits; UCL Upper confidence limits; \( \chi^2 \) Chi-square; ** Significant at \( P < 0.01 \); * degree of freedom 4

Table 2 (abstract P46). Larvicidal activity of different extracts of *V. anthelmintica* seeds against Late III/Early VI instar larvae of *A. aegypti*. No mortality was observed in the control.

<table>
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<th>Solvent used for extraction</th>
<th>Concentration of crude extract (ppm)</th>
<th>Mortality (%)</th>
<th>LC\textsubscript{50} (ppm) (LCL - UCL)</th>
<th>LC\textsubscript{90} (ppm) (LCL-UCL)</th>
<th>( \chi^2 )</th>
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LC\textsubscript{50} Lethal concentration that kills 50% of exposed larvae; LC\textsubscript{90} Lethal concentration that kills 90% of exposed larvae; LCL Lower confidence limits; UCL Upper confidence limits; \( \chi^2 \) Chi-square; * Significant at \( P < 0.05 \); ** Significant at \( P < 0.01 \); * degree of freedom 4
Background Threat of haemolytic anaemia in individuals with a deficiency in glucose-6-phosphate dehydrogenase (G6PD) enzyme activity prevents widespread use of primaquine, a vital drug for malaria elimination. Significant genetic variation underpins this human enzyme disorder, reflected as a spectrum of clinical severity following exposure to oxidative agents such as primaquine. Across malaria endemic countries (MECs), the prevalence and genetic basis of this predisposing condition vary greatly. An understanding of the relative risks presented by G6PD deficiency, in terms of prevalence and severity, is necessary to rationally determine primaquine drug policy.

Materials and methods We assembled a geo-referenced database documenting the spatial distribution of G6PD variants. A suite of maps were developed to illustrate this current state of understanding, including occurrence maps of the commonly reported variants and, where population samples were locally representative, maps detailing the relative prevalence of co-occurring variants. Countries were stratified according to the relative severity of the variants reported. These categorisations were combined with ranks of the overall frequency of deficiency in each country, resulting in an overall index of G6PD deficiency associated risk. A parallel index of uncertainty in the index was also developed.

Results The variant maps presented here make apparent the important contrasts between the spatial variants of G6PD deficiency between populations. As well as diversity among the variants identified within populations, there is great heterogeneity between the dominant variants identified between different populations. The Mediterranean variant was found to be the predominant variant in MECs across west Asia, where prevalence of deficiency was also high; these countries therefore ranked highest on the risk index. Across the Asian continent, risk from G6PD remained high due to the severity of reported variants and the disorder being relatively common. The low severity of variants reported from sub-Saharan African countries resulted in lower risk categorisation relative to Asia, though uncertainty in the data from this continent was high.

Conclusions Developing an evidence-base to support primaquine drug policy is essential. We present a cartographic suite and a framework for stratifying risk for regional comparisons. The risk index proposed...
here required several assumptions to overcome significant knowledge gaps. A deeper understanding of the common variants' primaquine sensitivity phenotypes is essential to maximise safe and effective use of primaquine.

**P50**
Consistently high baseline estimates for the proportion of human exposure to rural African malaria vector populations that occurred indoors
Bernadette Huhu1,2,3, Olivier Briët1,2, Aklilu Seyoum1, Chadwick Sikaala1, Nabie Bayoh1, John Gimnig1, Fredros Okumu1, Diadier Diallo1, Salim Abdulla1, Thomas Smith1,2, Gerry Killeen1,4
1Biomedical and Environmental Thematic Group, PO Box 78373, Dar es Salaam, United Republic of Tanzania; 2University of Basel, Petersplatz 1, Basel, CH-4003, Switzerland; 3Swiss Tropical and Public Health Institute, Basel, Switzerland; 4Liverpool School of Tropical Medicine, Vector Group, Pembroke Place, Liverpool L3 5QA, UK; 5National Malaria Control Centre, Chairama Hospital College Grounds, Off Great East road, PO Box 32509, Lusaka, Zambia; 6Centre for Global Health Research, Kenya Medical Research Institute, P.O. Box 1578, Kisumu, Kenya; 7Centers for Disease Control and Prevention, PO Box 1578, Kisumu, Kenya; 8Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, GA, 30333, USA; 9London School of Hygiene and Tropical Medicine, Disease Control and Vector Biology Unit, Nelson Street, WC1E 7HT, London, UK; 10Centre National de Recherche et de Formation Sur Le Paludisme (CNRFP), Ouagadougou, Burkina Faso

**Background**
Insecticide treated nets (ITNs) and indoor residual spraying (IRS) are highly effective options for controlling malaria transmission in Africa because the most important vectors, which are from the *Anopheles gambiae* complex and the *An. funestus* group, prefer biting humans who are indoors at night. It is feared that sustained large scale use of ITNs and IRS can cause these vectors to shift biting in place and time where ITNs and IRS are not effective.

**Materials and methods**
Matched surveys of mosquito and human behavior from six rural sites in Burkina Faso, Tanzania, Zambia, and Kenya with ITN coverage 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 maximum level of personal protection that ITN use can provide. The proportion of mosquitoes caught indoors ($P_i$) and between the first and last hours when most people are indoors ($P_e$) were also calculated as underlying indicators of vector preference for feeding indoors or at night, respectively.

**Results**
The vast majority of human exposure to *Anopheles* bites occurred indoors ($\pi = 0.90 – 1.00$). Neither *An. gambiae* s.l. nor *An. funestus* s.l. strongly preferred feeding indoors ($P_i = 0.46 – 0.63$ and $0.22 – 0.72$, respectively) but they overwhelmingly preferred feeding at times when most humans were indoors ($P_e = 0.84 – 1.00$ and $0.93 – 0.99$, respectively).

**Conclusions**
These quantitative summaries of behavioral interactions between humans and mosquitoes establish baseline values against which behaviour observed in rural vector populations exposed to high ITN or IRS coverage can be compared. Longitudinal monitoring of these quantities is vital to evaluate the effectiveness of ITNs and IRS and to evaluate the need for development of complementary measures targeting the outdoor-biting vectors.

**P51**
Olfactory drug delivery of artemether-curcumin combination for management of cerebral malaria
Kunal Jain1, Kuppusamy Gowthamarajan1, Sumeet Sood2, Kannan Elango2, Bhorjraj Suresh1
1Department of Pharmaceutics, J.S.S. College of Pharmacy (Constituent College of J.S.S. University, Mysore); 2Centre for Global Health Research, Kenya Medical Research Institute, P.O. Box 1578, Kisumu, Kenya

The objective of the present investigation was to explore the potential of nanoemulsion (NE) containing artemether-curcumin combination to accomplish the delivery of drugs to the brain via olfactory delivery system for management of cerebral malaria. The components for curcumin NE (C-NE) and artemether NE (A-NE) formulations were selected based on saturation solubility studies and were prepared by aqueous titration technique [1]. The mucoadhesive drug loaded nanoemulsions were made by adding chitosan (0.50% w/w) and stirred for 1 h. The nanoemulsions showed average globule size ranging from 32-70nm with polydispersity index of 0.221-1.000 and zeta potential in range of -12 to -28 mV. The formulations were subjected to thermodynamic stability tests like Heating-Cooling cycle, Freeze Thaw cycle and Centrifugation. All those formulations which passed the tests were subjected for further evaluation like transmittance, refractive index and electroconductivity tests. The percent transmittance of the formulations was >98% and refractive index was in range of 1.3-1.4 indicating transparent nature of nanoemulsions. Nanoemulsions exhibited high electroconductivity confirming that they were oil-in-water type. Transmission electron microscopy studies confirmed that globules were spherical in shape and size was in agreement with results obtained from globule size analysis. The nanoemulsions were evaluated for cytotoxicity studies using Vero cell lines by MTT assay. The CTC50 value of C-NE and A-NE was found to be 1950 mcg/ml and 2000mcg/ml respectively. Hemolytic activity was within the acceptable range revealing low toxicity risk of nanoemulsion. The ex vivo release studies of the formulated nanoemulsions were carried out using sheep nasal mucosa in comparison with drug suspension in simulated nasal fluid. The results revealed that release rate was biphasic and faster from drug suspension as compared to nanoemulsions. It can be concluded that nanoemulsions were prepared successfully having low particle size and can be used to deliver curcumin and artemether intranasally for management of cerebral malaria. The efficacy of the developed formulations either alone or in combination was evaluated further for antimalarial efficacy in *Plasmodium berghei* ANKA murine model of cerebral malaria in comparison with pure drug suspension administered intranasally and intravenously and showed promising results.

**Acknowledgements**
Kunal Jain would like to thank Council of Scientific and Industrial Research (CSIR), New Delhi, India for award of Senior Research Fellowship (SRF) number 8/484/0006/2012-EMR-I

**References**
PS3

Childhood imported malaria: could we have suppressed risk factors in some children?

Selva K Pillai1, Jean-Yves Siriez2, Eric Kendjo3, Sandrine Houzel4, Philippe J Guérin5, Jacques Le Bras6, Philippe J Guérin5, Jacques Le Bras6

1Centre National de Référence (CNR) du Paludisme, APHP, Paris, Ile de France, 75019, France; 2Centre de Référence du Paludisme, APHP, Paris, Ile de France, 75019, France; 3Centre National de Référence (CNR) du Paludisme, APHP, Paris, Ile de France, 75019, France; 4Centre National de Référence (CNR) du Paludisme, APHP, Paris, Ile de France, 75019, France; 5Centre National de Référence (CNR) du Paludisme, APHP, Paris, Ile de France, 75019, France; 6Ecole des Hautes Études En Santé Publique (EHESP), Paris, Ile de France, 75019, France

Background Malaria infection among child travellers need special attention as it rarely occurs in non endemic countries. Most studies dealt with risks in the paediatric population, but there is a need to justify age class choices and its direct implication of analysis for severity risk factors. In this study we demonstrated that analysing the multi factorial and caregiver dependent feedbacks for children <12 and <5 years old influenced their respective severity risk factors, that was not seen when analysed as a single continuous <18 years population.

Materials and methods Data collected from reported malaria cases of 2,357 children below 18 years old, from 2006-2011 to Centre National de Référence du Paludisme, throughout Metropolitan France were analysed for historically related travel habits, demography, practices and access to medical services. Multivariate logistic regression model with best maximum likelihood and Goodness of Fit were used to assess severity outcome risks within three separate groups (0-18 years as a single population, <12 years and those <5 years). Data was analysed using STATA 11.0.

Results Almost 97% (n=2287) travelled the African continent, of which 94% (n=2216) to African countries historically-linked with France. There were 101 severe cases with a case fatality ratio of 0.17% over the study period. The 0-18 years group analysis revealed children of expats/residents >6months had severity risks of OR 3.4 (95% CI: 1.39-8.30) and being born in endemic countries confirmed a protective factor against severity in all 3 groups analysed. However, evaluation of <12 years old, surfaced the risk of severity associated with declaration of chemoprophylaxis intake with OR 3.08 (95%CI: 1.31-7.24), thus not fulfilling its protective role. Declaration of inappropriate doxycycline use <12 years old was also detected. Being <5 years old and being born in France conferred a protective effect of OR 0.15 (95% CI: 0.02-0.97) were both reassuring and a new contribution in understanding imported childhood malaria. Medical services were promptly used by caregivers in France with diagnostic services and treatment initiated within the same day (mean 0.07 days, 95% CI: 0.05-0.95). Caregivers’ response accuracy of providing date of symptoms onset, were validated through an innovative approach.

Conclusion By improvising approach, we revealed both exclusive risk and protective factors for children <12 and <5 years old. Despite the presence of awareness among the caregivers to provide chemoprophylaxis to their children, possibility of unregulated or inappropriate chemoprophylaxis intake among child travellers became a risk factor for severe malaria, as it provided a false sense of security. Effective preventive medicine must include regulating chemoprophylaxis and community practices of them. Young children <5 were less likely to develop imported severe malaria. We hypothesized that having easy access and frequent contact to a good healthcare system and being young infants of possibly new immigrant mothers with immunity may partially explain this finding. For effective risk reduction, respect must be given to the unique age, developmental stage and characteristic of the paediatric population. Travel habits to countries historically-linked with France should be explored and leveraged to effectively reduce severe imported and endemic childhood malaria.

Acknowledgements French CNR Paludisme Study Group.

PS4

An observational field study of the barriers preventing successful treatment, control and prevention of malaria in the forest tribal area of Rampachodavaram in Andhra Pradesh, India

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1Student, Pleasant Valley High School, Bettendorf, Iowa, 52722, USA; 2Student, Topeka, Kansas, 66619, USA; 3Student, washburn University, Topeka, Kansas, 66619, USA; 4Student, Pleasant Valley High School, Bettendorf, Iowa, 52722, USA; 5Municipal Health Officer, Municipal Corporation Rajahmundry, AP, 533104, India; 6Graduate, Washburn University, Topeka, Kansas, 66619, USA; 7President/Founder, vedic international Institute for science and Arts, Bettendorf, Iowa, 52722, USA

Malaria Journal 2012, 11(Suppl 1):PS4

Background Rampachodavaram and its surrounding areas, a densely forested tribal location, tout one of the highest incidence rates of malaria in Andhra Pradesh, India [1]. The rural health care network seems sufficient, however, the prevalence of malaria suggests deficiencies. Government statistics report zero deaths in the forested tribal areas from malaria [2]; yet, field observation proves the contrary. The situation calls for focus, awareness and attention. This preliminary study focuses on uncovering the challenges and barriers for effective malaria control.

Materials and methods We travelled into the interior forest area to speak with and observe villagers, physicians and health officials at individual huts, rural governmental/non-governmental health centers and offices respectively. Structured interviews were conducted with all three groups and the responses were analyzed side by side. In addition, we reviewed official reports and records of malaria statistics to gain a holistic understanding of the procedures and policies regarding malaria control in the tribal area.

Results The tropical climate allows for a high mosquito density. Villages and surrounding forests are rife with hard-to-find stagnant pools that create accessible and favorable breeding places for mosquitoes. Villagers opt for practices, which have become woven into their tribal culture, that help them cope with the heat, tiring physical labor and poverty. Drinking jeelugu-kallu (indigenous alcoholic brew), misusing bed nets, sleeping without clothing, and disregarding malaria symptoms allow malaria to flourish. The general mistrust of government supplied medication, lack of education and heedlessness paid to government efforts exacerbate the problem. Current malaria initiatives include anti-larval operations, anti-adult operations, repellants and bed nets to prevent bites, and weekly surveillance and administration of medicine when needed by trained health workers [3]. While the protocols seem to encompass the entire problem, malaria continues to plague the tribal area. We found numerous communication gaps within malaria programs and between government and private health centers that can adversely affect program implementation. Also, these remote villages lack proper infrastructure and access to education. This coupled with the government’s lack of proper follow-up and continued care in villages creates loopholes for malaria to slip through.

Conclusions Our investigative surveys and fieldwork point to three major factors intertwined in the setback of Rampachodavaram’s malaria control: environment, culture, and infrastructure. The tribes’ primitive lifestyle supports an environment with antiquated drainage, disposal systems, architecture and agricultural apparatuses saturated with breeding places. Many of the practices in the tribes’ day-to-day
life have created a culture that is an easy target for mosquitoes. Lastly, a flawed infrastructure hinders efforts; irregularity in treatments and lack of follow-ups are common in the tribal area. If malaria is to be effectively combated in Rampachodavaram, it is imperative that more people become dedicated to the cause and strive to educate about precautions and treatment against malaria.

References
2. East Godavari District Epidemiological Data From 2006 To 2011: District Malaria Office Report; April 2012

P55
Anti-plasmodial action of de-novo-designed, cationic, lysine-branched, amphipathic, helical peptides
Naveen K Kaushik, Jyotsna Sharma, Dinkar Sahal
Malaysia Research Group, International Centre for Genetic Engineering and Biotechnology, Anura Asaf Ali Marg, New Delhi, 110067, India

Background
A lack of vaccine and rampant drug resistance demands new anti-malarials under such circumstances antibiotic peptides may offer a novel approach to tackle the parasite.

Methods
In vitro blood stage anti-plasmodial properties of several de novo-designed, chemically synthesized, cationic, amphipathic, helical, antibiotic peptides were examined against Plasmodium falciparum using SYBR Green assay. Mechanistic details of anti-plasmodial action were examined by optical/fluorescence microscopy and FACS analysis.

Results
Unlike the monomeric decapeptides \( \text{(Ac-GXRKXHKXWA-NH}_2 \) (X = F, D) \( \text{(Fm ΔFd IC}_50 \text{ >100 μM)}, \) the lysine-branched, dimeric versions showed far greater potency \( \text{(IC}_50 \text{ (μM)} \text{ Fd 1.5, ΔFd 1.39)} \). The more helical and proteolytically stable ΔFd was studied for mechanistic details. ΔFd, a K-K, dendraemer of ΔFm and (ΔFm), a linear dimer of ΔFm showed IC\(_50\) (μM) of 0.25 and 2.4 respectively. The healthy/infected red cell selectivity indices were >35 (ΔFd), >20 (ΔFm), and 10 (ΔFq). FITC-ΔFd showed rapid and selective accumulation in parasitized red cells. Overlaying DAPI and FITC fluorescence suggested that ΔFd binds DNA. Trophozoites and schizonts incubated with ΔFd (2.5 μM) egressed anomalously and Band-3 immunostaining revealed them not to be associated with RBC membrane. Prematurely egressed merozoites from peptide treated cultures were found to be invasion incompetent.

Conclusion
Good selectivity (>35), good resistance index (1.1) and low cytotoxicity indicate the promise of ΔFd against malaria.

P56
Inhibition kinetics of Plasmodium Lactate Dehydrogenase with herbal extracts suggest possible enzyme inhibitor molecular interaction
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Malaria Journal 2012, 11(Suppl 1):P56

Background
Resistance acquired by Plasmodium species (especially \( \text{P. falciparum} \)) to most of the present antimalarial drugs is the principal hindrance in controlling malaria. Thus, one of the major challenges towards elimination of malaria is development of novel and sustainable antimalarial drugs. In this milieu, herbs traditionally used to treat malaria are promising repertoires of antimalarial drugs. Earlier, lab studies have reported selective inhibition of \( \text{P. falciparum} \) and \( \text{P. vivax} \) specific Lactate Dehydrogenase (PfLDH and PvLDH) by \text{Phyllanthus amarus} aqueous extract and \text{Murraya koenigii} chloroform extract respectively. In the present investigation, we studied inhibition kinetics of PfLDH and PvLDH to explore molecular interactions between enzyme and inhibitor.

Materials and methods
Recombinant PfLDH and PvLDH, expressed in \( \text{E. coli} \), were used in enzyme assay. LDH activity was measured in the direction of pyruvate to L-lactate conversion. Steady state kinetic constants for substrate and cofactor as well as inhibition constants for plant extracts were measured by double reciprocal plot (Lineweaver and Burk plot). The enzyme inhibitor interactions were determined based on variations in the kinetic constants in presence of inhibitors, compared to control.

Results
Enzyme inhibition kinetics results are summarised in Table 1.

P57
Delivering two new treatments for malaria: a story of inventive partnerships
Graciela Diap1, Piero Olliaro2, Jean-René Kiechel2
1Drugs for Neglected Diseases initiative, 1202 Geneva, Switzerland; 2UNICEF/ UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR), 111 Geneva 27, Switzerland

The World Health Organisation (WHO) currently recommends the use of five artemisinin-based combination therapies (ACTs) for the treatment of uncomplicated \( \text{Plasmodium falciparum} \) malaria. ACT combines two effective antimalarial drugs and is the cornerstone of successful malaria control and ultimately, elimination. Fixed-dose combinations (FDC) of ACTs are preferred, as they promote adherence to treatment and reduce the risk of selecting for drug-resistant parasites.

In order to strengthen the ACT portfolio of FDCs, which at the time was minimal, the Drugs for Neglected Diseases initiative (DNDi), together with the WHO Special Programme for Research and Training in Tropical Diseases (TDR), launched the FACT (Fixed Dose Artemisinate Combination Therapy) project in 2002, with original funding from INCO/DEV. The FACT core group also included the Brazilian government-owned pharmaceutical company, Farmanguinhos/Fiocruz, the University of Bordeaux, Universit Sains Malaysia, Mahidol University and the Shoklo
Malaria Research Unit in Thailand, the Centre National de Recherche et de Formation sur le Paludisme in Burkina Faso and the University of Oxford, combining epidemiological and drug development expertise. Additional competencies in clinical studies, clinical supplies and regulatory matters strengthened the core team. Through innovative collaborations with this variety of partners, the FACT project delivered FDCs of Artesunate (AS) plus Amodialquine (ASAQ), and AS plus Mefloquine (MQ). Key contributions in scale-up, industrial production and regulatory filing were still needed to make the products available to patients, and different strategies were set up for each product. Both strategies were based on an initial development within the public and not-for-profit sector, with an extension to the private sector when a viable product was available, for registration, production and distribution.

Sanofi took over the last steps of industrial development and regulatory filing for ASAQ and the product was pre-qualified by WHO in 2008. Since then, ASAQ has been registered in over 30 countries, mostly in sub-Saharan Africa and over 120 million treatments have been delivered – making this the second most widely used ACT. Deployment was accompanied by a risk-management plan, together with an extensive training and educational programme developed by Sanofi. ASMQ FDC production was scaled up by Farmanguinhos/Fiocruz in Brazil showing the efficacy of the product in real-life conditions. Through a South-South technology transfer, ASMQ FDC production was transferred to the Indian pharmaceutical company Cipla to ensure availability in India and South East Asia. ASMQ FDC was registered in India in 2011 and in Malaysia in early 2012 and is under review for WHO prequalification. The FACT project demonstrates that alternative drug development strategies can make safe, affordable and sustainable treatments available to patients. Diverse partnerships gathering a wide range of expertise, from national programmes ensuring the most appropriate therapy is used in endemic areas to industrial partners for product implementation and wide distribution, were key to success. Both ASAQ and ASMQ FDCs are critical drugs for malaria control across Africa, Latin America and South East Asia.

Acknowledgements

Partners within the FACT project included:

- For the development of ASAQ: Sanofi, France; Medicines for Malaria Venture (MMV), Switzerland; National Centre for Research and Training on Malaria, Burkina Faso; Universiti Sains Malaysia; Oxford University, UK; Institute of Research for Development (IRD), Senegal; Université de Bordeaux Faculté de Pharmacie, France; Mahidol University, Thailand; Bertin Pharma, France; Médicins Sans Frontières (MSF); Epicentre, France; WHO-TDR; Kenya Medical Research Institute (KEMRI), Kenya; Indian Council of Medical Research (ICMR), India;
- For the development of ASMQ FDC: Farmanguinhos, Brazil; Cipla Ltd, India; Shoklo Malaria Research Unit, Thailand; Universiti Sains Malaysia; Oxford University, UK; WHO-TDR; Indian Council of Medical Research (ICMR), India; Médicins Sans Frontières, Holland; Centre Hospitalier Universitaire Vaudois (CHUV), Switzerland; National Institute of Medical Research, Tanzania; Kenya Medical Research Institute (KEMRI), Kenya; Centre National de Recherche et de Formation sur le Paludisme (CNRFP), Burkina Faso; Medicines for Malaria Venture (MMV), Switzerland

References


P59 Knowledge and practices of malaria prevention with ITNs in post- and near-elimination areas of Vanuatu

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Background Insecticide Treated Nets (ITNs) remain an important tool for sustained malaria control and play an integral part in malaria elimination strategies. As malaria incidence decreases in holodemic areas, however, proactive and regular use of ITNs may simultaneously decline if risk perception diminishes.

Data/methods In Summer 2012, we conducted a cross-sectional survey of three communities in Vanuatu: i) where malaria has been locally eliminated (Aneityum), ii) where malaria remains present but with rapidly declining incidence (Ambae), and iii) an urban area where malaria transmission may or may not occur (Efate). Respondents were asked a battery of questions regarding knowledge of malaria, ITN possession and use, and compliance with other anti-malaria interventions. Information on basic demographics, education levels, dietary habits and household economic activities were also recorded.

Results Residents of Aneityum (malaria eliminated) reported near universal use of ITNs, but uneven knowledge of malaria, particularly in younger individuals born around the time of malaria elimination. Residents in the other communities reported less consistent, though high levels of ITN use despite past individual malaria diagnoses.
Conclusions Results indicate that achieving sustained high levels of ITN use in near- and post-elimination contexts is possible, but that maintaining awareness could present a long-term challenge to prevent reintroduction and recrudescence. Sustained local community cooperation will be essential to maintaining elimination efforts worldwide.

P60 From compound to target: chemical proteomics and in silico screening identify Hsp90 and CDPK2 as putative targets in Plasmodium falciparum
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Background For several years now, Plasmodium falciparum is developing resistance to drugs in use. There is hence an urgent need for new treatments as well as new targets. By identifying the targets of novel or known active molecules with unknown mechanisms of action, it is possible to guide the development of new chemical entities towards their clinical application. This project aims at finding the putative target(s) of CP1, a new molecule in the development phase, and of triclosan, a well-known antibacterial and fungicide, by means of chemical proteomics and ligand based inverse virtual screening. Materials and methods The parasite lysate was incubated with the affinity matrix and retained proteins were analyzed by LC-MS/MS. Yeasts complemented with plasmoidal Hsp90 were grown in minimal media. Viability was calculated by comparison with untreated strains. Binding of CP1 derivatives to purified N-terminal PHsp90 was evaluated with Differential Scanning Fluorimetry. For the identification of potential triclosan binders, molecules were evaluated in silico using molecular docking program GOLD. Inhibition of PFCDPK2 and mechanism of action of triclosan were evaluated with a radiometric assay using myelin basic protein (MBP) as substrate.

Results For CP1, chemical proteomics identified heat shock protein 90 (Hsp90) as a putative binder. Subsequent assays confirmed that the viability of yeast cells where the wild-type Hsp90 has been substituted with the plasmoidal one was strongly reduced in presence of CP1. Moreover, CP2 was proven to bind the N-terminal domain of PHsp90. R triclosan, the in silico inverse screening proposed the calcium-dependent protein kinase 2 (PFCDPK2) as its potential binding partner. Enzymatic assays confirmed inhibition of PFCDPK2 with an IC50 of 48 μM. Furthermore, the mechanism of action was determined to be non-competitive towards ATP.

Conclusion This study shows that both chemical proteomics and in silico approaches are valuable tools for the identification of potential targets or binders of active molecules. The results obtained so far for PHsp90 point definitely towards an interaction with the protein, although a direct proof of inhibition is still needed. On the other side, the confirmation of the inhibition of PFCDPK2 by triclosan opens new perspectives in the use of this molecule and derivatives thereof against Plasmodium falciparum. In both cases, such results represent a starting point towards the optimization of the molecules and the development of new therapeutics against malaria.

P61 A new therapy for drug-resistant malaria using Plasmodium synthetic lethality inference
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Background If one gene of the Plasmodium (P) synthetic lethal (SL) gene pair has antimalarial drug resistance (ADR), a drug targeting the other gene of the SL pair can be used as an effective antimalarial drug for treating drug-resistant strains of malaria diseases. The approach introduced in this study is based on bioinformatic database integration. It employs a promising concept of synthetic lethality and has practical applicability in identifying gene targets to discover potential future drugs.

Materials and methods Potential antimalarial drug target genes were identified by integrating whole biological information from many databases, including BioGRID [1], KEGG SSDD database [2], GeneDB [3], PlasmoDB [4], and Ensembl [5]. The representations of the resulting networks were constructed by using Cytoscape (version 2.8.11) [6]. The distribution networks on gene ontology annotation (GOA) terms were statistically analyzed using BINGO 2.44 Cytoscape plugging [7]. The fitness data on the yeast homologous genes of the finally selected P genes were searched to find new drugs for clinical malaria treatment from Yeast Fitness DB [8].

Results A simple computational tool to analyze inferred SL genes of P species (P. falciparum, P. vivax and P.berghei) was established to identify SL genes that are possible drug targets. Information on SL gene pairs with ADR genes and their first neighbors from the yeast SL gene was inferred to search for pertinent antimalarial drug targets. We suggested that specific antimalarial drug candidates can be inferred by searching drugs that cause a fitness defect in the yeast SL gene.

Conclusions We suggest a new concept of drug-resistant malaria therapy in this study. The alternative antimalarial drug therapy consists of data integration and inference through the homology analysis of yeast-human P. Malaria parasites can be killed by mutating or blocking the SL partner of ADR genes. This concept is useful in selecting candidates as drug targets in antimalarial therapy. The methodology also provides not only drug target gene candidates for further experimental validation, but also information on new usage for already-described drugs. Drug candidates for targeting the suggested genes significantly benefit experimental validation in antimalarial drug discovery.

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References
1. BioGRID (Biological General Repository for Interaction Datasets) [http://thebiogrid.org]
2. KEGG SSDD database (Kyoto Encyclopedia of Genes and Genomes) [http://www.genome.jp/keg]
5. Ensembl [http://www.ensembl.org/index.html]

P62 Effect of farnesyltransferase inhibitor on the function of microchondria of Plasmodium falciparum
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Background Malaria is one of the world’s public health problems in terms of medical emergency with a high risk of mortality. The protozoan malaria parasites are transmitted by infected female mosquitoes [1].
The most pathogenic human malaria parasite, *Plasmodium falciparum*, has gradually expanded in last three decades [2]. Chloroquine is the cheapest and the most widely used drug in almost endemic countries. However, *Plasmodium falciparum* exhibits resistance to their drug. Resistance to the combination of sulfadoxine-pyrimethamine was also already emerged [3]. Farnesyltransferase have been identified in eukaryotic organisms, including pathogenic protozoa of the genera plasmodium [3, 4]. Therefore, the inhibition of farnesyltransferase has been suggested as a new strategy for the malaria treatment. However, the exact form of action of this class of agents is still unknown [5]. In addition, the effect of farnesyltransferase inhibitor on malaria mitochondria level is not fully understood. In this study, the effect of farnesyltransferase inhibitor on the function of mitochondria of *Plasmodium falciparum* were investigated experimentally. The oxygen distribution and the morphological shape of farnesyltransferase inhibitor-treated mitochondria were examined under *in vitro* condition. From this study, we found farnesyltransferase inhibitor is very important to understand the mitochondrial function of *Plasmodium falciparum* as an antimalarial drug.

**Materials and methods**

**Culture of malaria parasites**

Synchronization of *Plasmodium falciparum*

Determination of oxygen gradients in malaria parasites

Results In this study, farnesyltransferase inhibitor was treated to RBCs (Red blood cells) uninfected by *Plasmodium falciparum*. Farnesyltransferase inhibitor has noticeable effects on mitochondrial function of malaria parasites, compared to the control case for non-infected RBCs.

Conclusion

Oxygen distribution and morphological shape of farnesyltransferase inhibitor-treated mitochondria were investigated under *in vitro* condition. The farnesyltransferase inhibitor was observed to be very important to understand the mitochondrial function of malaria parasite as an effective antimalarial drug.

**Acknowledgment**

This research was supported by the World Class University (WCU) program through the National Research Foundation of Korea, funded by the Ministry of Education, Science, and Technology (MEST, R31-2008-000-10105 or R31-10105) and the Creative Research Initiatives (Center for Biofluid and Biomimic Research) from MEST and from the National Research Foundation (NRF) of Korea.

**References**


**P64**

**Specificity of malaria rapid diagnostic tests is affected by Trypanosoma brucei gambiense sleeping sickness**

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**Malaria Journal 2012, 11(Suppl 1):P64**

**Background**

In endemic settings, diagnosis of malaria increasingly relies on the use of rapid diagnostic tests (RDTs) instead of microscopic examinations. False positivity of such RDTs is poorly documented, although it may be particularly relevant in infections for which the differential diagnosis includes malaria, such as sleeping sickness, a fatal but treatable disease caused by *Trypanosoma brucei* parasite subspecies. We therefore examined the effect of *Trypanosoma brucei gambiense* sleeping sickness on the specificity of malaria RDTs.

**Materials and methods**

Blood samples of 117 sleeping sickness patients and 117 matched non-sleeping sickness controls were prospectively collected in the Democratic Republic of the Congo. Reference malaria diagnosis was based on microscopy corrected by a four primer real-time PCR. Ten commonly used rapid diagnostic tests for malaria were evaluated including three two-band tests and seven three-band tests, based on the detection of Pf-HRP-2, Pf-pLDH and/or pan-pLDH antigens of *Plasmodium*.

**Results**

Specificity of RDTs for diagnosis of malaria in controls was between 97.5 and 100% and was between 11.3 and 98.8% in sleeping sickness patients. For seven out of 10 RDTs, specificity was significantly lower in sleeping sickness patients compared to controls. Decreased specificity of malaria RDTs in sleeping sickness was mainly caused by false positivity of the pan-pLDH test lines, but also occurred frequently for the HRP-2 test lines. The Pf-pLDH test lines were not affected.

**Conclusions**

Specificity of some malaria RDTs in sleeping sickness is surprisingly low, and constitutes a considerable risk for misdiagnosis or delayed diagnosis of sleeping sickness.

**P65**

**SMS based external quality assessment of reading and interpretation of malaria rapid diagnostic tests: Preliminary results among more than 2000 end-users in the Democratic Republic of the Congo**

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Background Rapid diagnostic tests (RDT) are increasingly replacing microscopy for diagnosis of malaria in endemic settings. Although RDTs are simple and robust, errors in the post analytical phase i.e. in reading and interpretation of the RDT result, are not uncommon. In the Democratic Republic of the Congo (DRC) malaria is endemic, and malaria RDTs have been introduced since 2010. In June-July 2012, an external quality assessment (EQA) addressing correct reading and interpretation of the three band malaria RDT recommended by the National Malaria Control Programme was organized among end-users in DRC.

Materials and methods High resolution photographs of 10 patient RDT results representing a variety of malaria diagnosis, (combinations of) species, invalid or unreadable tests, faint positive test lines etc. were prepared. Answers were encoded in multiple choice format. Photographs were sent to focal points in 9 out of 11 provinces in DRC, who distributed them to malaria RDTs end-users. For each health facility, 1 questionnaire on availability, training and use of RDTs in the structure accompanied the photographs. End-users were requested to answer the multiple choice individually, by sending a short text message (SMS) to the study coordinator in Kinshasa who transferred SMS by blue tooth to an excel database. Questionnaires were recollected from the health facility by the focal points and sent back to Kinshasa.

Results Preliminary results are presented. In total, more than 2000 end-users participated in this EQA. Overall, about one out of 5 participants red and interpreted all 10 photographs correctly. Less than 1% had all 10 answers incorrect. For each individual photograph, between 50-90% of correct answers were received. In up to 40% of answers, the result represented a major error such as not recognizing an invalid test result, not recognizing negative test results or not recognizing a Plasmodium falciparum infection. Failure to detect faint or weak test lines was a common reason for wrong answers.

Conclusions The current EQA consisted of an innovative combination of a photograph-based approach, as being used in HIV and malaria RDT trainings, with communication by standard cell phone and SMS. Using this approach, we confirmed that errors in reading and interpretation of malaria RDTs are widespread in DRC. Data generated through this study, supported by feedback to the participants might as well improve end user performance in the structure accompanied the photographs. End-users were requested to answer the multiple choice individually, by sending a short text message (SMS) to the study coordinator in Kinshasa who transferred SMS by blue tooth to an excel database. Questionnaires were recollected from the health facility by the focal points and sent back to Kinshasa.

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and cost-effective addition to currently used control strategies. We previously used individual-based computer simulations (implemented in the openMalaria simulator) to analyze uncertainties in the predicted cost-effectiveness of introducing a malaria vaccine into the expanded programme on immunization in sub-Saharan Africa. We now extend these studies to include insecticide treated nets, a currently existing intervention. In addition, we also address model uncertainty in the current analysis.

Materials and methods We used techniques of probabilistic sensitivity analysis, involving randomly sampling the parameter vectors, to analyze the contributions of the different sources of uncertainty to the predicted cost-effectiveness. One specific aspect of these analyses of uncertainty is quantification of the value of acquiring additional information on these parameters by computation of expected value of perfect information (EVPI).

Results Among the most important predictors of the cost-effectiveness of a control program are the cost of the intervention program and the transmission intensity at the time of the start of the program. EVPI is shown to be substantial, and in particular the accrual of up-to-date information on local endemicity would seem an efficient way to inform decisions about local deployment.

Conclusions Probabilistic sensitivity analysis and value of information analysis using computer simulation models provide a powerful way to identify data gaps hindering rational resource allocation in malaria control.

P69 Abstract withdrawn

P70 Reduction of multiplicity of infections but no change in msp2 genetic diversity in P.falciparum isolates from Congolese children after introduction of artemisin-combination therapies

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Background In this first study conducted after the introduction of artemisin-combination therapies, we investigated the genetic diversity of P. falciparum isolates from children aged 1-9 years enrolled and followed up for one year to investigate clinical malaria cases. In addition, the msp2 profiles of P. falciparum isolates collected from successive malaria episodes in ten children who had four or more clinical episodes during the follow up were characterized. Three hundred and thirteen children residing in Southern part of Brazzaville participated in this study. Blood samples were obtained from all children at enrollment and checked for P. falciparum infection. Based on the one year follow-up data, two clinical groups were considered according to the number of malaria episodes presented over the follow up period: “protected” (children who did not experience any episode) and “unprotected” (those who experienced more than two episodes). Therefore, the msp2 genetic diversity of P. falciparum isolates collected at enrollment in the two groups was characterized by allele-specific nested PCR and compared. The msp2 profiles of P. falciparum isolates collected from successive malaria episodes was also characterized by allele-specific nested PCR. We found 43% FC27 and 57% 3D7 in protected vs 56% FC27 and 44% 3D7 in isolates from unprotected children. Seven and two alleles belonging to the FC27, and six and three alleles belonging to 3D7 families were distinguished in isolates from protected and unprotected children respectively. The mean MOI values at inclusion for the msp2 locus were 1.29 and 1.43 for protected and unprotected children respectively. 43 isolates were obtained from the ten children who had four or more clinical episodes during the follow up. A total of 63 alleles or fragments corresponding to 56% (36/63) FC27 and 44% (27/63) 3D7 were detected. The variant 400bp of FC27 was the most prevalent. 46% (20/43), 42% (18/43), 2% (1/43) and 2% (1/43) of isolates were found to have 1, 2, 3 and 4 parasite genotypes respectively and the mean MOI was 1.78.

Conclusion This study shows that the introduction of ACTs in the Republic of Congo has reduced the multiplicity of infection but not the genetic diversity of P. falciparum isolates from children living in Southern districts of Brazzaville.

P71 Towards implementation of rapid diagnostic tests for malaria case management in health centers of the Republic of Congo

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Malaria Journal 2012, 11(Suppl 1):P71

Since the introduction of artemisin-based combination therapies in the Republic of Congo, limited number of investigations have been conducted to evaluate the burden of the disease at the district level. The main goal of this study was to document laboratory-confirmed cases using rapid diagnostic tests of malaria in children and pregnant women attending health facilities located in Northern districts of Brazzaville and Pointe Noire which is the second main city of the Republic of Congo. As objective 2, the malaria diagnostic performance of each health facility has been assessed and as objective 3, genetic diversity, multiplicity of infection and the prevalence of P. falciparum resistance gene markers have been investigated during the malaria transmission October 2011 to February 2012. More than 1500 children and 700 pregnant women were recruited. The prevalence of malaria was comprised between 9% and 30% depending of the localization of the health center. It was found that acceptability of technicians to use rapid diagnostic tests was low and microscopy is still considered as the reference. A comparison between health centers and reference lab technicians showed similar level of in malaria diagnosis performance. The P. falciparum genetic diversity (20 msp2 gene alleles) and multiplicity of infection (1.7) do not show any reduction but parasite densities were lower than reported in studies conducted before the introduction of ACTs. As a conclusion: in order to improve quality of care and the acceptability of RDT, there is a need to provide a targeted training to heath workers.

P72 In-vitro studies on the sensitivity pattern of Plasmodium falciparum to antimalarial drugs and local herbal extracts

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The resistance of human malaria parasites to antimalarial compounds has become of considerable concern, particularly in view of the shortage of novel classes of antimalarial drugs. One way to prevent resistance is by using new compounds that are not based on existing synthetic antimicrobial agents. Sensitivity of one hundred (100) P. falciparum isolates to chloroquine, quinine, amodiaquine, mefloquine, sulphadoxine/pyrimethamine, artemisinin, Morndicida charanta (Ee6im), Diospyros monbuttensis (Ee6egun eja) and Morinda lucida (Oruwo) was determined using the in vitro microtest (Mark III) technique to determine the IC50 of the drugs. All the isolates tested were sensitive to Quinine, Mefloquine and Artesunate. Only 51% of the isolates were resistant to chloroquine, 13% to amodiaquine and 5% to sulphadoxine pyrimethamine respectively. Highest resistance to chloroquine (68.9%) was recorded among isolates from Ijebu zone while highest resistance to artesunate (30%) was observed in Ede zone. Highest resistance to sulphadoxine and pyrimethamine was recorded in Ede and Egba zones respectively. A significant positive correlation was observed between the responses to artemisinin and mefloquine (P = 0.001), artemisinin and quinine (P = 0.05), Quinine and mefloquine (P = 0.01). A significant negative correlation was observed between the responses to chloroquine and mefloquine (P = 0.05). Highest antiplasmodial activity
was obtained with the ethanol extract of *Diospyros monbuttensis* (IC$_{50}$ = 32 μg/ml) while the lowest was obtained from *Morinda lucida* (IC$_{50}$ = 250 μg/ml). Natural products isolated from plants used in traditional medicine, which have potent antiplasmodial action in vitro, represents potential sources of new antimalarial drugs.

**P73**

**Willingness of using rapid diagnostic tests for malaria and implications of concurrent availability of HIV tests in central Côte d’Ivoire**

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**Background**

Mortality due to malaria is largely driven by inadequate and/or delayed diagnosis and case management. The development and effective use of rapid diagnostic tests (RDTs) have contributed to reducing malaria mortality and morbidity, and hence RDTs have become essential tools for the control and elimination of malaria, particularly in areas where health services are deficient. We determined the knowledge, attitudes, practices and beliefs in relation to RDTs in two communities of central Côte d’Ivoire.

**Methods**

One hundred suspected malaria cases from Bozi and Yoho visiting the health centre in Bozi, were interviewed in April 2010 by administering a pre-tested questionnaire on current practice and perceptions of RDTs. The relationships between acceptance of RDTs and factors related to opinions were identified, using generalized linear mixed models. Qualitative data from open-end responses complemented the quantitative analysis.

**Results**

More than half of the people interviewed (54%) perceived blood as a “sacred body fluid”, while less than half complied with RDTs (44%). The concurrent availability and use of RDTs for HIV testing at the same health facility was associated with an unfavourable attitude towards RDTs for malaria (Fisher’s exact test, p < 0.001). The initial willingness of patients to accept malaria testing with RDTs was significantly related to general fear and wanting to know malaria infection status. For further and regular use of RDTs, an association was observed between the acceptance of RDTs and the wish to be tested for HIV (odds ratio (OR) = 16.6, 95% confidence interval (CI) = 1.03-236.5). Those thinking (OR) = 16.6, 95% confidence interval (CI) = 1.03-236.5). Those thinking

**Conclusions**

Socio-cultural factors might be barriers for accepting RDTs in general health services. There are social representations of malaria and HIV/AIDS, symbolic for blood or experiences in relation to blood sampling and blood-related diseases that might challenge the introduction and routine use of RDTs. These barriers must be given special attention to further promote RDTs for prompt and effective diagnosis and adequate management of malaria.

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**Beyond the nets: fighting malaria in the 21st century**

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**Background**

Malaria continues to be a major public health challenge, contributing to significant healthcare burdens and a negative impact on the economic and social development of affected countries [1]. With 20% mortality, malaria is Africa’s leading cause of death for children under the age of 5 [1-2]. In Ghana, it is 50% with approximately 7.3 million cases a year [3-4]. Between 1985 and 2003, the incidence of malaria increased from 31.8% to 44.7% [2-4]. Despite efforts at early diagnosis and treatment, insecticide treated net (ITNs) distribution, residual indoor and outdoor spraying; malaria remains a serious mortality and morbidity outcome in Ghana [5-7].

This research hypothesized that there is little understanding and acceptance amongst Ghanaians regarding the long and short term health effects in malaria vector transmission therefore fundamental prevention efforts are difficult to institutionalize. This research examined knowledge, attitudes and behaviors regarding malaria prevention and intervention in Greater Accra in 2010 and 2011 in a Family Practice health clinic. This work was based on the paramount belief that the effective containment of malaria needs an integrated systems approach which can only happen when there is a significant change in behaviors and belief systems [8].

**Materials and methods**

Patients from a family practice health clinic in Greater Accra, Ghana were surveyed in 2010 and again in 2011. Utilizing social research methods individuals were asked questions to determine their perceived risk of becoming infected with malaria and their knowledge, attitudes, and beliefs towards disease. Participants were selected based on the criteria of being patients of the clinic, residents of Ghana and being parents.

**Results**

In general participant responses indicated a good understanding of malaria transmission and prevention but on average participants believed that malaria transmission was inevitable suggesting that acceptance of prevention efforts is lacking. Males perceive their risk to be slightly higher by 0.5 compared to females; older age groups had slightly higher perceived risks, with an increase of 0.2 for every seven years of age and participant with higher levels of education perceived their risks to be lower by 0.3 per every degree earned. As age increased, the number of modes of protection increase. Participants were more open towards protection usage for children versus themselves. However, participants indicated they owned nets, thought they were effective but did not use them. Overall responses did not indicate a perceived need for systematic protective products or action planning nor was there a belief real prevention efforts by the government were taking place.

**Conclusions**

Findings from this work suggest that prevention efforts at the macro levels could more effective if tied to the current individual belief systems regarding malaria. Findings from this work suggest that the perception of malaria prevention is possibly linked to the lack of consistent, systematic and in-depth education regarding the disease and how it related to personal efficacy in which to effect change in one’s own environment and with medically appropriate and safe repellent solutions.

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Association of ABO blood group with severe falciparum malaria in adults: case control study and meta-analysis.
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Malaria Journal 2012, 11(Suppl 1):P75

Background
Erythrocyte-associated antigen polymorphisms or their absence have perhaps evolved in the human population to protect against malarial infection. Studies in various populations consistently demonstrate that blood group ‘O’ confers resistance against severe falciparum infection. In India, Odisha state has one of the highest malaria burden, are expected to be greater than the global mean, although there is currently less consensus between climate models on

Methods
A total of 353 P. falciparum infected subjects and 174 healthy controls were screened for ABO blood group. Falciparum-infected individuals were categorized as severe malaria and uncomplicated malaria. Severe malaria was further clinically phenotyped into cerebral malaria, non-cerebral severe malaria and multi-organ dysfunction. A meta-analysis was performed to assess the role of ABO blood group in severe malaria.

Results
Frequency of blood group ‘B’ was significantly higher in patients with severe malaria compared to the uncomplicated cases (P < 0.001; OR = 4.09) and healthy controls (P < 0.001; OR = 2.79). Irrespective of the level of clinical severity, blood group ‘B’ was significantly associated with cerebral malaria (P < 0.001; OR = 5.95), multi-organ dysfunction (P < 0.001; OR = 4.81) and non-cerebral severe malaria patients (P = 0.001; OR = 3.02) compared to the uncomplicated category. Prevalence of ‘O’ group in uncomplicated malaria (P < 0.0001; OR = 2.81) and healthy controls (P = 0.0003; OR = 2.16) was significantly high compared to severe malaria. Meta-analysis of previous studies, including the current one, highlighted the protective nature of blood group ‘O’ to severe malaria (P = 0.01). On the other hand, carriers of blood group ‘A’ (P = 0.04) and ‘AB’ (P = 0.04) were susceptible to malaria severity.

Conclusions
Results of the current study indicate that blood group ‘O’ is associated with reduced and ‘B’ blood group with increased risk of development of severe malaria in Odisha, India. Meta-analysis also supports the protective nature of blood group ‘O’ from severe falciparum infection.

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Understanding the role of climatic and environmental variables on the population dynamics of Anopheles gambiae s.s. and the implications for vector control strategies in different settings
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Malaria Journal 2012, 11(Suppl 1):P76

Background
The impact of weather, climate, and environmental conditions on malaria transmission have attracted increasing attention in recent years, although there remain significant uncertainties and debate regarding its precise role. Global mean temperatures are predicted to increase by several degrees over the coming century, although increases in Africa, accounting for over 90% of the global malaria burden, are expected to be greater than the global mean, with anticipated median increases around 3-4°C (depending on the emissions scenario). Global mean precipitation is also predicted to increase in most emissions scenarios over the coming decades, although there is currently less consensus between climate models on expected changes thereafter, particularly at low latitudes where the majority of cases arise and across many regions of Africa. Mathematical models provide powerful tools for understanding the impact of changes in environmental variables on transmission and intervention strategies, but this first requires realistic modelling of Anopheles population dynamics and its response to environmental variables.

Materials and methods
Experimental and field data are used to develop new parameterisations to model the relationships between key aspects of An. gambiae s.s. ecology and climatic and environmental conditions. These relationships are integrated into a deterministic model of Anopheles gambiae s.s. population dynamics to better understand mosquito response to changes in biotic and abiotic variables. This model is then calibrated against longitudinal vector abundance data from Tanzania, before applying the methods of matrix population modelling to analyse model behaviour (specifically, adult An. gambiae s.s. response to environmental conditions), and, hence, assess the implications for the design and implementation of local vector control strategies.

Results
A valuable modelling framework for assessing the effects of rainfall, cloudiness, wind speed, desiccation, temperature, relative humidity and density-dependence on vector abundance is developed, allowing ease of construction, analysis, and integration into malaria transmission models. Model calibration demonstrates excellent agreement with abundance data over a 40 month period, suggesting that recent malaria reductions in certain areas of Africa could be due to changing environmental conditions. Analysis of the model demonstrates the dependence on climatic conditions of An. gambiae s.s. persistence, resilience, stable stage distribution, and reproductive value of each of its life stages to fluctuations in local environmental conditions.

Conclusions
Mathematical models provide a valuable means of understanding the role of environmental variables on malaria vectors and hence for better understanding future malaria transmission under different environmental conditions, as well as the potential impacts of climate change. Modelling and analysis here provides an important contribution to this goal, enabling direct assessment of the implications of climatic and environmental conditions on the design of optimal local vector control strategies, as well as evaluating the potential impact of climate change on the likely success of these interventions. The work also highlights research gaps and priorities that should be targeted if we are to more reliably understand future malaria risk in regions predicted to experience significant changes in climatic variables over the coming decades.

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Can the recycling of LLIN reduce their coverage and use? Social, cultural and ethical aspects of LLIN life cycle management:
exploratory qualitative data from Madagascar
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Malaria Journal 2012, 11(Suppl 1):P77

Background
There is growing awareness of the likely impact of increased numbers of LLIN on the environment, if not disposed of or recycled appropriately. The WHO and UNEP initiated a pilot study to identify and assess the feasibility of environmentally-sound and cost-effective options for collection, recycling and disposal of LLIN. In this context, several studies were conducted in rural Madagascar where 22,559 used bed nets were collected for recycling. A social science study was carried out to provide preliminary data on socio-cultural factors related to the collection and replacement of LLIN for disposal or recycling.

Methods
Exploratory qualitative research was carried out following the pilot study in Betioky, Tsihombe, Fenerive Est and Ambanja,
triangulating participant observation, interviewing and group discussions. Data analysis was a continuous, flexible and iterative process concurrent to data collection. Final analysis was carried out using NVivo 9.

Results It cannot be a priori excluded that the collection of LLIN that are being used in any form/way (sleeping, alternative and secondary uses) from households for recycling purposes can, under certain conditions, lead to lower LLIN coverage and use. Several factors account for this. (i) Net preference. LLIN use for malaria prevention is expected to decrease when the nets distributed after the collection do not meet local requirements, additionally leading to alternative uses. Consequently, community members were often not willing to hand over old nets before confirming that new nets were appropriate for their intended use. (ii) Public/Private Sphere. The collection campaign brings net use out of the private and into the public sphere, in certain cases leading to lower net use and presenting an additional problem for collection. Users stated feeling ashamed at having to present dirty, ripped or bad smelling nets in public. Such concerns can lead to users refraining from relinquishing nets and/or to reducing net use in order to keep nets presentable for future collection. (iii) Net Lifecycle. The economic value placed on nets, for both sleeping and alternative/secondary uses, along with the sense of individual ownership of the nets, raises the question whether it is feasible to recycle nets during this stage of the net’s lifecycle. More so, given the fact that people will receive new nets based on epidemiological criteria and not in relation to their willingness to hand over used nets. Collecting nets at the stage of “waste” (when they are no longer used for any purposes) was locally more acceptable.

Conclusion The collection of used bed nets can be expected to be most feasible (i) for LLIN without locally perceived economic value, preferably at the stage of waste, (ii) when assessing users that net preference criteria are met by new LLIN, (iii) when the collection strategy is planned and appropriately explained to the community upon distribution of the LLIN. Given the possible concerns regarding net coverage and use, the collection strategy ought to be defined prior to net distribution and based on in-depth data on the local context.

P78 Forecasting malaria: ensemble modelling and predicting the impact of interventions
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Malaria Journal 2012, 11(Suppl 1):P78

Background Over the past decade many new mathematical models for malaria have been developed to assess the impact of interventions. Given sufficient data to inform them, these models can be useful tools for policy planning and forecasting the state of malaria in an affected region or to investigate important questions pertaining to controlling and eliminating malaria. But how accurate are these models? And importantly, how do the predictions from different models compare, and what are the uncertainties surrounding their predictions?

Materials and methods We present the Swiss TPH malaria modelling group’s initial approach for combining and evaluating a small ensemble of models. The ensemble comprises 14 individual-based stochastic simulation models of P. falciparum dynamics, with varied assumptions about immune decay, transmission heterogeneity, and access to treatment. Models parameters were fit to an extensive library of field data. We present a principled methodology for selecting and using an ensemble of models, which takes the accuracy and uncertainty of single-model predictions into account. The accuracy of individual models, evaluated against clinical data, is used to weight their predictions. The methodology can be applied to an ensemble composed of structural different models, potentially combining the Swiss TPH models with models from other groups. The uncertainty of the stochastic models in the ensemble is used to derive a range of reasonable values for model outcomes, such as predictions of the impact of interventions.

Results The proposed methodology has been pilot with an ensemble of Swiss TPH models to investigate the pre-erythrocytic vaccine RTS,S. Clinical trial data informs the models of the vaccine profile and the ensemble provides predictions of the impact of a pre-erythrocytic vaccine when scaled to national levels.

P79 Estimating transmission intensity from P. falciparum serological data using antibody density models
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Malaria Journal 2012, 11(Suppl 1):P79

Background Serological data are increasingly being used to monitor malaria transmission intensity and have been demonstrated to be particularly useful in areas of low transmission where traditional measures such as EIR and parasite prevalence are limited. The seroconversion rate is usually estimated using catalytic models in which the measured antibody levels are used to categorise individuals as seropositive or seronegative. One limitation of this approach is that the cut-off between positive and negative is arbitrary. Furthermore, the continuous variation in antibody levels is ignored thereby potentially reducing the precision of the estimate.

Material and methods To overcome these limitations we developed a series of age-specific density models which mimic antibody acquisition and loss. These were fitted to antibody titre data from 12 villages at different altitude in Northern Tanzania to estimate the rate of acquisition of antibodies as a measure of transmission intensity for multiple P. falciparum endemic settings.

Results Our results indicate that a model in which the boost in antibodies following exposure depends on the existing antibody level (with a decline in the size of the antibody boost with higher levels of circulating antibodies) and that includes variation between individuals in the size of the response fits the data well. We obtained a high correlation between our new estimates of the force of infection and estimates of the seroconversion rate obtained from the original catalytic model (r=0.95). Our estimates were also highly correlated with the estimated EIR (r=0.83) and parasite prevalence (r=0.67) in these 12 villages. The precision of the estimates obtained using the density model was greater than those obtained using the catalytic model.

Conclusion This approach, if validated across different epidemiological settings, could be a useful alternative model to estimate transmission intensity from serological data which avoids the need for an arbitrary cut-off value.

P80 Human lysozyme as a potential diagnostic marker in malaria: a mechanistic study of haemozoin-induced monocyte degranulation
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Background Lysozymes are antibacterial proteins defined by their ability to hydrolyse beta-1,4-glycosidic linkage between N-acetylmuramic acid and N-acetylglycosamine of peptidoglycan in the cell wall of bacteria [1]. In the recent years, little evidence on their involvement in malaria pathogenesis has emerged. In Anopheles gambiae and stephensi, lysozyme was shown to bind to oocysts of Plasmodium
berghei and falciparum, thereby facilitating their development within the mosquito [2]. In human patients, lysozyme plasma levels correlated significantly to parasitaemia degree, suggesting its potential role as marker of disease severity [3]. In this context, phagocytosis of haemozoon (HZ, malarial pigment) was shown in a previous work to induce in vitro lysozyme release from human monocytes [4]; here, the underlying mechanisms were investigated.

Materials and methods Human adherent monocytes from healthy donors were allowed to phagocytose for 2 h natural HZ isolated from Plasmodium falciparum cultures; after the end of phagocytosis, cells were incubated for 2 additional h in the presence or absence of: anti-TNFalpha/IL-1beta/MIP-1alpha blocking antibodies; recombinant TNFalpha/IL-1beta/MIP-1alpha; p38 MAPK inhibitor (SB203580); NF-kappaB inhibitors (quercetin, artemisinin, and parthenolide). Thereafter, lysozyme levels in cell supernatants were evaluated by measuring lysis of Mycobacterium Lysodeikticus suspensions through spectrometry, and TNFalpha, IL-1beta, and MIP-1alpha levels by ELISA. In cell lysates, p38 MAPK and NF-kappaB pathways were investigated by Western blotting or EMSA.

Results HZ promoted a time-dependent release of lysozyme, along with TNFalpha, IL-1beta and MIP-1alpha. HZ-induced lysozyme release was abrogated by anti-TNFalpha/IL-1beta/MIP-1alpha antibodies, and mimicked by all three recombinant cytokines. Moreover, HZ early activated either p38 MAPK or NF-kappaB pathways by inducing: p38 MAPK phosphorylation; cytosolic i-kappaBalpha phosphorylation and degradation; NF-kappaB nuclear translocation and DNA-binding. Inhibition of both routes prevented HZ-dependent lysozyme release.

Conclusions These data suggest that the HZ-triggered overproduction of TNFalpha, IL-1beta and MIP-1alpha mediates induction of lysozyme release from human monocytes through activation of p38 MAPK and NF-kappaB pathways. Therefore, the present work provides new evidence on the mechanisms underlying HZ-enhanced monocyte degranulation in falciparum malaria, supporting the hypothesis that lysozyme could be used as a new affordable marker in severe malaria.

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References

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Abstract not submitted for online publication

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Effects of foreign immigrants on malaria situation in cleared up and potential foci in one of the highest malaria burden district of southern Iran
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Malaria Journal 2012, 11(Suppl 1):P81

Background Objective: The One of the main objectives of malaria elimination program is protection and expansion of cleared up foci, towards and its final goal which is zero is to cut off the indigenous malaria cases. This study is aimed to assess the effect of foreign immigrants on malaria incidence in some clear up and potential foci in Konarak District, south east of Iran.

Material and methods In this descriptive-analytic study, the numbers of malaria patients in clear up and potential foci were analyzed in Jahiliyan region, located on the route of Pakistani and Afghan migration immigrants, during the 2005 to 2009. Data were described using frequency tables and analyzed by paired T-test. Also some of the development indicators were investigated in order to make sure that they did not change during the years of the study period.

Results The Annual Parasite Incidence (API) increased from a range of “30 to 142.9” after presence of immigrants in 2007, while it was “0 to 49” three years before their presence. The paired T-test showed a significant difference between the number of malaria cases in the villages from 2006 to 2008 and also 2007 to 2008. Development indicators didn’t have dramatic change during the five years, 2005-09 years.

Conclusions According to this research, the major cause of increasing malaria in the villages was the presence of foreign immigrants that led to increasing API index in 2008; so, cross border movement foreign immigration is a critical issue point to be considered in the malaria elimination program especially in the cleared up foci.

Acknowledgments We are extremely grateful to all who facilitated our field work. Particular thanks are expressed to the officials and respected staff in Konarak District Health Center.
P83 Extended haematological follow-up after parenteral artesunate in African children with severe malaria

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Malaria Journal 2012, 11(Suppl 1):P83

Background Mortality of severe malaria is significantly lower in patients treated with parenteral artesunate when compared to quinine. No significant side effects have been described from previous studies but follow-up periods have been limited. First reports from Europe have shown cases of post-treatment haemolysis occurring two weeks after the first dose of parenteral artesunate. Furthermore, haemolysis was accompanied by a delay in adequate reticulocytopoiesis for up to two weeks. All patients developing post-treatment haemolysis had hyperparasitaemia. The aim of this study was to gain information about the incidence, clinical impact and pathophysiological background of this effect in the hyperendemic setting of sub-Saharan Africa.

Materials and methods This study was conducted as a substudy to the ongoing Severe Malaria in African Children Follow-up study and was implemented in Lambaréné, Gabon and Kumasi, Ghana. 50 patients were recruited per study site.

Patients were randomized to different treatment regimes of parenteral artesunate (3 doses of 4 mg/kg iv, im or 5 doses of 2.4 mg/kg im respectively). Haematological parameters and serum markers of haemolysis and erythropoiesis (lactate dehydrogenase, haptoglobin, bilirubine, soluble transferrin receptor and erythropoietin) were determined on days 0, 7, 14 and 28. The clinical impact of changes in haemoglobin levels was assessed.

Results and conclusions The preliminary results show a delay in the reticuloipoietic response after treatment of severe malaria, which is accompanied by a slower recovery of haemoglobin levels in hyperparasitaemic patients (>100,000 parasites/μl) when compared to low parasitaemic patients. Hyperparasitaemic patients had a mean rise of 1.1 g/dL in Hb between days 0 and 28, while patients with low parasitaemia had a mean rise of 2.1 g/dL between days 0 and 28. First results on the potential pathophysiological background including haemolysis and / or impaired erythropoiesis will be presented. In conclusion, there seems to be an association between parasite levels and recovery of haemoglobin levels. Whether or not this effect is associated with malaria disease, treatment with parenteral artesunate or both is subject of further investigations.

P84 Abstract not submitted for online publication

P85 Plasmodium translationally repressed gene products are essential for parasite development and malaria transmission

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Background The sexual and ookinete development of Plasmodium relies on the translation of mRNAs supplied maternally in the macrogametocyte as translationally repressed transcripts [1]. Translational repression depends on the interaction of mRNAs and RNA binding proteins such as DOZI and CITH; their absence results in mRNA destabilisation and developmental arrest of the parasite in the mosquito midgut [2]. Among the repressed mRNAs ~40 encode for potential surface molecules or adhesins. While some are well-characterised (e.g. P25 and P28), most are putative with no known function or homology. pb25/pb28 gene disruption severely impairs parasite development [3] while the presence of anti-P25 and anti-P28 antibodies in a blood meal reduces mosquito infection [4,5]. A P25-based transmission blocking vaccine (TBV) has reached human phase 1 clinical trials but results have not been fully satisfactory [6]. For this reason, novel antigens are being pursued as targets of malaria TBVs.

Materials and methods RIP-Chip and RT-PCR of immunoprecipitated DOZI and CITH mRNPs from the rodent malaria parasite P. berghei were used to identify molecules with clear surface targeting signals that are associated with P body-like mRNPs. Targeted gene deletion and GFP-tagging was used to identify the function, sub-cellular localisation and expression patterns of these proteins. To study the development of knock-out parasite lines, Anopheles stephensi mosquitoes were allowed to feed on infected mice and infection was quantified at the oocyst and sporozoite stages. In silico analysis identified highly immunogenic peptides in each protein; they were concatenated as codon-optimised, chimerical His6-tagged fusion proteins for heterologous expression to be used in transmission blocking assays.

Results 22 mRNAs encoding for surface proteins were shown to be associated with both DOZI- and CITH-defined mRNPs. These include
pb25, pb28, the entire pb-fam-5 family, as well as 12 uncharacterised gene products with orthologs in *P. falciparum* and *P. vivax*; the latter were targeted for gene deletion. Several of the knock-out mutants show a clear impairment in mosquito stage development, either at the oocyst or sporozoite levels. Some of these completely fail to transmit to naive mice. Full length and concatenated versions (superantigen) of these proteins were expressed in *Escherichia coli* BL21. Superantigen expression was achieved more easily and in higher yields than full-length proteins.

Conclusions Here we identify novel *P. berghei* translationally repressed mRNAs that encode for mosquito stage surface proteins and are important for parasite development within its vector. Parasites lacking some of these proteins fail to transmit to naive hosts, and are therefore attractive targets for novel transmission blocking vaccines. Heterologous expression of *Plasmodium* protein is frequently a challenging task due to their disordered nature and the A/T richness of the genome. We used codon optimisation to compensate for A/T rich genes and developed a superantigen strategy to combine the most immunogenic regions of several proteins while avoiding their hydrophobic domains. The results show that our superantigens are more easily expressed in bacteria, and in higher amounts. This will enable us to use them in transmission blocking assays, targeting more than one parasite surface protein at the same time.

References

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Reducing the spread of artemisinin resistant malaria through community-level directly observed therapy in Western of Cambodia

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Background Artemisinin resistant parasites have been documented recently in the Cambodia-Thailand border area, which has been the epicenter of parasite resistance to anti-malarials in the past. In September 2010, the USAID funded Malaria Control in Cambodia (MC2) project, in collaboration with National Malaria Control program (CMN) and with technical and financial support from WHO, conducted community Day-3 surveillance for Pf (+) to gather evidence of drug resistance in the area. From June, 2011, the community surveillance expanded follow up to Day-28.

Methods Twenty-six village malaria workers (VMWs) were trained on malaria screening and treatment through direct observed therapy (DOT). VMWs use RDT for all suspected malaria cases on Day-0, smears on Day-0 and Day-3, filled the case investigation form, and completed follow up on days 1-3, Day-7, and Day-28 if Day-3 (+). Both malaria smears are sent to the health center laboratory for reading on Day-3. Malaria patients are treated with DHA-PiP for three days with DOT. An SMS alert system is sent to a higher level if Day-3 (+) is confirmed by lab staff. Cases identified positive on Day-7/Day-28 are referred for second line treatment with Quinine + Tetracycline for 7 days.

Results Between June 2011 and June 2012, 327 Pf positive cases were enrolled by VMWs. Over half (54%) were adult males between the ages of 15-49 years. A quarter (22%) were mobile and migrant workers. Twenty-seventy out of 327 cases (9%) remained positive on Day-3 and received further investigation and treatment. In addition, one case remained positive on Day-7, and 8 cases (30%) were positive on Day-28 as detailed in Table 1. The proportion of Day-3(+) cases decreased dramatically in the second phase of the pilot.

<table>
<thead>
<tr>
<th>Time frames</th>
<th>D0 (%)</th>
<th>D3 (%)</th>
<th>D7 (%)</th>
<th>D28 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sep 2010-May 2011</td>
<td>200</td>
<td>48 (24%)</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Jun 2011-Jun 2012</td>
<td>327</td>
<td>27 (9%)</td>
<td>1 (4%)</td>
<td>8 (30%)</td>
</tr>
<tr>
<td>Total</td>
<td>527</td>
<td>75 (15%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Early warning signs of drug resistance detected by VMWs should be treated with second line treatment at the health facility, but only three referred cases were accepted for 2nd line treatment. A challenge to seven-day treatment with Quinine + Tetracycline is the accessibility of health facilities. The CNM decided to implement other interventions such as IRS, FSAT, and screening surrounded index cases but they also have challenges.

Conclusion and discussions This pilot proves that malaria community DOT and follow up is vital to reduce the spread of potential multi-drug resistance. Identification of early warning signs of drug resistance and treatment by DOT for first line and second line by VMWs is a feasible approach. To ensure high quality results, strong monitoring, supervision, real-time feedback results are needed. Further discussion is needed to: (1) develop a comprehensive strategy to expand surveillance on a large scale including improving malaria case management and follow-up, (2) develop harmonized guidelines for promoting cross-border treatment and contact tracing, and (3) simplify the strategy for second line treatment and screening surrounding an index case.

Acknowledgements These results would not have been possible without the support from CNM, USAID, WHO, and other stakeholders.

P87

Lipid profile modifications of the lung tissue and surfactant in a murine model of malaria associated ARDS

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Malaria Journal 2012, 11(Suppl 1):P87

Background One of the lethal complications of malaria is acute lung injury and in its more severe form, acute respiratory distress syndrome (ARDS). In the murine model of malaria-associated ARDS (C57BL/6 infected with *Plasmodium berghei* PbnK65) the cytokine profile of infected animals is significantly altered in relation to the percent parasitemia. Preliminary data from our laboratory have shown alterations of the fatty acid profile in lungs of mice infected by PbnK65. However, it is not known if the molecular organization and lipid composition of pulmonary surfactant change during malaria ARDS. Surfactant once secreted forms organized lipid structures referred as large aggregates (LA). During respiration an inactive form of surfactant is also produced, the small aggregate form (SA). We explored the lipid profile both of the aggregates and of the lung tissue from non infected and infected animals with PbnK65 and with *Plasmodium chabaudi* (PcA5), a Plasmodium strain that does not induce lung pathology.

Materials and methods C57BL/6 mice were infected by PbnK65 or PcA5 parasites and sacrificed 6, 8 and 10 days after infection. Cell-free BAL was centrifuged to obtain the LA and SA fractions. The right lung
was perfused and homogenate. PL content was quantified according to Bartlett and PL pattern by HPLC or HPLC analysis.

Results An increase in the total content of PL of BAL in all the infected groups from day 8 post infection and increased levels of total proteins from days 6, were observed. Unexpectedly, the percentage of LA is significantly increased in NK65 mice as well the protein content and protein/PL ratio. These alterations are absent in the AS groups. The LA fraction of the NK65 shows a significant increase in the relative amounts of sphingomyeline and decrease of phosphatidilylycerol. The same changes were observed in the SA fraction accompanied by significant increase of lysophosphatidylcholine (LPC). The membrane enriched fractions of the lungs from NK65 mice are characterized by a significant increase of phosphatidylcholine and phosphatidylethanolamine. No differences are present in the other classes of PL and in the AS group.

Conclusions The increase in PL in the lung tissue is a common response to alveolar inflammation. This modification, absent in AS mice, appears to be correlated with malaria ARDS and consistent with the eosinophilic hyaline membrane deposition and cell infiltration observed in the alveoli of NK65 mice. The BAL fluid of NK65 mice is characterized by a high increase of protein levels indicative of oedema and alveolar leakage due to the lung pathology. On the contrary, the total PL increase present also in AS groups, seems related to malaria infection but not to lung pathology. Increased total protein levels are present in the LA fraction of NK65 mice, probably due to blood-derived proteins being incorporated into or associated with these microstructures in the alveolar hypophase. The increase of LPC, a known inhibitor of surfactant activity, in the SA fraction of NK65 mice is consistent with the action of phospholipases which are known to be present in the lungs during inflammatory injury.

P88 Entomological inoculation rates in 2009 on Bioko Island, Equatorial Guinea
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Background The Bioko Island Malaria Control Project (BIMCP) in Equatorial Guinea started in 2004 with the goal to reduce malaria transmission and associated morbidity and mortality on Bioko Island. In 2009 the project was extended by a second 5-year term from 2009-2013. To achieve these goals a set of integrated interventions were implemented combining vector control, effective case management, improved management of malaria during pregnancy, public communication, monitoring and evaluation, and operational research. Vector control consists of indoor residual spraying (IRS) with bendiocarb and long-lasting insecticide treated bed-nets. Here we report the results from the human landing collections of 2009 on vector density, biting times, species composition, sporozoite rates, and entomological inoculation rates (EIR).

Materials and methods Regular adult overnight mosquito collections were carried out by the human landing method at three sites on the island.

Results In the northwestern Punta Europa area the outdoor and indoor EIRs were 1028 and 738, respectively (883 combined). In this area close to 100% of captured mosquitoes were *Anopheles gambiae* s.s. The outdoor and indoor EIRs in southeastern Riaba were 301 and 196, respectively (248 combined). Here species composition consisted of 46% *An. gambiae* s.s. and 54% *An. melas*. In southwestern Arena Blanca the indoor and outdoor EIRs were 162 and 127, respectively (145 combined) with almost 100% being *An. melas*.

Conclusions Large spatiotemporal variations in mosquito density, biting activity, species composition and sporozoite rates indicate that pockets of high malaria transmission remain despite a largely successful control program. These hotspots require additional specific targeted vector control interventions, e.g. larval control or environmental management.

P89 Abstract not submitted for online publication
P90
Reactive surveillance methods used for malaria elimination in Asia and the Pacific: Results from a 12 country survey
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1Anti-Malaria Campaign, Ministry of Health, Sri Lanka; 2Global Health Group, University of California, San Francisco, USA; 3Asia Pacific Malaria Elimination Network, Joint Secretariat: University of Queensland, Australia and the UCSF Global Health Group, USA; 4Disease Control Division, Ministry of Health, Malaysia; *World Health Organization, Philippines
Malaria Journal 2012, 11(Suppl 1):P90

Background Moving from malaria control to elimination requires a strong surveillance system, one able to detect all malaria infections, including those without symptoms. Active case detection is designed to do this. One such active surveillance method is reactive case detection. Reactive case detection is the process that a malaria control program undertakes in response to a confirmed case of locally transmitted infection or an imported case that is found in a receptive area. The goal is to find additional cases of malaria infection and halt transmission through treatment of cases and targeted vector control. Although recommended as a tool for malaria elimination, there is little guidance on how to implement this tool. Different triggers are used, and there is substantial evidence to guide programs in what type of strategy might work in different epidemiological settings. The Asia Pacific Malaria Elimination Network, or APMEN, is a regional group of 12 country partners with a goal of malaria elimination. One of the main objectives of APMEN is to build the evidence base on malaria elimination, of which active surveillance methods are an important component. This survey aims to provide information on the different strategies in use and will help form the foundation for future studies on reactive case detection in the Asia Pacific.

Materials and methods A survey was developed to identify the strategies employed by countries in the areas of: index case investigation, additional screening measures taken in response to a locally transmitted case, training & monitoring of surveillance officers in these activities, reporting structures, SOPs, and additional vector control or entomological surveillance measures used. Analysis of the survey was conducted in Excel by identifying the proportion of positive responses for each question. Some survey respondents required follow up for clarification.

Results Nine of 12 countries responded to this survey, all of which were part of the Asia Pacific Malaria Elimination Network. Preliminary results are presented here. The majority of respondents report that any case triggers a case investigation. All countries report that a visit to the index case is conducted as part of the case investigation, but the other measures used vary greatly from country to country. For example, only four countries collect information on history of malaria when the index case is collected, whereas eight supervise treatment measures used vary greatly from country to country. For example, only four countries collect information on history of malaria when the index case is collected, whereas eight supervise treatment.

Conclusions All countries in the survey employ reactive case investigation, although the scale of this intervention is different for all countries. Also different is the approach of the index case, the additional screening measures, and whether vector measures are employed as part of the procedures. Countries employ these methods without a rigorous evaluation of their effectiveness and without assessing the cost of measures per newly identified case. In order to make evidence-based decisions about surveillance measures, there needs to be more detailed, context-specific guidance on the most effective reactive case detection methods and subsequent screening and vector control.

P91
An in silico drug treatment model to assess the robustness of regional age-based dosing regimens for artemisinin-based combination therapies
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Malaria Journal 2012, 11(Suppl 1):P91

The standard drug development process for antimalarials and other drugs uses weight-based dosing (mg/kg) to predict blood concentrations of the drug, and hence their effect. Consequently, the current World Health Organization Guidelines for the treatment of malaria [1] provide target doses and therapeutic dose ranges in mg/ kg/day. However, in resource-poorn settings, age-based dosing is often employed instead of weight-based dosing because of the scarcity of correctly functioning weighing scales outside of clinical settings. Due to the wide variation in weight by age this approach inevitably results in over- and under-dosing of a proportion of the population.

We have recently developed a modelling method to create statistically robust global and regional malaria-specific weight-for-age references representative of the malaria-endemic countries [2] and employed it to predict optimized age-based regimens for artemisinin-based combination therapies (ACTs) for case management of uncomplicated malaria (unpublished). The presented work now assesses the robustness of these age-based regimens using an in silico model of antimalarial drug treatment to predict treatment outcome based on individual infection parameters such as parasite numbers, variation in patient pharmacokinetics, and parasite variation in their drug sensitivity [3]. This extended pharmacokinetic/pharmacodynamic model for ACTs allowed us to investigate extreme treatment scenarios in a large number of patients over long follow-up periods that for ethical reasons could not be applied in clinical trials: typical examples include poor adherence (e.g. delayed, reduced or missed doses) or administration of doses above or below recommended therapeutic dose ranges and particularly in most vulnerable individuals such as infants and young children. Pharmacological modelling of antimalarial treatment cannot replace the gold standard of clinical trials, but the model outputs can identify patient groups that are at higher risk of treatment failure due to under-dosing or adverse events due to over-dosing.

We acknowledge the Medical Research Council for funding of this work.

References

P92
Challenges in diagnosing pediatric malaria in Dar es Salaam, Tanzania
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Background Malaria is a major cause of pediatric morbidity and mortality. No clinical features clearly differentiate malaria from other causes of pediatric febrile illness. Lack of laboratory equipment and expertise makes malaria diagnostics a challenge, leading to overdiagnosis and overtreatment of malaria. Molecular methods (PCR) are emerging but have so far not proven practical for routine clinical use. Emerging antimalarial and antibiotic resistance calls for precise diagnostics and treatment of febrile illness, and has led WHO to recommend laboratory confirmation of malaria in children before starting treatment.

We acknowledge the Medical Research Council for funding of this work.

References
Methods Children admitted with fever were recruited consecutively among admissions at the general pediatric wards at Muhimbili National Hospital (MNH) in Dar es Salaam Tanzania from January-June 2009. Clinical, demographic and laboratory features were registered. Microscopy of thick blood smears was done as part of the routine at MNH, and thin blood smears were stained and examined later. Retrospectively, genus-specific PCR of Plasmodium mitochondrial DNA was performed on DNA extracted from whole blood for all patients and species-specific PCR was done on samples positive by genus-specific PCR. Univariate and multivariate statistical analysis was performed using IBM SPSS Statistics version 19 (SPSS Inc, IBM Company).

Results The study included 304 children. Within four weeks before admission 62.6% had received antimalarials. Forty children had positive routine thick blood smears upon admission and twenty had positive research thin blood smears upon retrospective examination. Twenty-five percent had positive PCR, all positive for P. falciparum. PCR results confirmed positive routine microscopy in only 52.5% and research microscopy in 100%. Almost every fifth febrile child (55/304) had positive PCR but negative research microscopy. High parasitemia on routine microscopy was associated with positive research microscopy and positive PCR. The true prevalence of malaria in the population remains unknown as none of the diagnostic methods can be interpreted as a true gold standard. Palmar pallor, low hemoglobin and low platelet count were significantly associated with both positive PCR and positive research microscopy (p<0.001). In hospital, 65.1% received antimalarial treatment. Clinically determined severity of palmar pallor was clearly associated with hemoglobin level.

Conclusions The study identified discrepancies between routine malaria microscopy, research malaria microscopy and PCR. Almost half of routine microscopy positive cases were negative on PCR, indicating prevalent overdiagnosis of malaria. PCR was positive for many research microscopy negative cases. This may in part be due to prevalent treatment with antimalarials before admission. Palmar pallor and low hemoglobin levels were predictors for malaria in this study. The current routine diagnostic method for malaria appears to lead to overdiagnosis of malaria and, consequently, overseuse of antimalarials. Conversely, children with false positive malaria diagnosis may die because they do not receive treatment for the true cause of their illness. Malaria is still a prominent health issue in Tanzania, and the uncertainty of both clinical and routine laboratory tests may lead to misuse of antimalarials and antibiotics and consequently contribute to emerging drug resistance. Diagnostic algorithms employing new methods such as rapid diagnostic tests (RDTs) may contribute to improving malaria diagnosis and treatment.

Table 1 (abstract P93). The estimation of longevity of An. sundaicus in Nongsa Pantai Village, Batam City, Riau Islands Province during July-October

<table>
<thead>
<tr>
<th>Observation (month)</th>
<th>Porous rate</th>
<th>Gonothropic cycle (days)</th>
<th>p**</th>
<th>Longevity (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>July</td>
<td>0.66</td>
<td>3</td>
<td>0.8707</td>
<td>7.22</td>
</tr>
<tr>
<td>August</td>
<td>0.70</td>
<td>3</td>
<td>0.8849</td>
<td>8.41</td>
</tr>
<tr>
<td>September</td>
<td>0.71</td>
<td>3</td>
<td>0.8921</td>
<td>8.76</td>
</tr>
<tr>
<td>October</td>
<td>0.72</td>
<td>3</td>
<td>0.8962</td>
<td>9.12</td>
</tr>
<tr>
<td>Mean</td>
<td>0.695</td>
<td>3</td>
<td>0.8857</td>
<td>8.39</td>
</tr>
</tbody>
</table>

*The average of PR in July and September is 0.70%; **p = daily survival rate; equvalen with square root of proportion of female gravid.

P93

The longevity of Anopheles sundaicus in a small area: Nongsapantai Village, Batam City, Indonesia

Dewi Susanna, Tris Eryanmd

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Malaria Journal 2012, 11(Suppl 1):P93

Background Mosquito lifespan is one component of the lifetime transmission potential of an individual mosquito. The length of life or lifespan (longevity) of an adult Anopheles may affect its power of transmitting malaria [1]. This study was to analyze the longevity of Anopheles sundaicus in small area, Nongsapati Village, Batam City, Indonesia.

Method Research used time trend design which investigated within 3-4 months (July-October) in Nongsapantai Sub-villages, Nongsapantai Village, Batam City, Indonesia. The estimation of An. sundaicus’s longevity used a formula based on the Parous Rate and its gonothropic cycle, that is the duration of time the mosquito matures its eggs [2]. The gonothropic cycle of An. sundaicus is 3 days.

Results The life span (longevity) of An. sundaicus in Nongsapantai Village ranged from 7.22 to 9.39 with 8.39 in average as shown in Table 1.

P94

Imported malaria cases in Sukabumi District-West Java Indonesia, in 2012

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Background Sukabumi District is located in the southern area of West Java province. In 2004, a malaria outbreak occurred, 785 cases were reported and 8 of them died. During the last 3 years, the incidence of malaria has been constantly high. In 2009, 290 cases were reported while in 2010 and 2011, there were 316 and 273 cases reported. The malaria cases occurred in 14 sub districts out of 47 sub districts in
Sukabumi District area.[1] The malaria endemic in Sukabumi District indicates a very low impact of the malaria elimination program in the district. Therefore it is a necessity to identify the characteristics and transmission of malaria in Sukabumi District as basis for the future malaria elimination program.

Materials and methods The research was conducted in 4 sub district out of 14 malaria endemic sub district in Sukabumi District West Java. Using a cross-sectional study, interviews were carried out for all malaria incidences that were reported during the period of January 2011- April 2012 from Health Centers in 4 sub-districts, consists of 17 villages with stratification of MC1 to HCI with API 1–<5%. The total respondents were 204 people, which were visited at home.

Results The malaria cases in 4 subdistricts in Sukabumi were mostly import cases (71%) not indigenous cases. The respondents were mostly infected from the areas outside of Java Island. They were sent back home when they were found to be ill and they got treated in the Health Centre located in their homeland. The majority of the cases were people who worked in Sumatra Island (88.3%), Sulawesi (5.5%), Nusa Tenggara (3.4%) and Papua (2.8%). Most of the respondents were male (95%), in the productive age or 15-54 years old (93%). They worked in the mining sector, mostly working in night shifts (69%). After recovering from malaria, around 64.2% of the respondents return to their previous work location, where they got malaria. The types of plasmodium found in the study area were Plasmodium vivax (88.2%), P. falciparum (7.4%) and Mix (4.4%). As much of 80.9% of the respondents received an ACT (Artemisinin Combination Therapy), due to the resistance of chloroquin and SP (sulfadoxin-Primethamin), which follows the rules of Ministry of Health for malaria elimination programme.[2] Due to the strategy of malaria eradication strategy [3-4] to achieve low transmission and substantial reductions in mortality and morbidity from malaria,[5] it is necessary for the people who travel to malaria-endemic areas and eventually settle in those areas to take anti-malaria drugs,[6] and avoid mosquito bites at night by using mosquito nets and or repellent.

Conclusion The most malaria cases in Sukabumi were imported malaria cases from outside of Sukabumi. They were in productive age male migrants who worked in mining sectors outside Java Island. It is necessary to educate, control environment, empower the community and to coordinate multi-sector in preventing malaria, by not letting the malaria cases transferred to another area or their homeland but to be treated in the endemic area.

References
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P95
Improving LLIN utilization and coverage through an innovative distribution and malaria education model: a pilot study in Okavango Sub-District, Botswana

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Background The Botswana Malaria Indicator Survey in 2007 indicated remarkably low ITN coverage and usage rates of 9.4% and 5.3%, respectively. To achieve universal coverage of LLINs, optimize uptake, and catalyze Botswana toward its 2015 goal of elimination, Botswana tested a new delivery model to replace its revolving fund scheme of selling subsidized nets to communities at risk.

Materials and methods Okavango Sub-District experienced the highest malaria burden in the country (36% of total burden) and was thus chosen as the pilot site. After comprehensive training, sub-district health staff distributed 32,000 LLINs free of charge door-to-door in 2009 and an additional 6,500 LLINs in 2010 and offered hanging assistance with kits of hooks and strings to households. All nets distributed were mapped by household. Both distributions were supported by health education campaigns, including household visits, roadshows, and calendar posters. Two surveys in 2009 and 2010 evaluated pilot outcomes using a structured questionnaire to interview 557 and 362 randomly selected households in Okavango. Findings from the pilot study informed scale up of LLINs throughout Botswana.

Results The pilot successfully increased LLIN ownership in Okavango from 13% of households owning at least one ITN in 2007 to 89% of households owning at least one LLIN in 2009 and 94% in 2010. LLIN usage also increased markedly from 5.3% of residents sleeping under an ITN in 2007 to 38% of residents sleeping under an LLIN in 2009 and 46% in 2010. In 2009, 73% of LLINs were immediately hung with the assistance of distributors, and the probability of using an LLIN was 13% higher if households were assisted in hanging the LLINs. Households with a visible poster were 26% more likely to use an LLIN, and subsequent health education visits were significantly associated with higher usage (p=0.0005) – after no visits, 64% of nets were used while after three visits, 83% of nets were used. The majority of respondents received their malaria messages from LLIN distributors, health talks at clinics, and visits by health educators, indicating the critical role of door-to-door visits in increasing awareness about malaria and LLINs.

Conclusions An intra-household analysis found that smaller households generally owned enough LLINs to cover all household members but larger households did not receive enough nets, indicating unequal distribution of LLINs. Moreover, in households with 100% coverage, 67% of residents used an LLIN in 2010 – a substantially higher rate than the district-wide rate of 46% that does not take into account whether households actually owned enough nets to use them.

Clinical malaria cases declined from 6,446 in 2008 to 22 in 2011 and confirmed cases declined from 183 to 15. Alongside ongoing IRS, more robust implementation of parasitological diagnosis, and other interventions, the pilot was associated with a substantial decline in malaria cases in Okavango.

Acknowledgements We are grateful to Malaria No More US, Malaria No More UK, and UNICEF for funding this pilot project.

P96
Toward malaria elimination in Botswana: a pilot study to improve malaria diagnosis and surveillance using mobile technology

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Background identifying every suspected malaria case, correctly diagnosing those cases, and reporting cases accurately in a timely matter are critical in Botswana's move toward malaria elimination. Based on current evidence on the use of mobile phones for reporting disease incidence from health facilities in several African countries for HIV/AIDS, TB, and malaria[1,2,3], mobile phone-based malaria case reporting in Botswana was piloted to understand the true incidence of malaria and markedly improve surveillance and response for elimination.
Materials and methods The Botswana Ministry of Health conducted a pilot study in 14 health facilities in malaria-endemic Chobe District to test a Malaria Early Epidemic Detection System using GPS-enabled smartphones with customized menus to monitor, map, and trigger response to malaria cases. Nearly all health workers from the district were trained centrally and onsite to effectively build capacity on the phone-based reporting system, malaria case management, and active case detection. Activities were reinforced by regular visits by supervisors to each health facility over 15 months.

Health workers diagnosed patients using RDTs and collected blood smears and dried blood spots for malaria PCR for all RDT-positive cases. Data was entered in the smartphones per confirmed case (immediate case-based notification), weekly (aggregate data), and per case investigation. The system then sent data in real-time to a secure website developed for the pilot with automated graphical and tabular analyses. GPS coordinates were also collected from households during case investigation that were then projected onto maps on the website. Simultaneously, the system sent a weekly email to district and national officials with aggregated data for monitoring, as well as an SMS immediately to the same officials when a single case was notified through the smartphones.

Results The pilot clearly demonstrated the importance of parasitological diagnosis to reveal the true burden of malaria in a district that was assumed to have endemic malaria – the number of clinical cases reported in Chobe declined from 2,092 cases in 2010 to 164 cases in 2011 and 22 cases in 2012 through the end of the pilot in June. The proportion of suspected cases tested markedly increased from an estimated 11% to 98% during the pilot project. Just over 97% percent of all those tested were found negative, yielding a positivity rate of 2.6%. All positive cases were notified through mobile phones within 48 hours of diagnosis. An average of 77% of health facilities sent reports on key indicators through the phones each week against a set target of 80%. Select positive malaria cases were successfully mapped by residence, investigated, and monitored for 28 days to field test the new active case-based surveillance tools and system.

Conclusion The pilot significantly improved the accuracy, timeliness and geographic pinpointing of confirmed malaria cases, critical in an elimination programme to identify and clear transmission foci. Onsite training of health workers in case management and smartphone technology greatly improved diagnostic practices and response to positive cases. Combined, these activities will substantially increase Botswana’s ability to target and treat remaining cases, prevent onward transmission, and advance the country toward its elimination goal.

Acknowledgements We are grateful to Malaria No More UK, Malaria No More US, and the Global Health Group for funding the pilot project. We are also grateful to Hewlett-Packard for donating the Palm Pre 2 smartphones and to Mascom for contributing SIM cards and data transfer.

References
Results PvTTRAgs binds to the human erythrocytes in a concentration dependent manner and not to the human lymphocytes. The binding was specific as competition with untagged proteins inhibits the erythrocyte binding to 50% at equimolar concentration. Antibodies raised against rabbits and produced by infected patients inhibit the binding of respective PvTTRAgs at different dilutions. PvTTRAgs and PvATRAgs74 was sensitive to chymotrypsin only and not to the trypsin and neuraminidase while all other PvTTRAgs were resistant to all of these proteases.

Conclusions Six out of fifteen PvTTRAgs bind specifically to the human erythrocytes and were inhibited by rabbit anti PvTTRAgs and also by the antibodies produced during natural course of P. vivax infection. There may be more than one RBC receptor for these six PvTTRAgs where only PvTTRAgs38 and PvATRAgs74 are sensitive to chymoprysin while others using those molecules which are other than glycophorin and sialoglycoproteins. Studies are in progress to investigate the respective RBC receptors for these PvTTRAgs and their role in erythrocyte invasion process.

Acknowledgements Department of Biotechnology, Government of India for financial support. Dr. Chetan Chitnis for providing DBP region II (PvRII) and Dr. S. S. Chauhan for his help in critical evaluation of the data.

References
P101
Findings of the literature review on mobility, infectious diseases and malaria
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Background Imported malaria is no longer a challenge only for malaria free countries, for countries implementing in malaria elimination strategies and seeking to address cross-border transmission. Mobility is frequently mentioned as a risk factor and a barrier to elimination by malaria researchers and policy makers. However, only a small body of research has engaged in a detailed analysis of the links between mobility and malaria transmission, and attempts to incorporate these findings into policy are rarer still. This paper presents the findings of a literature review on malaria and human mobility supported by the Asia Pacific Malaria Elimination Network (APMEN). It attempts to shift the agenda from identifying human mobility as a risk factor, to finding strategies to work collaboratively with mobile populations towards the goal of malaria elimination.

Materials and methods The objectives of the literature review were to highlight key lessons in the ways in which the published malaria literature discusses human mobility identify lessons to be learned from the ways that other infectious disease control programmes such as HIV/AIDS and polio have addressed human mobility identify potential ways forward, so that it becomes possible to address human mobility within malaria elimination initiatives. The review focused upon published, peer-reviewed literature on malaria and mobility sourced through PubMed and ProQuest, and grey literature sourced through Google Scholar.

Results The paper will present a brief taxonomy of mobility, since there are many different form of behaviour that are often grouped together as ‘mobility’. It then discusses the three key themes that most frequently recur within the published malaria literature: namely: mobility, land use and economic change; borders; and accessing mobile populations. The paper then discusses the ways in which other infectious disease control programmes such as HIV/AIDS and polio have addressed human mobility, and to identify the key lessons to be learned from these programmes.

Conclusions Recommendations, methodologies, and areas that APMEN partners and other organisations may consider for future work, in order to move towards a more productive engagement with mobile populations.

P102
Findings of the literature review on larviciding in elimination environments in Asia Pacific
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Background The Vector Control Working Group of Asia Pacific Malaria Elimination Network posed the question “Do we know enough about the use of larviciding as a vector control method in elimination environments to provide evidence to APMEN Country Partners?”. This paper summarises our approach to addressing these question and the findings from this review.

Materials and methods From October 2011 – March 2012, a web based search using the key words: vector control, elimination, malaria, guidelines, standard operating procedures, larviciding, vector management, biological control, was conducted using Google Scholar, PubMed and Scopus. In addition, grey literature was sought through the World Health Organization (WHO) library. A database of literature collected by a research group undertaking a Cochrane Systematic review of vector control was shared with the group. Articles were sought in any language, with abstracts of materials in languages other than English translated by colleagues and members of the vector working group fluent in the required languages, to see if it fulfilled the criteria for inclusion. The date range used for the search was from 1955 – 2012, in order to allow earlier references and manuals regarding larviciding and the use of vector control in the eradication period to be included in the review. In total, 347 articles, books and manuals (12) were reviewed of which 117 met the inclusion criteria.

Results There is a large body of literature on a range of larvicides and their suitability for a range of environmental and vectoral contexts that occur in the Asia Pacific region. Very few have been explicitly tested or referred to as suitable in elimination settings nor in many of the Asia Pacific regional countries. Some of these articles, books and guidelines provide useful operational data on the use of larvicides, their safe handling and storage, and other operational details. Only a few discussed monitoring and evaluation aspects of the use of larvicides in programmes. None of the literature reviewed discussed detailed costs, compared cost effectiveness or made cost comparisons between different larvicides and/or between different vector control methods.

Conclusions The recent Interim position paper on larviciding in Sub-Saharan Africa noted that “in general larviciding should be considered for malaria control (with or without other interventions) only in areas where the breeding sites are few, fixed and findable”. Although in SSA many of the larval breeding sites were noted not fulfil these three basic criteria for success, in the APMEN region there are some vectoral species that do have these characteristics and are knowledge vector targets for larval source management. Challenges identified are the lack of published literature operational aspects of larval control/environmental management, although larvicides may not have been considered cost effective in control environments, when moving towards elimination, these remaining larval sources of primary and sometimes the secondary incriminated vectors becomes the “last push”. Without firm evidence it will be hard to convince policy makers and funders to invest in larval source reduction as an elimination strategy.

Acknowledgements This work has been an output of the Vector Control Working Group of APMEN, and all members of that group have contributed to the information provided in the note and its analysis and review. The contributions of all those involved is appreciated. Special acknowledgment is made of the library database shared with the APMEN and which was developed to undertake a Cochrane Review of vector control. This library was compiled by Julie Thwing and Lucy Tusting, with the support of the Centers for Disease Control and Prevention Library Services, the London School of Hygiene and Tropical Medicine Library Services, the Armed Forces Pest Management Board, the Interim position paper April Geneva; WHO page 3

References
1. WHO 2012 The role of larviciding for malaria control in sub-Saharan Africa

P103
Findings of the literature review on larviciding in elimination environments in Asia Pacific
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Malaria Journal 2012, 11(Suppl 1):P103

Background The Vector Control Working Group of Asia Pacific Malaria Elimination Network has been working with countries to identify vector control capacities, needs and activities to assist the sharing of knowledge between partner countries.

Materials and methods A comprehensive literature review and a survey of malaria vector control was conducted in early 2011 amongst the then eleven APMEN countries, of these eight countries responded. The survey was conducted through a questionnaire sent via email to all APMEN Country Partner representatives at that time. The literature review addressed the question: What vector control tools are APMEN countries using; how are these tools selected and how are they being used in the context of malaria elimination? What else is needed to eliminate the remaining low-levels of transmission? Is there any data to indicate what methods are working or not working? What are the challenges (technical and operational) facing the use of vector control interventions for elimination available in Asia-Pacific region?
Integrated approaches to malaria control – addressing new challenges to malaria research

**P104**

**Title:** Integrated approaches to malaria control – addressing new challenges to malaria research

**Authors:** Martin Wiese

**Affiliations:** International Development Research Center, IDRC, Ottawa, Ontario, Canada, K1G 3H9

**Malaria Journal 2012, 11(Suppl 1):P104**

While significant progress in understanding and controlling malaria has been made, extensive regions of sub-Saharan Africa remain hotspots of transmission, burden and impacts from malaria [1]. The pathogenicity of falciparum malaria and the high vectorial capacities of Anopheles gambiae vector systems compound in Africa with poor and unsecured livelihoods, weak health and disease surveillance systems, and agro-ecological transformations on an almost continental scale [2]. Moreover, the patterns of risks and vulnerabilities to malaria remain dynamic under global and climate changes.

Today’s efforts against malaria rely on innovation of tools and funding mechanisms, and the progressive scaling up of intervention packages especially targeting the elimination of malaria at its current transmission limits to ‘shrink the malaria map’ [3]. However, delivery modalities for intervention packages are challenged by poor, remote and unstable conditions and weak health systems. Thus, new approaches are needed for developing, delivering and maintaining malaria control in areas where high or unstable disease transmission compounded with systemic vulnerabilities cause the world’s most significant malaria burden [4-6].

Synergy harvesting should open new options to tackle the root causes of malaria risks and vulnerabilities linked to health systems, livelihoods, and ecosystems conditions. Such integrated research will require collaboration of researchers across sectors and disciplines. However, we currently lack evidence on the added values of integrated malaria research and control from combined investments into health systems strengthening, livelihoods improvements and, for example, agro-ecosystems interventions.

The rationale for a new initiative dedicated to develop integrated research partnerships on malaria in Africa stems from the need for a consolidated evidence base, enhanced capacities, and stronger advocacy for outcome oriented malaria research that transcends disciplinary and sectoral boundaries [7].

Integrated research requires systems thinking, outcome-oriented monitoring and evaluation approaches, collaboration - across disciplines, sectors and regions -, multi-stakeholder engagement, and sensitivity to social equity. The complexity of these challenges currently outpaces the existing capacities of research teams, especially in regions most affected by malaria. These same regions also have commonly weak research capacities and compartmented institutional landscapes. With this in mind and based on four decades of investments into applied malaria research in developing regions across the globe, Canada’s International Research Development Centre, IDRC, is currently supporting the formation and consolidation of multi-national, multi-disciplinary research consortia in high-transmission and high-burden countries of Sub-Saharan Africa. Since 2011, 3 multi-country consortia, selected through an open competitive process, mobilize early-career researchers from research institutions of 10 countries in this region (Benin, Burkina Faso, Cameroon, Kenya, Mali, Niger, Rwanda, Tanzania, Togo, Uganda). Teams develop research, build collaboration and alliances, advocate and leverage support for integrated malaria research and control. They seek to identify and assess synergy opportunities for malaria control from investments into environmental, livelihoods and health systems improvements.

**Acknowledgements**

The abstract is being presented on behalf of the Ecosystems and Human Health and the Governance for Equity in Health Systems Program teams at the International Development Research Centre, Canada, which funds the Integrated Research Partnerships for Malaria Control in Africa (IPMA) initiative.

**References**


**P105**

**Title:** Concomitant Plasmodium falciparum and intestinal helminth infections in a rural community of southern Côte d’Ivoire

**Authors:** Richard B Yap1,2, Eveline Hufurmans3,4, Kigbafori D Silué1,2, Clarisse A Houngbedji1,5, Chammartin Frédérique3,4, Martin Wiese6,7, Jürg Utzinger3,4, Eliezer K N’Goran1,2, Eveline Hürlimann3,4,

**Affiliations:** 1Departement Environnement et Santé, Centre Suisse de Recherches Scientifiques, Abidjan, Côte d’Ivoire; 2BP 1030 Abidjan 01; 3Unité de Formation et de Recherches en Biosciences, Université de Cocody, Abidjan, Côte d’Ivoire; 22 PB 582 Abidjan 22; 4Department of Epidemiology and Public Health, SwissTropical and Public Health Institute, Basel, PO 4002 Basel, Switzerland; 5Unité de Formation et de Recherche en Biologie de la reproduction Animale, Université d’Abobo-Adjamé, Abidjan, Côte d’Ivoire; 01 BP 801 Abidjan 01 Malaria Journal 2012, 11(Suppl 1):P105

**Background**

Despite efforts to control the disease, malaria is still threatening the life of millions of people in sub-Saharan Africa [1]. In addition the distribution of malaria often overlaps in space with so called neglected tropical diseases (NTDs). People in endemic areas can therefore host more than one parasite species infection at the same time, hence making polyparasitism a common phenomenon [2-4]. The consequences of these diseases are manifold and can include impairment of cognitive development and anemia, school aged-children and pregnant women representing the most vulnerable groups with particular risk of morbidity [4-6]. In Côte d’Ivoire, these
diseases are widely prevalent but vary in their spatial distribution and present different patterns of associations. Risk factors such as distance to water bodies and socio-economic status have been identified among the underlying causes for this heterogeneity [7,8]. As a result of this heterogenous occurrence of multi-parasite infections related morbidity and burden due to polyparasitism will vary as well between areas. For control activities decision making usually takes place at global and/or national level but for integrated, cost-effective and sustainable control efforts better understanding of co-infection dynamics at different spatial scales are urgently needed. The main goal of this study was therefore to describe the pattern of concomitant infections with *Plasmodium* and intestinal helminths in a rural setting in southern Côte d’Ivoire.

**Method**

A cross-sectional study was conducted in a hamlet of Azaguié, named Ancien Carrefour, located 40 km from Abidjan, in September 2011. Blood and faecal specimens were collected to identify *Plasmodium spp, Schistosoma mansoni,* soil-transmitted helminths (hookworm, *Ascaris lumbricoides*, *Trichuris trichiura*), and intestinal protozoan infections by microscopy using standardized quality-controlled procedures. The study involved 413 persons from 85 households. Data analysis was done using logistic and multinomial regression models taking into account household effects.

**Results**

*Plasmodium falciparum* overall prevalence was 60.53% (Table 1), which means a parasitemic index (PI) of 57.55% that characterises a hyper endemic malaria area. Predominant NTD parasites were *Schistosoma mansoni* (27.36%) and hookworm (31.23%) (Table 1).

<table>
<thead>
<tr>
<th>Parasite</th>
<th>People infected (%)</th>
<th>Independent variable</th>
<th>People infected (%)</th>
<th>OR (95%CI)α</th>
<th>P -value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. falciparum</em></td>
<td>250 (60.53)</td>
<td>Age (years)</td>
<td>0-5</td>
<td>57 (68.67)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5-16</td>
<td>96 (82.05)</td>
<td>2.08(1.06;4.08)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&gt;16</td>
<td>97 (45.54)</td>
<td>0.38(0.23;0.63)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sex</td>
<td>Female</td>
<td>129 (60.28)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Male</td>
<td>121 (60.80)</td>
<td>1.03(0.68;1.58)</td>
</tr>
<tr>
<td><em>S. mansoni</em></td>
<td>113 (27.36)</td>
<td>Age (years)</td>
<td>0-5</td>
<td>1 (1.20)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5-16</td>
<td>28 (23.93)</td>
<td>25.80(3.08;173.73)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&gt;16</td>
<td>84 (39.44)</td>
<td>53.81(7.21;401.50)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sex</td>
<td>Female</td>
<td>28 (22.43)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Male</td>
<td>65 (32.66)</td>
<td>1.69(1.10;2.60)</td>
</tr>
<tr>
<td>Hookworm</td>
<td>129 (31.23)</td>
<td>Age (years)</td>
<td>0-5</td>
<td>4 (4.82)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5-16</td>
<td>39 (33.33)</td>
<td>9.88(3.50;27.80)</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>&gt;16</td>
<td>86 (40.38)</td>
<td>13.21(4.03;5.40)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sex</td>
<td>Female</td>
<td>54 (25.23)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Male</td>
<td>75 (37.69)</td>
<td>1.77(1.23;2.54)</td>
</tr>
</tbody>
</table>

**Table 2 (abstract P105). Multinomial logistic regression for *P. falciparum* and *S. mansoni* mono and co-infection with age and sex as independent variables, accounting for household effects**

<table>
<thead>
<tr>
<th>Infection</th>
<th>Positive for infection (%)</th>
<th>Sex</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. falciparum</em> mono-infection**</td>
<td>140 (33.90)</td>
<td>1.20(0.71;2.02)</td>
<td>0.495</td>
</tr>
<tr>
<td><em>S. mansoni</em> mono-infection**</td>
<td>20 (4.84)</td>
<td>2.31(1.21;4.40)</td>
<td>0.011</td>
</tr>
<tr>
<td><em>P. falciparum-S. mansoni</em> co-infection</td>
<td>35 (8.47)</td>
<td>1.77(0.91;3.42)</td>
<td>0.092</td>
</tr>
</tbody>
</table>

**Table 3 (abstract P105). Multinomial logistic regression for *P. falciparum* and Hookworm mono and co-infection with age and sex as independent variables, accounting for household effects**

<table>
<thead>
<tr>
<th>Infection</th>
<th>Positive for infection (%)</th>
<th>Sex</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. falciparum</em> mono-infection**</td>
<td>140 (33.90)</td>
<td>1.16(0.68;1.98)</td>
<td>0.581</td>
</tr>
<tr>
<td>Hookworm mono-infection**</td>
<td>27 (6.54)</td>
<td>2.11(1.09;4.05)</td>
<td>0.026</td>
</tr>
<tr>
<td><em>P. falciparum-Hookworm</em> co-infection</td>
<td>44 (10.65)</td>
<td>1.77(1.01;3.09)</td>
<td>0.045</td>
</tr>
</tbody>
</table>

α Odds Ratio

**Table 1 (abstract P105). Logistic regression for single parasite species infections regardless of any other parasite species infection, accounting for household effects**

<table>
<thead>
<tr>
<th>Covariates</th>
<th>Sex</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. falciparum</em> mono-infection**</td>
<td>1.20(0.71;2.02)</td>
<td>0.96(0.95;0.98)</td>
</tr>
<tr>
<td><em>S. mansoni</em> mono-infection**</td>
<td>2.31(1.21;4.40)</td>
<td>1.01(1.00;1.03)</td>
</tr>
<tr>
<td><em>P. falciparum-S. mansoni</em> co-infection</td>
<td>1.77(0.91;3.42)</td>
<td>0.99(0.98;1.01)</td>
</tr>
</tbody>
</table>

**Table 2 (abstract P105). Multinomial logistic regression for *P. falciparum* and Hookworm mono and co-infection with age and sex as independent variables, accounting for household effects**

<table>
<thead>
<tr>
<th>Covariates</th>
<th>Sex</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. falciparum</em> mono-infection**</td>
<td>1.16(0.68;1.98)</td>
<td>0.97(0.95;0.99)</td>
</tr>
<tr>
<td>Hookworm mono-infection**</td>
<td>2.11(1.09;4.05)</td>
<td>1.02(1.01;1.04)</td>
</tr>
<tr>
<td><em>P. falciparum-Hookworm</em> co-infection</td>
<td>1.77(1.01;3.09)</td>
<td>0.99(0.98;1.01)</td>
</tr>
</tbody>
</table>

α Odds Ratio

**Table 2. People infected only with this parasite**

**Table 3. People infected only with this parasite**

"Relative risk ratio"
These parasites overlapped with *P. falciparum*. The co-infection prevalences of *P. falciparum-S. mansoni* and *P. falciparum-hookworm* were 15.98% and 18.16%, respectively. Participants older than 5 years are at higher risk of co-infection compared to their younger. Multinomial analysis of co-infection of *P. falciparum-S. mansoni* (Table 2) reflected no significant association of age and sex to the co-infection risk. However, age was negatively related to the *P. falciparum* mono-infection risk, while female and age were negatively associated to the *S. mansoni* mono-infection risk. Multinomial analysis of co-infection of *P. falciparum-hookworm* (Table 3) showed that female were positively associated to the co-infection risk, while female and age presented a positive association to the hookworm mono-infection risk and age presented a negative association to *P. falciparum* mono-infection risk. 

**Conclusion** This study confirms that polyparasitism is common in rural settings. However, implications of polyparasitism on morbidity and quality of life are not well understood. Further research should focus on understanding co-infection dynamics on the purpose of designing and implementing a sustainable integrated control strategy.

**Acknowledgments** The study received financial support from Swiss National Foundation (Project No 32003B 132949/1).

**References**

P107
Therapeutic efficacy of artemether-lumefantrine combination in the treatment of uncomplicated malaria among children under 5 years in 3 ecological zones in Ghana
Kwadwo Koram
Epidemiology Department, Noguchi Memorial Institute for Medical Research, College of Health Sciences, University of Ghana, P.O. Box LG581, Legon, Ghana

Background
In 2008 artemether - lumefantrine and dihydroartemisinin-piperaquine were added to amodiaquine - artesunate as first line drugs for uncomplicated malaria in Ghana. The introduction of new drugs calls for continuous monitoring of these drugs to provide timely information on trends of their efficacy and safety to enhance timely evidence-based decision making by the National Malaria Control Programme. In this regard, we monitored the therapeutic efficacy of artemether - lumefantrine from September 2010 to April 2011 in 4 sentinel sites representing the 3 main ecological zones of the country.

Materials and methods
The study population involved all children aged between 6 and 59 months presenting at the Out-Patient Department (OPD) of a study site clinic with symptoms suggestive of malaria. Using the 2009 WHO protocol for surveillance of antimalarial drug efficacy, primary outcomes for the study were treatment outcomes on Day 14 and Day 28 for the different ecological zones whilst secondary outcomes were patterns of fever and parasite clearance as well as gametocyte carriage and haematological responses. The Institutional Review Board of the Noguchi Memorial Institute for Medical Research, University of Ghana, reviewed and approved the study.

Results
Per-protocol analysis showed that the overall PCR-corrected cure rates on day 14 and day 28 were 96.5% (95% CI: 92.1, 98.6) and 95.4% (95% CI: 90.3, 98.0), respectively, with statistically significant differences between the ecological zones. The 90.4% day-28 cure rate observed in the savannah zone (95% CI: 78.2, 96.4) was significantly the lowest compared with 100% (95% CI: 93.2, 99.9) in the forest zone and 93.8% (95% CI: 77.8, 98.9) in the coastal zone (P=0.017). Fever and parasite clearance were slower among children enrolled in the savannah zone. The proportion of children still febrile on day 1 post-treatment was significantly highest in the savannah zone (42.9%; 95% CI: 30.0, 56.7) compared with the forest zone (16.7%; 95% CI: 9.5, 27.2) and the coastal zone (10.5%; 95% CI: 3.4, 25.7) (P=0.000). Additionally, 14.5% (95% CI: 6.9, 27.2) of the children enrolled in the savannah zone were parasitaemic on day 2 post-treatment whilst no child was parasitaemic on the same day in the forest and coastal zones. Gametocytaemia after day 3 post-treatment was rare in all the zones. Mean haemoglobin concentration significantly increased only in the forest zone from 10.1g/dl (95% CI: 9.6, 10.5) on day 0 to 11.0g/dl (95% CI: 10.6, 11.4) on day 28 (p=0.004).

Conclusions
We conclude that AL remains efficacious in Ghana with significant ecologic zonal differences. The savannah zone may be a potential zone for any emergence of resistant alleles as a result of the slower parasite clearance observed in the zone.

Acknowledgements
We acknowledge the contributions of Dr Constance Bart Plange (National Malaria Control Programme Manager), Dr Felicia Owusu-Antwi (WHO, Ghana), Dr Jackson Silah (WHO-AFRO), Dr Marian Warsame (WHO, Geneva), and study teams. The study received financial support from the Global Fund to fight AIDS, Tuberculosis and Malaria (GFATM) and the US President's Malaria Initiative (PMI).

P108
Intermittent preventive treatment for children (IPTC) combined with timely home treatment for malaria control
Collins K Abariga, Kwadwo A Koram
Department of Epidemiology, Noguchi Memorial Institute for Medical Research, University of Ghana, Box LG581, Legon, Ghana

Background
Malaria is estimated to cause between 300 and 500 million clinical cases with about 700,000 to 1.6 million deaths every year, most of these deaths occur in sub-Sahara Africa. In Ghana malaria accounts for about 32.5% of all Out Patient attendances and about 24.6% of deaths in children under 5 years. The World Health Organization recommended the use of artemisinin based combination therapy (ACT) for the treatment of uncomplicated malaria to provide effective treatment against Plasmodium falciparum and slow down the spread of drug resistance. Ghana had adopted the use of Artesunate + Amodiaquine for the treatment of uncomplicated malaria in the country since 2005. Malaria control in Ghana, like elsewhere in sub-Sahara Africa, relies on early diagnosis and prompt treatment of suspected cases and the home is where early recognition and in most cases prompt treatment is initiated. However, the current combination therapy is not widely available for home management as a result of the fear that making these drugs available may lead to abuse and therefore lead to the emergence of Plasmodium falciparum resistance to these drugs. Intermittent preventive treatment (IPT) has now been accepted as an important component of the malaria control strategy but has not been implemented in combination with timely home management to measure their impact on malaria prevalence in a target population.

Methods
This paper reports finding from a two year implementation of a combined intermittent preventive treatment for children (IPTC) and timely home management of malaria using Artesunate + Amodiaquine. All children aged six to 60 months received home-based delivery of intermittent preventive treatment using Amodiaquine + Artesunate, delivered at home by community assistants every four months (six times in 24 months). Malaria parasite prevalence surveys were conducted before the first and four months after the third and sixth IPTC rounds to serve as baseline, year-one and year-two evaluations.

Results
Results showed a significant reduction in malaria prevalence from 25% at baseline to 1% at year-two evaluation. At baseline, 13.8% of the children were febrile (axillary temperature of ≥37.5°C) compared to 2.2% at year-one-evaluation while about 2.0% were febrile at year-two-evaluation. The improved access to ACT drugs for the treatment of suspected malaria in children aged six to 60 months could be one of the ways to achieve the Abuja target of getting 60% of under five suspected malaria cases into treatment within 24 hours of symptom onset, which was not achieved by 2005 as was targeted.

Conclusion
IPTC combined with timely treatment at home could be an effective tool for malaria control in sub-Saharan Africa, especially in difficult to reach areas and this must be looked favourably by policy makers if malaria elimination should be a reality in the nearest future. If policy makers may be bold to initiate policy to allow adults to participate in IPT at least once or twice in a year, this could further accelerate the reduction in malaria prevalence to a level that will reduce the public health burden of malaria.

P109
The affordable medicines facility - malaria in Ghana: baseline and endline survey findings
John H Amusa,1-3 Samuel Blay Nguah1, Daniel Ansong2, Graciela Diap1
The Independent Evaluation Team1
1University of Minnesota School of Public Health, Division of Health Policy and Management, Minneapolis, MN 55455, USA; 2Komfo Anokye Teaching Hospital, Kumasi, Ghana; 3Drugs for Neglected Diseases initiative, Geneva, Switzerland

Background
The AMFm Independent Evaluation team: ICF International (Fred Arnold, Yazoume Ye, Ruhl Rien) and London School of Hygiene and
The Affordable Medicines Facility - malaria (AMFm), hosted by the Global Fund to Fight AIDS, Tuberculosis and Malaria, is a financing mechanism which subsidizes quality-assured Artemisinin-based Combination Therapies (ACTs) for distribution to the public and private sectors, complemented by supporting interventions to promote rational drug use. AMFm has been in operation since mid-2010 in eight national-scale operational pilots in Ghana, Kenya, Madagascar, Nigeria, Tanzania mainland, Uganda and Zanzibar. By March 2012, over 220 million co-paid ACT treatment doses had been ordered.

The Independent Evaluation (IE) of AMFm Phase 1 was commissioned by the Global Fund to assess the impact of AMFm on availability, price, market share and use of quality-assured ACTs in all the operational pilots as part of evidence gathering needed to inform decisions regarding the future of the AMFm. The assessment is based on a pre- and post-test design with detailed documentation of the implementation process and context, treating each pilot independently. In each pilot, a nationally representative survey of outlets stocking antimalarial medicines was conducted at the baseline (2009/10) and the endline (2011).

In Ghana, the IE was carried out by a team comprising collaborators from the Komfo Anokye Teaching Hospital, Drugs for Neglected Diseases initiative, ICF International and London School of Hygiene and Tropical Medicine. As part of the IE, national level baseline and endline outlet surveys were conducted in Ghana involving the collection and analysis of primary data to answer three questions related to the availability, affordability and market share of quality-assured ACTs using a cluster sampling approach. In-depth key informant interviews were also conducted to provide the necessary context information to help in the interpretation of the survey results.

In Ghana, 1,241 and 1,093 outlets were enumerated for the baseline and endline outlet surveys respectively. For the baseline survey, 57.3% (CI: 50.5-64) of outlets with any antimalarials in stock at the time of the survey visit carried artemisinin monotherapies, while only 30.7% (CI: 26.1-35.8) of interviewed outlets had quality-assured ACTs in stock at the time of the survey visit. At baseline, the median cost to patients of one adult equivalent treatment dose of quality-assured ACTs was US$ 3.42 (IQR: 2.4-7.53) for 1,092 products. Detailed results on changes in quality-assured ACT availability, price and market share over a 14-month period between baseline and endline surveys in Ghana will be presented.

P110 Feasibility and acceptability of insecticide treated plastic sheeting (ITPS) for vector control in Papua New Guinea

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Malaria Journal 2012, 11(Suppl 1):P110

Background This study assessed the feasibility and acceptability of utilising insecticide treated plastic sheeting (ITPS) as a malaria control intervention in Papua New Guinea (PNG). Method ZeroVector® ITPS was installed in 40 homes across four study sites representing a cross section of malaria transmission risk and housing style. Structured questionnaires were completed at the time of ITPS installation (n=40) and at four weeks post installation (n=40) with the household head. Similarly, focus group discussions (FGDs) with the male and/or female household heads were completed at installation (n=5) and four week follow-up (n=4).

Results ZeroVector® ITPS was successfully installed in a range of homes employing traditional and/or modern building materials in PNG. The ITPS installations remained intact over the course of the four week trial period and were highly acceptable to both male and female household heads. No dissatisfaction with the ITPS product was reported at four week follow-up; however, the installation process was time consuming, participants reported a reduction in mosquito net use following ITPS installation and many participants expressed concern about the longevity of ITPS over the longer term.

Conclusion ZeroVector® ITPS installation is feasible and highly acceptable in a diverse range of PNG contexts and is likely to be favourably received as a vector control intervention if accessible en masse. A longer-term evaluation is required before firm policy or public health decisions can be made regarding the potential application of ITPS in the national malaria control program. The positive study findings suggest a longer-term evaluation of this promising malaria control intervention warrants consideration.

P111 Specialist technician-entomologists adjustment for malaria control in endemic setting of Iran

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Background Effective malaria control programmes prevent malaria transmission by promoting personal protection measures and effective vector control strategies, and providing appropriate case management with early diagnosis and effective treatment. The objective of the study was to understanding of malaria entomologist in relation to current malaria control in their area of activities and technical disciplines.

Methods A qualitative approach was adopted based on a semi-structured interview with 30 individuals working at community-level in malaria endemic areas. Thematic content analysis was used to analysis the data.

Results Although most of participants have been concern with the distribution and causation of problems of malaria control, few of them have addressed the issue of solutions. Participants had positive perceptions on their basic activities and opinions in relation to biologic and epidemiologic factors in their field work. In contrary, their perceptions in relation to malaria control policy and integrated management of vector control were rather negative.

Conclusion There was not feedback mechanism on malaria control activity among practitioners. Therefore, any problems due to the mismatch between the institutional tasks and individual role performance should be feed back into the health system and adjustment should be made. Greater emphasizes should be given to the choice of solutions.

References

P112 Lost in translation? Bridging the preclinical / clinical divide

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Malaria Journal 2012, 11(Suppl 1):P112

Establishing the relevance of preclinical data to the clinical situation is a problem that has plagued drug development in the majority of
therapeutic areas. Typically a drug development cascade relies on preclinical data assuming the in vitro and in vivo data are consistent and predict some elements of clinical efficacy. Quantitative translation from preclinical to clinical is model based and requires PK (pharmacokinetics) and PK/PD (pharmacokinetic/pharmacodynamics) models linking drug exposure with effect.

The first modeling step undertaken for anti-malaria drug development at MMV has been to develop a model capable of linking preclinical in vitro to preclinical in vivo data to:

- Derive efficacy parameters for scaling including additional parameters that put them in context (parasite growth rates and maximum rates of killing)
- Determine the relationship and consistency between the in vitro and preclinical in vivo data.

This approach, whilst using data from a broader range of compounds and mechanisms, does assume that preclinical data is relevant replicating, at least partially, the clinical disease.

The in vitro data characterized the effects of fixed concentrations of compound on the reduction of P. falciparum parasite concentrations and estimated IC50s. The in vivo assessment in SCID mice determined the compound’s dose-dependent reduction of P. falciparum parasite concentrations and estimated an ID90. The in vivo data was modelled using a nonlinear mixed effects PK/PD model in Nonmem and its parameter estimates compared to those from the in vitro data.

The modeling was performed in a step-wise approach where a PK model was fitted to concentration time course data from a single dose in order to simulate the time courses used in the efficacy study (4 doses, 24 hours apart). The PK/PD model was then fitted to the observed parasitemia data using the simulated concentrations. The PD model estimated baseline parasitemia, rate constants for parasite growth and death, and the concentration dependent modulation of parasite death. The model determined that the parasite concentration expanded by approximately 3-4 times every 48 hours (in contrast to approximately 10 times in humans). For the two compounds modeled to date, model-estimated IC50 values from the in vivo data matched the in vitro estimates of IC50. The model, using the estimated parameters was also able to accurately simulate the decline in parasitemia and subsequent recrudescence following a single dose in the SCID P. falciparum model providing some mechanistic validation.

This modeling is at an early phase requiring more data, preclinical and clinical, for validation including evaluating methods for the prediction of clinical PK. The agreement between in vivo and in vitro parameter estimates is encouraging and will be confirmed as Agreement between in vitro and in vivo parameters is encouraging as is the model’s ability to capture parasitemia dynamics. A simple approximation of this model is being evaluated for use in assessing MMV’s preclinical candidates. In addition to confirming the model’s preclinical utility, clinical data is being sought to test whether the model is equally able in predicting drug effects on P. falciparum in humans, confirming the relevance of preclinical data.

Acknowledgements: The Translational Medicine and Discovery departments, MMV, Diseases of the Developing World Biology and DMPK departments, GlaxoSmithKline, Tres Cantos.

**P114**

Abstract not submitted for online publication

**P113**

Fragmented population structure of *Plasmodium falciparum* in Papua New Guinea: Implications for malaria control

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Malaria is being controlled in Papua New Guinea (PNG) where the epidemiology of the disease ranges from highly endemic in low-lying regions to epidemics in the highlands. Analyses of microsatellite haplotypes have revealed that populations of *Plasmodium falciparum* on the north coast of PNG are genetically isolated. If this fragmented population structure is found throughout PNG it will provide a unique opportunity for planning malaria control strategies and focusing efforts on regions where they are likely to have the greatest impact. We are working towards defining a high-resolution population genomic map of parasite networks and migration patterns throughout PNG using single nucleotide polymorphisms. Our approach, preliminary data and the practical implications of this research will be discussed in context with the national malaria control program.
Background Estimating the changing burden of malaria disease remains difficult due to limitations in health reporting systems in those countries with the largest burden of disease. Methods extrapolating from parasite prevalence data are therefore often employed.

Materials and methods We present an approach to estimating disease incidence from prevalence data accounting for the changing age distribution of cases that occurs as transmission declines. We use a transmission model to capture the shifting age-pattern of disease at different transmission intensities through dynamically modelling the acquisition and loss of immunity. The model is fitted to age-stratified data on the incidence of uncomplicated malaria due to Plasmodium falciparum from 24 sites in 9 sub-Saharan African countries. We used nested Bayesian methods, and accounted for variation in treatment rates and reporting methods (active versus passive case detection).

Results We estimate that passive case detection picks up 33% (95% credible interval (Crl): 19-59%) as many cases as daily active detection, and weekly detection 76% as many (95% Crl: 61-88%). However, there was wide variation in incidence between studies that cannot be explained by differences in case-finding or case definitions such as parasitaemia thresholds, and so substantial uncertainty remains in the incidence at any given transmission intensity. We estimate that at a parasite prevalence in 2 to 10 year-olds of 60%, 55% of cases occur in under-fives and 14% in over 15s; at a prevalence of 20%, 21% are in under-fives and 41% are in over 15s; and at a prevalence of 5%, 10% are in under-fives and 60% in over 15s.

Conclusion These estimates allow us to predict the incidence of clinical malaria in any age group, based on an estimate of the parasite prevalence in a possibly different age range. As the results are based on a transmission model, we can also predict the impact of interventions on incidence and its age pattern.

Linking the incidence and age patterns of clinical malaria to parasite prevalence using a mathematical model

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Comparative Immunogenicities of Full-Length Plasmodium falciparum Merozoite Surface Protein 3 and a 24-Kilodalton N-Terminal Fragment

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Background Merozoite surface protein 3, a leading malaria vaccine candidate, is expressed in schizont stage as 48 Kda non GPI anchored protein which remains associated with merozoite surface after egress. Role of MSP3 in parasite growth is not well defined.MSP3 is abundantly expressed on the surface of merozoites and is released as a soluble protein. Recently suggested nomenclature has placed MSP3 in a new MSP3 multigene family and termed it MSP3.1. MSP3.1 has been shown to be the least cross-reactive among the members of the MSP3 family. Affinity-purified MSP3 antibodies from the sera of P. falciparum-exposed individuals and antibodies from mice vaccinated with MSP3 peptides exhibited ADCI activity (15, 21). Aotus monkeys vaccinated with yeast (Pichia pastoris) expressed full-length MSP3 (MSP3F) were partially protected from a challenge with P. falciparum parasites. Antigenicity and functional assays have identified a 70-amino acid conserved domain in the N-terminal region of MSP3 to be a target of biologically active antibodies. Long synthetic peptides based on...
the conserved N-terminal sequences, including the 70-amino-acid sequence, have been developed for vaccine trials in humans. 

Materials and methods Recombinant *Plasmodium falciparum* merozoite surface protein 3 (PfMSP3F) and a 24-kDa fragment from its N-terminal (MSP3N) that includes the essential conserved domain, which elicits the maximum antibody (Ab)-dependent cellular inhibition (ADI), were expressed as soluble proteins in *Escherichia coli*. Both proteins were found to be stable in both soluble and lyophilized forms. Immunization with MSP3F and MSP3N formulated separately with two preservative-free adjuvants, aluminium hydroxide (Alhydrogel) and Montanide ISA 720, produced significant antibody responses in mice and rabbits. Polyclonal Abs against both antigens recognized native MSP3 in the parasite lysate. These two Abs also recognized two synthetic peptides, previously characterized to possess B cell epitopes from the N-terminal region. Antibody depletion assay showed that most of the IgG response is directed toward the N-terminal region of the full protein. Anti-MSP3F and anti-MSP3N rabbit antibodies did not inhibit merozoite invasion or intraerythrocytotic development but significantly reduced parasitemia in the presence of human monocytes. The ADI demonstrated by anti-MSP3N antibodies was comparable to that exhibited by anti-MSP3F antibodies (both generated in rabbit).

Conclusions: Our work in this study has demonstrated that although immunizations with the full-length MSP3 leads to a substantial antibody response to the epitopes present in the N-terminal region, the N-terminal polyepptide fragment itself elicits a strong and effective immune response that makes it a strong vaccine candidate.

**P118**

Differences in the pharmacokinetics of currently approved antimalarial drugs in uncomplicated malaria patients compared to healthy subjects

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Malaria Journal 2012, 11(Suppl 1):P118

For an effective antimalarial therapy, maintenance of antimalarial drug concentrations well above minimum parasitidal concentration for certain time duration is required in the target compartments (eg., blood, liver). On the other hand, malaria parasite infection may affect few physiological functions (eg., hepatic metabolism, protein binding) that may impact the pharmacokinetics and tissue disposition of antimalarial drugs. The altered PK in turn can affect the concentrations of the drugs and consequently their efficacy. Therefore, this work was undertaken to understand whether there is any difference in the pharmacokinetics (PK) of currently approved/available antimalarial drugs between malaria patients and healthy subjects.

The pharmacokinetic data of approved drugs was obtained from various public resources and the data was compiled for absorption, distribution, metabolism and elimination (ADME) properties. These ADME properties were further analyzed against the information available on reported physiological changes (e.g. reduced hepatic blood flow in patients, changes in CYP enzyme levels, etc.) to understand the possible contributing factors for potential alterations in the pharmacokinetics of drugs in patients. Pre-clinical information available for these drugs were also retrieved and used for the present investigation.

The results indicated that there was a significant alteration in the pharmacokinetic properties of most of the currently available/ approved antimalarial drugs in malarial patients compared to healthy subjects. The correlation analysis indicated that physiological changes such as hepatic blood flow, CYP enzyme expression/activity and protein binding may be the potential reasons for the observed differences. This analysis could be useful to envisage changes in the PK properties of the drugs based on their ADME properties and further aid in the development of future antimalarial drugs.

**P119**

Multiplex multi-antigen, multi-species, microsphere-based ELISA to detect antibodies to three human *Plasmodium* species

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Background: Multiplex ELISA that detect antibodies against more than one *Plasmodium* species while allowing species differentiation would be highly valuable for epidemiology and vaccine studies in areas of mixed infections and identification of malaria-exposed blood donors in non-endemic countries. Here we report a highly sensitive, multiplex ELISA based on recombinant proteins from *Plasmodium falciparum*, *P. vivax* and *P. malariae* malaria for pan-species and species-differentiating detection of antibodies in malaria-positive reference samples and in samples from individuals living in a malaria endemic region in Ghana, Africa.

Materials and methods: Multiplex ELISA was developed utilizing the Luminex xMAP technology that allows the simultaneous detection of antibodies of different specificities that react with antigenic epitopes on multiple beads (microspheres) of different dye intensity. Seven recombinant Plasmodium antigens (*P. falciparum*: CSP, AMA-1, LSA-1 and MSP1_10, *P. vivax*: AMA-1 and MSP1_10, and *P. malariae*: MSP1_1) were covalently coupled to carboxylated magnetic beads. The dilutions of human plasma/serum were incubated with 3000-5000 antigen-conjugated beads in 96-well plate. Following incubation with a biotin-labeled human anti-IgG conjugate, a streptavidin-PE conjugated fluorescent substrate was added and the plates were read on Bio-Rad BioPlex 200 reader. The reader was set to read a minimum of 50 beads with identical unique detection signal, the results were expressed as median-fluorescent intensity and cut-off titer were established using a pool of normal human serum samples from the US blood donors.

Results: Multiplex ELISA detected 100% of the confirmed malaria reference samples belonging to *P. falciparum*, *P. vivax* and *P. malariae* infected patients. The inclusion of multiple antigens in the multiplex assay makes the test more sensitive than the conventional plate ELISA. The assay was capable to detect differential antibody reactivity to seven Plasmodium antigens in serum samples from 75 adults from malaria endemic area in Ghana who had no demonstrable parasitemia by microscopy. The assay also successfully distinguished between the mixed *P. falciparum* and *P. malariae* infections in imported malaria samples obtained in United States.

Conclusions: We have developed a highly sensitive multiplex ELISA that detects the antibodies specific to *P. falciparum*, *P. vivax* and *P. malariae* in a single test format. This assay is being further improved to incorporate the fourth human Plasmodium – *P. ovale*. We think that this test may be of high value in epidemiological surveys to determine species-specific malaria exposure in areas of mixed infections and vaccine efficacy studies.

**P120**

Do topical repellents divert mosquitoes within a community?

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Background: Repellents are compounds which interfere with the mosquito’s olfactory system hindering them to identify their hosts and succeeding in taking a blood-meal [1]. However, repellents do not
eliminate the host-seeking mosquitoes, they simply reduce human-vector contact. Consequently, there is a possibility that individuals, who do not use repellents, experience more bites than usual because mosquitoes are diverted from the repellent users. The objective of this study was to measure if diversion occurs from households that use repellents to those that don’t within a community with incomplete topical repellent coverage.

**Materials and methods** An interventional study was performed in three villages of southern Tanzania using 15%-DEET (N,N-Diethyl-meta-toluamide) and a placebo lotion. Three coverage scenarios were investigated: complete repellent coverage (all households were given 15%-DEET), incomplete repellent coverage (80% of households were given DEET-15% and 20% were given a placebo lotion) and no repellent coverage (all households were given a placebo lotion). The coverage scenarios were rotated between villages. Mosquito densities were measured through aspiration of indoor and outdoor resting mosquitoes respective to each enrolled household. Data was analysed using mixed-effects models and the no coverage scenario was used as reference.

**Results** Placebo users living in a village where 80% of the households used 15%-DEET were likely to have nearly three times more mosquitoes (p<0.001) resting in their dwellings in comparison to households in a village where nobody uses repellent (Table 1).

<table>
<thead>
<tr>
<th>No coverage</th>
<th>Complete coverage</th>
<th>80% Coverage (repellent users)</th>
<th>80% Coverage (non repellent users)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IRR Mean 1</td>
<td>4.97 [3.77 – 6.16]</td>
<td>0.69 [2.83 – 4.06]</td>
<td>0.83 [1.94 – 9.76]</td>
</tr>
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</table>

**Conclusions** There is strong evidence that mosquitoes are diverted between households that use repellent to those that don’t. This study arises questions on health equity associated with repellent usage. Policy makers should take into consideration these results while devising vector control programs, as less privileged individuals are likely to suffer more mosquito bites and therewith be more exposed to vector-borne diseases if universal coverage is not reached.

**Acknowledgments** We would like to thank the people of Sanje, Upper and Lower Matete for their support during the project.

**References**


**P121**

Insecticide-treated durable wall lining for malaria control: multicentre studies from Africa and South-East Asia

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**Background** Indoor residual spraying (IRS) is a primary method of malaria vector control but its potential 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 wall lining (DL) can be considered a novel form of long-lasting IRS, which gradually releases insecticide over a period of three to four years when used to cover interior house walls. DL is designed to overcome the logistical constraints associated with repeated rounds of spraying whilst retaining the most attractive feature of IRS, the protection of all members of the community [1-3]. To establish DL as a viable substitute it must demonstrate equivalent or superior levels of bioefficacy, acceptability, durability and logistical feasibility to currently available products.

**Materials and Methods** To identify a desirable material to develop into a durable wall lining, a one year preliminary trial was conducted among rural and urban households in Angola and Nigeria (n=258) comparing three deltamethrin-treated prototype materials (polyethylene shade cloth, laminated polyethylene sheeting and mosquito wall netting) [4]. The most popular lining material (shade cloth polyethylene, henceforth DL) was then evaluated in comparison with conventional IRS during a one year multicentre trial conducted in rural households in malaria endemic Equatorial Guinea, Ghana, Mali, South Africa and Vietnam (n=220).

**Results** During the preliminary trial a dichotomy between rural and urban participants emerged. Rural households favoured wall adornments and accepted wall linings because of their perceived decorative value and entomological efficacy, whereas urban households preferred minimal wall decoration and objected to the materials aesthetics and installation feasibility. Of the prototype lining materials assessed, polyethylene shade cloth DL was the most popular because of its ease of installation, aesthetics and resemblance to locally available materials. During the multicentre field trial, DL demonstrated consistently higher levels of bioefficacy compared to IRS, with no significant loss of bioactivity after 12 months. Field samples of DL retained on average 78% of their original insecticide content after one year. The majority of 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 product at the end of trial, the majority of participants chose DL regardless of the earlier household allocation.

**Conclusions** These two trials represent the largest field evaluation of DL to date [4]. The high level of acceptability among rural inhabitants identifies these communities as the ideal target consumer group for DL. DL remained fully efficacious against mosquito vectors, demonstrated minimal loss of insecticide content over 12 months of field use and was unequivocally more popular than IRS and other long-lasting vector control products. Together these results demonstrate that DL has the potential to overcome many of the operational challenges associated with IRS and may represent a viable long-lasting alternative, a scenario not dissimilar to the advantages and superiority shown by long-lasting insecticidal nets when introduced in place of conventional insecticide-treated nets.

**References**


**P122**

Nigerian malaria: the problems and the fight

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Malaria is one of the world’s top killer diseases, especially for the young children. Malaria has remained a major threat to public health and
economic development in the tropical and subtropical regions of the world. Attempts to control or completely eradicate the disease have failed massively as a result of well-known resistance to drugs for the malaria parasite and to insecticides for the vector and the situation has become life-threatening. Though there is collaborative energy both at the international, national and individual level to fight the disease by developing vaccines and new drugs, no-one has produced any permanent result yet. In the field of science, education about understanding the disease has recorded a tremendous progress.

After HIV/AIDS, malaria is the second leading cause of death in Africa. It is believe that nearly 1 in every 5 deaths among kids in Africa is as a result of malaria. In my country Nigeria, malaria is a major public health problem where it accounts for more cases and deaths than any country world over. About 97% of my country’s population is at risk for malaria because of their location. It is only 3% of Nigeria’s population live in the malaria free zones. Malaria alone accounts for more than 300,000 deaths each year in Nigeria. This estimate is well above the 215,000 deaths each year from HIV/AIDS. If the above accounts for Nigeria alone how about other 29 Sub-Saharan African countries which together accounts for 90% of the world wide malaria deaths?

The vaccine, which has been promised to be ‘just round the corner’ for many years, remains elusive. It is important to ask why this is so, when effective vaccines exist for many other infectious diseases. What are the reasons for the slow rate of progress, and what has been learned from the first clinical trials of candidate malaria vaccines? What are the remaining challenges, and what strategies can be pursued to address them? The remaining major challenge is poverty. About 70% of Nigeria population lives in poverty. Should any permanent remedy be found any time soon, these Sub-Saharan African countries like Nigeria should be the starting point in the fight to eliminating malaria!

P123
Abstract not submitted for online publication

P124
Targeting PfRh5 on Merozoites to Prevent Basigin Binding
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The goal of the research study was to shed light on a promising research area which may prove to be a safer more specific vaccine candidate; PfRh5 on merozoites. Erythrocyte invasion is paramount to the pathogenesis of P. falciparum requiring a series of extracellular recognition between the erythrocyte receptors and merozoites ligands. Of the known receptor-ligand interactions, one has been found essential to erythrocyte invasion in all P. falciparum strains; P. falciparum reticulocyte-binding protein homologue, PfRh5. This ligand binds to erythrocytes via basigin, an Ok blood group antigen and a type I integral membrane receptor consisting of many ligands. Basigin, also known as CD147, is a protein receptor on red blood cells, and a member of the immunoglobulin superfamily (Crosnier et al. 2011). It has further been found that a cysteine-rich protein called P. falciparum Rh5 interacting protein, Pfrip, forms a complex with PfRh5 on merozoites, where one study found antibodies to Pfrip1 to effectively inhibit merozoites attachment and invasion in erythrocytes (Chen et al, 2011).

The most effective targeting strategy seems to be preventing PfRh5 on merozoites from binding the receptor basigin on red blood cells (RBC). This receptor is essential for Plasmodium falciparum to enter RBC by binding basigin via PfRh5 protein on the malaria parasite. Basigin is a member of the immunoglobulin superfamily, a group of cell surface soluble proteins involved in the binding, adhesion, and recognition processes of cells. Since PfRh5 is specific to merozoites, a vaccine based on PfRh5 would not affect RBCs or other parts of the body, likely not initiating an auto-immune response. This presents the best opportunity due to the specificity of PfRh5 which makes it a ‘safe’ and effective vaccine targeting strategy.

Due to the specificity of the attack method used by P. falciparum, it seems evident that the vaccine must target a specific receptor of one of the parasites many stages. The focus of the study is to shed light on this newly found research and to create a vaccine in the future that develops antibodies against the parasite ligand of Basigin, Rh5, since PfRh5 protein on the parasite binds to basigin. Due to the need of merozoites to enter and replicate in RBC, this seems like the most plausible vaccine development. Since humans are both the carriers and “spreaders” to mosquitos, it is paramount to find a vaccine for humans to stop the spread of gametocytes to mosquitos which would prevent sporozoites formation and the re-infection of other humans. The author believes that developing antibodies against the parasite ligand for basigin will prevent merozoites invasion into erythrocytes.

P125
The repertoire diversity of the Plasmodium falciparum stevor multigene family in complicated and uncomplicated malaria in India
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Background The deep vascular sequestration of parasitized erythrocytes is a central pathological event in falciparum malaria. Variant surface antigens are encoded mainly by three multi-copy
gene families, namely, var, stevor, and rifin. Var is one of the most important families that plays a crucial role in antigenic variation and immune evasion. Clinical and epidemiological studies have shown that severe or complicated malaria is manifested in a limited number of patients. This indicates that a subset of these multigene families could be determinants in the manifestation of different malaria phenotypes. Recent studies have indicated the possible role of stevor (sub-telomeric variable open read), a multi-gene family, in erythrocyte invasion, antigenic variation and host cell modification, of infected erythrocytes. In this study, we describe the repertoire and diversity of members of the stevor multigene family in patients with complicated and uncomplicated malaria in India.

Materials and methods Plasmodium falciparum complicated isolates (n=8) from Odisha and uncomplicated isolates (n=7) from Assam, Madhya Pradesh and Goa were collected. Members of the stevor multigene family were amplified using degenerate PCR primers. Amplified PCR products were cloned and a total of 35 clones per cloning experiment were sequenced. A maximum likelihood phylogeny was constructed in order to understand the genetic repertoire of members of the stevor multigene family in severe and non-severe isolates and extent of stevor repertoire in Indian isolates.

Results A range of 21-31 unique sequences was obtained out of 35 clones sequenced: for each of the 15 isolates. Nucleotide diversity analysis shows extensive genetic polymorphism that supports the hyper-variability nature of stevor multigene family in field isolates. The repertoire and diversity of the stevor multigene family varied between all four geographical regions of the Indian subcontinent. Phylogenetic tree analysis showed clustering of sequences from complicated isolates, and suggests that the stevor genetic repertoire is less diverse in comparison to uncomplicated isolates.

Conclusions This study suggests an extensive genetic diversity of stevor in Indian P. falciparum isolates, however the genetic repertoire from complicated cases was less diverse. The high degree of stevor diversity has important implications for the design of effective anti-malaria control measures.

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P127
Modeling the relationship between precipitation and malaria incidence in Mpumalanga, South Africa
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Background Climatic or weather-driven factors such as rainfall have considerable impact on vector abundance and the extrinsic cycles that parasites undergo in mosquitoes [1]. Climate models therefore allow for a better understanding of the dynamics of malaria transmission [2]. While malaria seasons occur regularly between October and May in Mpumalanga, there is considerable variation in the starting point, peak and magnitude of the season. The relationship between rainfall and malaria incidence may be used to better model the variation in the malaria season. As a first step, this study seeks to explore the complex association between rainfall and malaria incidence through time series methods.

Materials and methods The statistical relationships between weekly malaria case data and accumulated weekly rainfall were explored for Mpumalanga in the period 2002 and 2010. Two analyses were performed; namely, cross correlations of the raw data series and cross correlations of the pre-whitened data series. Pre-whitening is achieved by using the Box Jenkins approach to fit Seasonal Autoregressive Integrated Moving Average (SARIMA) models.

Results Cross correlation analysis of the raw rainfall and case series yielded significant negative correlations between lag -20 and lag -40 and significant positive correlations between lags -4 and -10. The cross correlations analysis of the transformed rainfall and case series (with stabilized variance) yielded significant negative correlations between lag -40 and lag -18 and significant positive correlations between lag -13 and lag 2. The analysis of the pre-whitened series showed that many of these correlations were spurious. A SARIMA (1,1,2)(0,1,1)\_2 model was fitted to the transformed rainfall series and applied to the transformed case series and a cross correlation analysis of the residuals of these two SARIMA models showed significant positive correlations at lags -5 and -6.

Conclusions The relationship between rainfall and malaria incidence is non-direct and complex. The consequence of pre-whitening is to reduce unassociated autocorrelations in the time series before the cross correlations are computed, thereby reducing the number of spurious correlations. Lagged rainfall data has the potential to be used in place of trigonometric functions to model the variable seasonality component in mathematical models of malaria transmission.

Acknowledgements The author would like to acknowledge her supervisors A/Prof F Little, Prof K Barnes and Dr L White and thank the Mpumalanga Provincial Department of Health and the South African Weather Services for the provision of data.

References

P128
The changed occupation and behavioral among imported malaria cases 2009-2011 in Sukabumi District-West Java, Indonesia
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Malaria Journal 2012, 11(Suppl 1):P128

Background A malaria outbreak occurred in the Sukabumi District in 2004, 785 cases were reported and 8 of them died. The imported cases are sometimes blamed as the trigger of the outbreak. Till now malaria has been endemic in Sukabumi. This research aimed to discover the characteristic of the migrants’ cases before and after they had got malaria.

Materials and methods The subjects were imported malaria cases that were collected from the Health Center in 4 Sub-district in Sukabumi District for the year 2009 to 2011, and 145 subjects were interviewed in their house using structured questionnaires. The data were analyzed descriptively to describe the distribution of the cases in terms of sex, occupation and the practice in preventing the transmission of malaria.

Results The majority of import malaria cases in Sukabumi were male and of productive age (more than 15 years old). They worked mostly in mining, and the rest were in plantation, merchant, and other type of labour or housewives. After they got infected by malaria they went or were sent back to their home land (Sukabumi). After they have got treatment and got well, some of them went back to their previous occupation in the same location and some in different location with the same activity. Few of them did not go back and stay unemployed in their home land.

In relation to the risk factors in malaria transmission, workers who were treated in Sukabumi mostly worked as miners and had experienced night shift, or some of them worked as “ojek” (motorcycle cab) and worked till late at night. Some of them had experienced night activities such as hanging out or watching television and some of them were not aware of malaria transmission. Around 56.6% used a mosquito net or repellent.

Table 1 (abstract P128). The distribution of characteristic malaria import cases in Sukabumi, West Java, Indonesia 2009-2011

<table>
<thead>
<tr>
<th>Total cases=145</th>
<th>Variable</th>
<th>Categories</th>
<th>Number</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Male</td>
<td>139</td>
<td>95.9%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>6</td>
<td>4.1%</td>
<td></td>
</tr>
<tr>
<td>age</td>
<td>&lt;15 years</td>
<td>0</td>
<td>0.0%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>15-54 years</td>
<td>138</td>
<td>95.2%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt; 54 years</td>
<td>7</td>
<td>4.8%</td>
<td></td>
</tr>
<tr>
<td>Occupation (before ill)</td>
<td>Plantation Growers</td>
<td>4</td>
<td>2.8%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Merchants</td>
<td>4</td>
<td>2.8%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Miners</td>
<td>130</td>
<td>89.7%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Housewives</td>
<td>2</td>
<td>1.4%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Casual Laborers</td>
<td>1</td>
<td>0.7%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Other</td>
<td>4</td>
<td>2.8%</td>
<td></td>
</tr>
<tr>
<td>Occupation (after ill)</td>
<td>Moved out and change of occupation</td>
<td>35</td>
<td>24.1%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Moved out and same occupation</td>
<td>10</td>
<td>6.9%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Same location and change of occupation</td>
<td>12</td>
<td>8.3%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Same location and same occupation</td>
<td>85</td>
<td>58.6%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Unemployed</td>
<td>3</td>
<td>2.1%</td>
<td></td>
</tr>
<tr>
<td>Night Behaviors</td>
<td>Toilet activity</td>
<td>1</td>
<td>0.7%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hangout, watch television</td>
<td>13</td>
<td>9.0%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Patrolling</td>
<td>5</td>
<td>3.4%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fishing</td>
<td>4</td>
<td>2.8%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Night shifts (miners, ojek)</td>
<td>82</td>
<td>56.6%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No outside activity</td>
<td>4</td>
<td>2.8%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Other</td>
<td>13</td>
<td>9.0%</td>
<td></td>
</tr>
<tr>
<td>The use of mosquito net and repellent</td>
<td>Use mosquito net and repellent</td>
<td>85</td>
<td>58.6%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Do not use mosquito net and repellent</td>
<td>60</td>
<td>41.4%</td>
<td></td>
</tr>
</tbody>
</table>
repellent, and the rest did not take any protection to control malaria transmission.

Conclusion The imported malaria cases in Sukabumi were dominated by males of productive age, worked as miners experienced in night shift, without proper protection to avoid malaria transmission and they consistently looked to return to their previous occupation in the same location. So this is important for the district health office either in the home land or the work destination to promote malaria transmission protection among workers.

Reference

P129
Sustainable planning in a malaria vector control program: a study in Pesawaran, Indonesia

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Background One effective way to control malaria is through vector control [1, 2]. Pesawaran is a malaria endemic area with a number of Annual Parasite Incidence (API) of 2.97. [3] The formulation of vector control programs in endemic areas should consider the principles of sustainability [4]. The purpose of this study is to formulate the concept of the development of malaria vector control programs in Pesawaran considering sustainability principles.

Method The study was conducted in the area of Pesawaran, Indonesia which has malaria receptive areas along the coast [4]. Data was obtained from a study of literature, in-depth interviews and questionnaires to experts. The experiment was conducted in April-June 2012. The analysis method used is descriptive qualitative as well as quantitative and Analytic Hierarchy Process (AHP). AHP can be used to solve problems related to tangible and intangible factors. Data, ideas, and intuition can be set by using a logical hierarchy structure. Hierarchy is the arrangement of factors / elements of the existing problems that can be set / controlled [5]. Data processing fees expert AHP using software version 11.

Results District Pesawaran has endemic malaria receptive areas. Approximately 68.0% of the total patients in health centers Hanura Malaria, 16.9% were in health centers kike and the rest, 15.1% were in health centers Padang Mirr or. There were high numbers of cases of malaria in both these areas, because of the many mosquito breeding places such as abandoned farms [4]. Hierarchical model alternative malaria vector control programs as recommended by WHO and in accordance with the conditions of the research area is the management of abandoned farms from becoming mosquito breeding places, chemical and biological larvicide, Insecticide outdoor residual spraying and insecticide Indoor residual spraying (IRS indoor and outdoor) [2, 6]. Based on a literature study into consideration in the selection of an alternative is the social, economic, environmental, technological, and institutional [2, 4, 6]. Data processing by expert software choice v. 11 shows that the best course of malaria vector control that is able to maintain the quality of the environment in Pesawaran is turned off so as not to be neglected pond breeding places of mosquitoes (62%), chemical and biological larvicide (23%), and the IRS indoor and outdoor (15%).

Data processing by expert software choice v. 11 shows that the best course of malaria vector control that is able to maintain the quality of the environment in Pesawaran is turned off so as not to be neglected pond breeding places of mosquitoes (62%), chemical and biological larvicide (23%), and the IRS indoor and outdoor (15%). The order of aspects to be considered in the selection of alternative vector control is the social aspect (0.312), the environment (0.258), economic (0.201), technology (0.131) and institutional (0.097).

The social aspect (sub criteria: community participation, involvement of other stakeholders, employment, and minimal conflicts in society) tops the list to be considered in the selection of malaria vector control program. Cooperation stakeholder and public participation to determine the success of vector control in an endemic area [4, 6].

Conclusion Priority malaria vector control in endemic areas Pesawaran, considering the principle of sustainability, namely environmental management on farms neglected in order not to become breeding places and the biggest aspects to be considered a priority selection is the social aspect.

References
Table 1 (abstract P130). Mean Center and SDE

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sub Variable</th>
<th>X</th>
<th>Y</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Center</td>
<td>Minimum</td>
<td>106.487241</td>
<td>-7.232089</td>
</tr>
<tr>
<td></td>
<td>Maximum</td>
<td>106.7401</td>
<td>-7.018312</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>106.602712</td>
<td>-7.11819</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>0.041497</td>
<td>0.047272</td>
<td></td>
</tr>
<tr>
<td>Geometric Mean</td>
<td>106.602713</td>
<td>-7.118034</td>
<td></td>
</tr>
<tr>
<td>Harmonic Mean</td>
<td>106.602705</td>
<td>-7.117879</td>
<td></td>
</tr>
<tr>
<td>SDE</td>
<td>SD along new axis</td>
<td>7968.41m</td>
<td>5836.57m</td>
</tr>
<tr>
<td></td>
<td>axis length</td>
<td>15936.83m</td>
<td>11673.13m</td>
</tr>
</tbody>
</table>

### P131

Abstract withdrawn

### P132

Using the cultural model to plan intervention for malaria control in immigrants and native communities in endemic area, earmarked for malaria elimination southeastern Iran

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Background To improve malaria control measures, taking into account local beliefs and practices are essential. Recently, Iran has been earmarked for malaria elimination while the majority of malaria patients are imported cases from eastern neighbouring countries. In the present study, we employed the culture model as a theoretical framework to examine how health beliefs, behaviors and practices associated with improving access to prevention measures, early diagnosis and treatment of malaria in two communities, immigrants and native residents in a malaria endemic region located in southeast of Iran.

Materials and methods A mixed-methodology was designed by means of two quantitative surveys and qualitative focus groups. In total, 380 participants volunteered to take the cross-sectional survey, with 185 immigrants, 195 native residents completing quantitative surveys and also 40 participating in the qualitative focus groups.

Results A significant association between education level and knowledge on malaria transmission was also observed within both communities. Although the majority respondents associated the disease transmission with mosquito bites only 16.5% immigrants as compared to 63.4% native residents reported to use mosquito net. Data from focus group emerged three themes includes similarity in perception about malaria, difference in type of treatment and decision making and, finally resemblance to prevention of malaria in both communities.

Conclusions Matching the cultural characteristics of immigrants and native residents' culture to malaria interventions and services will improve receptivity to, acceptance of, and salience of these efforts.

References

### P133

Development of dynamical weather-disease models to project and forecast malaria in Africa

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Malaria Journal 2012, 11(Suppl 1):P133

Background Weather and climate play an important role in the spread of malaria. Suitable weather conditions for malaria are found in sub-Saharan Africa, where most of the worldwide malaria cases and deaths are found. For this reason, integrated weather-disease malaria models are useful tools to project the malaria future and to provide monthly- to-seasonal forecasts.

Methods Malaria projections and forecasts are undertaken by two dynamical mathematical-biological malaria models: (i) the LMM (London Malaria Model) [1-3] and (ii) VECTRI (VECtor-borne disease community model of the International Centre for Theoretical Physics, Trieste). Both models are driven by daily temperature and precipitation values. An improved version of the LMM was introduced by [2], which was calibrated by malaria field observations from West Africa [3]. Regarding the assessment of the impact of climate change on malaria [4], the LMM was driven by data from the Regional Model (REMO) including the effect of land surface changes.

For the QWeCI (Quantifying Weather and Climate Impacts on health in Developing Countries) project, a seamless weather prediction system has been developed at ECMWF by appending the first 25 days of the forecast to the 120-day lead time prediction. The forecast is calibrated to correct for displacement errors of West African monsoonal temperatures over the Kericho tea estates: revisiting the climate in the tropical highlands of Kenya. [5], [6]

Results and outlook The malaria projections up to 2050 [4] based on the integrated REMO-LMM reveal a southward shift of the epidemic malaria area in West Africa due to the precipitation decline. The increased temperatures lead to an increase of transmission in highland
territories. Formerly, malaria free areas become epidemic, whereas the epidemic risk is decreased in lower-altitude regions. Actual research within the EU Seventh Framework Programme (FP7) QWeCI and HEALTHY FUTURES projects is underway to exploit the feasibility of monthly-to-seasonal malaria forecasts. QWeCI is currently developing prototype seamless malaria forecasts for Malawi (http://nwmstest.ecmwf.int/products/forecasts/d/inspect/catalog/research/qweci/). The LMM and VECTRI neglect various important malaria factors like immunity, malaria control activities, or different vector characteristics. Further development of VECTRI will be undertaken to include other relevant malaria factors. Note that VECTRI represents a community model meaning that the model and code is publicly available (http://users.icsp.toronto.edu/~jmorris/dervectri/). The LMM is included in the so-called Disease Model Cradle (DMC) that is downloadable (http://www.liv.ac.uk/qweci/project_outputs/). Open-access web versions of both models are applicable for point data (see http://qweici.uni-koeln.de/).

References

P134
Some methodological issues in the development of public health recommendations from field trials of new malaria vector control interventions
Jo Lines
London School of Hygiene & Tropical Medicine, Keppel St, London WC1E 7HT, UK

In this paper, I consider some technical aspects of the process by which public health recommendations are inferred from the results of epidemiological field trials and other research on new vector control products and technologies. I argue that the conventional Cochrane Collaboration methods for summarising clinical trials may need to be modified and extended in order to be valid when applied to vector control.

With medical interventions, for which the Cochrane methods were originally designed, the primary mode of action of the intervention works through biological processes within an individual, and the individual person is a natural experimental unit. This makes it easier to assume that a series of trials from different locations are more or less replicates of each other: they are asking more or less the same question, and in the absence of bias and sampling error they should produce more or less the same answer. In the case of vector control, on the other hand, the intervention has its most direct effects on mosquito, and the causal chain that leads to health benefits mostly occurs outside people, in the external environment. For this reason, in order to summarise a series of vector control trials, it is necessary to standardise not only the human populations, the interventions and the observed outcomes, but also the local vector populations and the broader ecological characteristics of the settings where the trials were carried out. Only with this additional matching is it valid to assume that vector control trials carried out in different settings are asking the broadly same question about the same intervention.

The same applies to the process of generalising from a set of field trials in specific settings to broader public health recommendations. We know from first principles that a vector control intervention that is effective in one setting may not be so in another. Moreover, the field trials of a new intervention can include only a small subset of the likely range of settings where the intervention might eventually be used. Thus we need systematic methods for asking not just “whether” an intervention will be effective, but also “where and when” it will be effective. In the past, malarialogists have used “eco-epidemiological stratification” to deal with this problem: this approach now needs to be made more systematic and integrated.

Particular attention therefore needs to be given to the “epidemiological mode of action” of entomological interventions. This is the causal chain by which effects on mosquitoes lead to reduced infective biting for at least some people (e.g. “personal protection” and “the mass effect” caused by ITNs). This useful new concept, which has emerged from current discussions within the vector control product development community, appears to play a central role in the interpretation of evidence from vector control field trials for public health purposes.

P135
Malaria in Croatia: from eradication until today
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Background According to the World Health Organization, in Croatia malaria was officially eradicated in 1964. Since then a certain number of cases of imported malaria is registered every year, but this has seen a declining trend throughout the years.

Materials and methods A retrospective study about the incidence of imported malaria in Croatia in the period from 1987 to 2011 based on the official data of the Croatian National Institute of Public Health.

Results Thus at the beginning of the observed period there were 12 cases of imported malaria per year, whilst in the last five years that number dropped to a yearly average of 6. Between 1987 and 2011, Croatia recorded a total of 233 cases of imported malaria. The disease still most commonly affects seafarers and workers temporarily employed in malaria endemic countries. During 2011, of the 7 cases of imported malaria in Croatia, only 1 was a seafarer. It is very likely that the number of imported malaria cases is somewhat higher in seafarers but the disease often goes unnoticed among Croatian health services due to the fact that seafarers get treatment in ports around the world. The predominant causative agent for imported malaria is Plasmodium falciparum, which has been found in 61.8% (144/233) of patients. The disease is still mostly acquired during visits to Africa: 187 out of 234 (80.25%); visits to Asia account for a smaller portion (41/233; 17.6%), while South America has not been recorded as a source of imported malaria cases in the past 10 years. Although almost all travellers and seafarers are advised to use chemoprophylaxis and ship management companies must provide chemoprophylaxis for their seafarers, irregular or non-existent application of chemoprophylaxis is the cause of imported malaria contraction. However, data on chemoprophylaxis should be considered with caution since these were obtained via patient polls and depend on their memory at that particular point in time.

Conclusion Malaria movements worldwide, the reoccurrence of autochthonous malaria cases in countries where the disease had been eradicated, the existence of malaria-transmitting mosquitoes and a certain number of imported malaria cases in Croatia are all alarming facts. Therefore, health surveillance including a mandatory and adequate chemoprophylaxis for travellers to endemic areas remains a binding measure of public health care aimed at controlling malaria in Croatia.

References
2. Perić D, Škrobonja I, Škrobonja A: Malaria in Croatia in the period between 1987 to 2006.
The discovery of new chemotypes to feed the pipeline of antimalarial drugs remains a constant challenge, particularly in light of emerging resistance to current therapies. Recently, phenotypic screenings have been successfully used for antimalarial hit generation where the biological target(s) may often not be clearly identifiable. To catalyse malaria research by both filling the pipeline and having a better understanding of ligand-target relationships, a unique screening tool has been elaborated: the Malaria Box. The Malaria Box is a set composed of 400 commercially available chemical entities derived from a selection of more than 20,000 hits from the screening of corporate and academic libraries [1,2,3]. The originality of the Malaria Box relies in its composition of 200 lead-like and 200 probe-like compounds that have confirmed activity on blood-staged Plasmodium falciparum and that have been assessed for cytotoxicity. Lead-like compounds have been validated, and the presence of known toxicophores has been reviewed. Conversely probe-like compounds are intended to represent the broadest cross-section of structural diversity. Significantly, the scope of the Malaria Box goes beyond the Malaria field as active compounds may have utility in other parasitic or neglected diseases. It is well documented that artemisinin was initially discovered from helmint research and is currently a gold standard drug against Malaria. Also, the presence of orthologues of various molecular targets may lead to new therapeutic applications in orphan diseases or for example oncology. Ultimately, the data collection resulting from the Malaria Box would enable the community to better understand similarities and differences between parasite diseases or orphan diseases by mining data sets that were previously considered separately [4]. Herein we disclose the selection process applied to assemble the Malaria Box as well a preliminary results.

References


Microsatellite characterization of Plasmodium vivax in pregnant women on the Thai–Myanmar border

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1Department of Clinical Tropical Medicine, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand; 2Department of Molecular Tropical Medicine and Genetics, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand; 3Shoklo Malaria Research Unit, Mae Sai, Tak, Thailand; 4Mahidol-Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand; 5Centre for Clinical Vaccinology and Tropical Medicine, Churchill Hospital University of Oxford, Oxford, UK; 6Institut National de la Santé et de la Recherche Médicale, Unité Mixte de Recherche 94S,Paris,France; 7Université Pierre & Marie Curie, Faculté de Médecine Pitié–Salpêtrière, Paris, France


Background Plasmodium vivax infections in pregnant women are associated with low birth weight and anaemia. Genotyping of P. vivax is useful to study P. vivax in pregnancy, though it is still remains challenging to distinguish between relapse from hypnozoite stage and recrudescence from blood stage following treatment. A genetic investigation of P. vivax in pregnancy on the Thai-Burmese border, comparing the genotype between at follow up with the day of admission for pregnant patients and non pregnant patients was undertaken in this study.

Materials and methods One hundred and sixteen blood samples infected with P. vivax were isolated from 18 pregnant women with ≥2 episodes obtained from whole blood (12 women had 2 reappearances, 4 and 2 women had 3 and 4 reappearances, respectively) and 18 non-pregnant women with 2 consecutive episodes collected on dried blood spot. All samples were genotyped with eight microsatellite markers. Analyses were performed for genetic diversity, multiplicity of infection (MOI), and comparison of genotypes for individual episodes detected in samples.

Results Eight microsatellite loci, 6 to 15 alleles were found at each locus. The mean number of alleles per locus was 1.40 and 1.17 (P < 0.001) for pregnant and non-pregnant patients, respectively. The overall mean expected heterozygosity (He) was 0.845 in both groups. In pregnant patients, the multiplicity of infection (MOI) was 1.85 while it was 1.44 (P = 0.028) in non-pregnant patients. The greater number of minor alleles in pregnant patients may either the nature of the sample. Combined genetic data from days of follow up and day of admission showed that genotypes were different 57 % (25/44) of those pregnant patients and 58 % (21/36) of non-pregnant patients (P=0.891).

Conclusions This study confirmed that different P. vivax genotypes were found during follow up when compared to day of admission in both groups.

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Malaria elimination strategy and challenges in People’s Republic of China

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Background Malaria remains an infectious disease of foremost public health importance in the People’s Republic of China. Historically, high malaria incidence rates have been reported from 24 provinces of PR. China with more than 30 million cases annually reported. With the significant reduction of malaria incidence, the national malaria elimination was launched in 2010.

Methods The risk factors related to malaria transmission in China was reviewed based on the previous literature reviewing, and the capacity of malaria elimination was analysed based on the readiness in surveillance and response system. The challenges and future research priorities related to the elimination strategy were put forward.

Results Owing to large-scale control activities facilitated through primary healthcare networks and community participation, the infection rate of Plasmodium vivax has been reduced to under 0.01% in most areas of China, and P. falciparum malaria has been eliminated in most provinces, except Yunnan and Hainan. The elimination strategy formulation and its readiness analysis were performed with discussion on the challenges for the national malaria elimination programme in China, while the southern border areas in Yunnan will be the one of most hard issue to elimination the disease. Finally the recommendation on surveillance and response approaches based on currently satus leading to malaria elimination in China were put forward.

Conclusion The national malaria elimination programme launched in 2010 is able to achieve its optimal goal to eliminate malaria in whole country by 2020, if facilitated by surveillance and response system.
Insecticide resistance: a challenge to malaria vector control in Ethiopia

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Dereje Olana4, Richard Rehinger5, William Brogdon6


Background
In Ethiopia, indoor residual spraying (IRS) and insecticide-treated bed nets form the main malaria vector control. As the two tools rely on synthetic insecticides, it was found necessary to document the up-to-date distribution and levels of insecticide susceptibility of *Anopheles arabiensis*.

Materials and methods
Between 2008 and 2011, insecticide susceptibility tests were carried out in 39 localities out of which 12 were repeatedly visited from 2 to 4 years. Tests were conducted using WHO test kits and procedures [1] on non-blood fed, 48-72 hours old female An. arabiensis which were reared from field collected larvae and pupae. The insecticides were discriminating doses of DDT, malathion, fenithrothion, propoxur, bendiocarb, deltamethrin and lambdacyhalothrin. Controls were exposed to insecticide free oil impregnated papers. The WHO recommendations were applied to classify the population as susceptible, acquiring possible resistance and resistance [1]. The presence and frequency of the target site insensitive resistance mechanisms, *kdr* (L1014F mutation) and ace-1 (G1195 mutation) were investigated from vector populations of nine localities following the procedures described in [2,3].

Results
All results depicted very low mortalities of *An. arabiensis* due to DDT, implicating wide distribution of resistance to this insecticide (Table 1). Resistance is also significantly high to deltamethrin, lambdacyhalothrin and malathion. Bendiocarb resistant populations were also detected from a few localities. The vector populations are susceptible to propoxur-methyl and propoxur, susceptibility was also very high to fenithrothion. Of 229 *An. arabiensis* more than 95% were found to carry the *kdr* gene (both homozygous and heterozygous genotypes) while 47 tested specimens were without the ace-1 allele mutation.

Conclusions
Similar studies in the past by other workers [4,5,6,7] together with this one showed increased resistance of *An. arabiensis* to insecticides belonging to the four major classes. This would pose a serious challenge to vector control in the coming years. Given the small number of insecticides for IRS and LLINs, the Federal Ministry of Health of Ethiopia should take timely measure by formulating a policy as well as implementing insecticide resistance management within the frame work of integrated vector management.

Acknowledgements
The assistance of regional MOH staff including those retired is greatly acknowledged. The study obtained financial support from the President’s Malaria Initiative and World Health Organization.

Table 1 (abstract P139). Mortality results of *Anopheles arabiensis* and number of localities with susceptible and resistant populations (2008-2011)

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>Percentage mortality</th>
<th>Number of localities with <em>An.arabiensis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average</td>
<td>Range</td>
</tr>
<tr>
<td>DDT</td>
<td>15.2</td>
<td>0-85.0</td>
</tr>
<tr>
<td>Deltamethrin</td>
<td>72.7</td>
<td>18.8-100</td>
</tr>
<tr>
<td>Lambdacyhalothrin</td>
<td>49.9</td>
<td>3.0-94.0</td>
</tr>
<tr>
<td>Malathion</td>
<td>86.4</td>
<td>38.0-100</td>
</tr>
<tr>
<td>Fenithrothion</td>
<td>98.2</td>
<td>76.5-100</td>
</tr>
<tr>
<td>Primiphos-methyl</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Propoxur</td>
<td>99.5</td>
<td>96.0-100</td>
</tr>
<tr>
<td>Bendiocarb</td>
<td>95.0</td>
<td>53.0-100</td>
</tr>
</tbody>
</table>

References
confirmed by cloning of amplicons and genotyping of different colonies (mean of 10 colonies per PCR product).

**Results** The majority of parasites showed distinct haplotypes in relapses compared to primary infection. It was demonstrated a high frequency of multiple-clone infections both in primary infection and relapse. A variation of predominant alleles among distinct markers in different malaria recidives of the same individual was observed. Therefore, haplotypes in relapse could also be identified in primary infections as a rare allele.

**Conclusions** Altogether our findings suggest that mechanisms involved in hypnozoites activation might not be based only on parasites genetic programming but also on host/environment factors.

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**P141**

**Peripheral blood cell signature and inflammatory responses during pregnancy-associated malaria**

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**Background** Placental malaria (PM) is caused by sequestration of *Plasmodium falciparum* infected erythrocytes into the intervillus space of the placenta, resulting in pathological alterations. During PM, mononuclear cells infiltrate the placenta inducing several immunological events, which cause pathological alterations in the placenta, impairing materno–fetal interaction. Several studies have shown transient depression of cell-mediated immunity of the woman during pregnancy and their modulation during PM. In this study, we investigated the impact of *P. falciparum* infection during pregnancy on inflammatory immune responses.

**Methods** We conducted a longitudinal, prospective study in Benin, in which we enrolled ~1000 pregnant women with a gestational age of 24 weeks or less, and followed them up until delivery. Immunophenotype and activation levels *ex vivo* of peripheral blood mononuclear cells (PBMC), as well as plasma concentrations of a panel of cytokines and chemokines, were assessed in subgroups of 132 women at inclusion and 111 at delivery, using flow cytometry, standard cytometric bead arrays and ELISA. *P. falciparum*-infected women were matched to uninfected controls based on age, gestational age and gravidity.

**Results** Both at inclusion and at delivery *P. falciparum* infection was associated with significantly increased frequencies both of B cells overall and of activated (CD86 hi) B cells. Infection-related profiles were otherwise quite distinct at the two different time-points, characterized by, for example, fewer T regulatory cells (Treg) at inclusion but more T effector (Teff) cells at delivery. Independent associations with an increased risk of maternal anaemia were found for altered antigen-presenting cell frequencies at inclusion, but for an increased frequency of Teff at delivery. *P. falciparum* infection was also associated with increased IL-6, IL-10, MIG and IP-10 plasma levels both at inclusion and at delivery.

**Conclusions** The timing and/or duration of *P. falciparum* infections during pregnancy – chronic at inclusion but acute/recently-acquired at delivery – is reflected by similar (e.g. B cells) but also distinct (e.g. Treg/Teff, immature monocytes) variations in PBMC populations. The data suggest that innate immune responses as well pro- and anti-inflammatory mediators play important roles in pregnancy-associated malaria pathogenesis.

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**P142**

**Factors associated with utilization of community health workers in improving access to malaria treatment among children in Kenya**

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**Background** Information on the success of community case management in improving access to effective malaria treatment for young children relies on broad utilization of community health workers (CHWs) to diagnose and treat fever cases. A better understanding of the factors associated with CHW utilization is crucial in informing national malaria control policy and strategy in Kenya. Specifically, little is known in Kenya on the extent to which CHWs are utilized, the characteristics of families who report utilizing CHWs and whether utilization is associated with improved access to prompt and effective malaria treatment. This paper examines factors associated with utilization of CHWs in improving access to malaria treatment among children under five years of age by women caregivers in two malaria endemic districts in Kenya.

**Methods** This study was conducted in 113 hard-to-reach and poor villages in Malindi and Lamu districts in the coastal region classified as having endemic transmission of malaria. A cross-sectional household survey was conducted using a standardized malaria indicator survey.

**Table 1 (abstract P142).**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Baseline</th>
<th>Endline</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size (N)</td>
<td>269</td>
<td>345</td>
<td></td>
</tr>
<tr>
<td>Women caregiver education level</td>
<td></td>
<td></td>
<td>0.125</td>
</tr>
<tr>
<td>None</td>
<td>53.2 (143)</td>
<td>57.7 (199)</td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>43.5 (117)</td>
<td>41.2 (142)</td>
<td></td>
</tr>
<tr>
<td>Secondary</td>
<td>3.4 (9)</td>
<td>1.2 (4)</td>
<td></td>
</tr>
<tr>
<td>Woman caregiver age category</td>
<td></td>
<td></td>
<td>0.332</td>
</tr>
<tr>
<td>&lt;=20 y</td>
<td>21.2 (57)</td>
<td>15.7 (54)</td>
<td></td>
</tr>
<tr>
<td>21-30 y</td>
<td>44.6 (120)</td>
<td>47.1 (162)</td>
<td></td>
</tr>
<tr>
<td>31-50 y</td>
<td>26.7 (72)</td>
<td>29.4 (101)</td>
<td></td>
</tr>
<tr>
<td>51+ y</td>
<td>1.1 (4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>7.4 (20)</td>
<td>6.7 (23)</td>
<td></td>
</tr>
<tr>
<td>Male household head</td>
<td>84.1 (216)</td>
<td>81.7 (282)</td>
<td>0.285</td>
</tr>
<tr>
<td>Household owns radio</td>
<td>40.9 (105)</td>
<td>32.6 (113)</td>
<td>0.041</td>
</tr>
<tr>
<td>Household owns bicycle</td>
<td>56.4 (145)</td>
<td>48.1 (166)</td>
<td>0.044</td>
</tr>
<tr>
<td>Household owns mosquito nets</td>
<td>81.3 (209)</td>
<td>89.3 (308)</td>
<td>0.006</td>
</tr>
<tr>
<td>Village size</td>
<td></td>
<td></td>
<td>0.531</td>
</tr>
<tr>
<td>&lt;= 60 households</td>
<td>21.9 (59)</td>
<td>18.6 (64)</td>
<td></td>
</tr>
<tr>
<td>61 to 100 households</td>
<td>24.5 (66)</td>
<td>27.8 (96)</td>
<td></td>
</tr>
<tr>
<td>101 to 200 households</td>
<td>31.6 (85)</td>
<td>32.2 (111)</td>
<td></td>
</tr>
<tr>
<td>&gt;200 households</td>
<td>21.9 (59)</td>
<td>21.3 (74)</td>
<td></td>
</tr>
<tr>
<td>Household wealth rank</td>
<td></td>
<td></td>
<td>0.335</td>
</tr>
<tr>
<td>Most poor</td>
<td>22.3 (60)</td>
<td>23.8 (82)</td>
<td></td>
</tr>
<tr>
<td>Poor</td>
<td>54.3 (146)</td>
<td>57.7 (199)</td>
<td></td>
</tr>
<tr>
<td>Least poor</td>
<td>23.4 (63)</td>
<td>18.6 (64)</td>
<td></td>
</tr>
</tbody>
</table>
There was an increase in reported utilization of CHWs as source of advice/treatment for child fevers from 2% at baseline to 35% at end-line, accompanied by a decline in care-seeking from government facilities (from 67% to 48%) and other sources (26% to 2%) including shops.

The most poor households and poor households reported higher utilization of CHWs at 39.4% and 37.9% respectively, compared to the least poor households (17.0%). Households in villages with less than 200 households reported higher CHWs utilization as compared to households in villages having >200 households. Prompt access to timely and effective treatment was 5.7 times higher (95% CI 3.4-9.7) when CHWs were the source of care sought. Adherence was high regardless of whether source was CHWs (73.1%) or public health facility (66.7%).

Conclusion: The results of this study provide evidence that use of trained and supervised community health workers in community case management improved management of uncomplicated child fever cases in hard to reach villages in Malindi and Lamu District in Coastal Province of Kenya. In addition to this, poverty seems to be closely linked to child caregivers seeking services of community-based service providers, highlighting the impediment of poverty towards accessibility of cost sharing services widely practiced in Kenyan public health facilities. Policy actions to address barriers to effective utilization of CHWs in healthcare delivery should be scaled up in such hard to reach communities. The government and partners should, therefore, invest more in mechanisms which support CHW utilization especially the roll out of the Community Health Strategy 2006 as part of successful control of malaria and other infectious diseases.

The potential for utilization of CHWs in improving access to malaria treatment at the community level is promising. This will not only enhance access to treatment by the poorest households but also provide early and appropriate treatment to vulnerable individuals, especially those living in hard to reach areas.