Summary

The Malaria Centre's Epidemiological Studies range from the molecular diagnosis of *Plasmodium ovale* sub-species to programmatic monitoring and evaluation. Many of the activities in this section relate to the science of surveillance of infection and disease, complemented by modelling activities to inform our understanding of the potential impact of interventions. Other studies have developed molecular assays to discriminate between *Plasmodium ovale curtisi* and *Plasmodium ovale wallikeri,* then used them to explore the differences in latent periods between the two sub-species.

Malaria Centre members are contributing epidemiological expertise to a WHO-led project on the impact of insecticide resistance and, through this, the evaluation of the added benefit of Indoor Residual Spraying on insecticide-treated mosquito nets in Sudan, Kenya, Cameroon, Benin and India. Statistical analysis and data modelling have been used to inform an understanding of where Intermittent Preventive Treatment in Infants (IPTi) should be considered and the potential effects of broadening the age group receiving Intermittent Preventive Treatment (IPT).

Staff are working in Kenya to follow up on studies of school-based IPT to explore the potential of Intermittent Screening and Treatment to improve the health outcomes and educational achievement of school children. School-based screening programmes may also have the potential to identify transmission hotspots which could then be targeted with malaria interventions. The impact of malaria in West African school children is also being evaluated in a range of epidemiological settings.

It is evident that hotspots of transmission sustain transmission of malaria in some areas. The stability of hotspots is not entirely clear and school staff are exploring the potential of surveillance-based interventions to disrupt transmission in these hotspots. As control improves still further, the importance of reliably identifying gametocyte infections is thrown into sharp focus, as is the need to understand how best to collect and store filter paper samples. It has also been confirmed that sub-microscopic gametocyte infections are an important source of infections to the mosquito population with implications for surveillance in pre-elimination settings.

High level control in some countries also draws attention to the importance of monitoring cross-border malaria and being able to distinguish between *Plasmodium falciparum* and *Plasmodium vivax* infections in areas of low *Plasmodium falciparum* transmission. Encouraging work has shown the potential of anti-body responses to mosquito antigens to identify areas at high risk of malaria transmission.

Many of these activities will contribute to the de-

velopment of new approaches and tools for use in rational surveillance and response systems. However, it remains important to strengthen surveillance with the tools available today and LSHTM staff are working in West and East Africa to understand the role of different strategies for monitoring and evaluation of malaria control programmes.

Global project on the impact of insecticide resistance.

***LSHTM Investigators**: Immo Kleinschmidt.*

***External Investigators/Collaborators:** World Health Organization; Liverpool School of Tropical Medicine, UK; Ministries of Health of Sudan, Kenya, Cameroon, Benin & India.*

***Funding Body:** The Bill & Melinda Gates Foundation through the World Health Organization Global Malaria Programme.*

This study will assess the impact that insecticide resistance has on the effectiveness of malaria vector control tools Long Lasting Insecticide-treated Nets (LLINs) and Indoor Residual Spraying (IRS). In two countries this is done by cluster randomised trials of universal coverage LLINs versus universal coverage LLINs in combination with IRS, with levels of insecticide resistance of the main vector balanced between the two study arms.

In each cluster resistance to the insecticide used on LLINs is monitored, and malaria incidence is estimated from cluster specific cohorts of children followed up over the duration of the study. In three countries, clusters are established and malaria vector mosquitoes in each cluster assessed for resistance to the insecticide used. Clusters with either very high susceptibility or very high resistance to the insecticides in use are retained for the study, and cohorts of children recruited for follow-up and estimation of malaria incidence. Resistance impact will be assessed from the ratio of incidence rates in clusters with high compared to those with low resistance. Resistance mechanisms will be determined in subsets of study clusters. Initial findings from one country may be available by the end of 2012.

Combined use of vector control methods for malaria control.

***LSHTM Investigators**: Immo Kleinschmidt.*

***External Investigators/Collaborators:** Martin Donnelly (Liverpool School of Tropical Medicine, UK); Khalid Elmardi (Ministry of Health, Sudan); Josh Yukich (Tulane University, USA).*

***Funding Body:** Global Environmental Facility/ United Nations Environment Programme through the World Health Organisation Regional Office of the Eastern Mediterranean.*

The project will assess whether there is added benefit if Indoor Residual Spraying (IRS) and Long Lasting Insecticide-treated Net (LLINs) are used in combination, compared to the use of LLINs alone, in a large cluster randomised trial in four study areas of Sudan. Malaria incidence through passive case detection is estimated in cohorts of children in each study cluster for comparison between the study arms study as the primary endpoint. Clusters were randomised to study arms, balanced on a number of criteria including insecticide resistance at baseline. Vector control interventions according to the random allocation have been implemented at high coverage in 2011, cohorts recruited, community health workers trained and appointed, and follow-up of about 6,000 person years of observation completed during the malaria season. Interim results will be available in early 2013.

Children at Pokukrom primary school, Mankranso, Ashanti region, Ghana.

Intermittent Preventive Treatment (IPT) for African Children: where and how should IPT be applied?

***LSHTM Investigators**: Ilona Carneiro, Arantxa Roca-Feltrer, Lucy Smith, Joanna Armstrong-Schellenberg, Brian Greenwood & David Schellenberg.*

***External Investigators/Collaborators:** Tom Smith, Marcel Tanner & Amanda Ross (Swiss Tropical and Public Health Institute, Switzerland).*

***Funding Body:** The Bill & Melinda Gates Foundation and the UK Department for International Development.*

Intermittent Preventive Treatment in infants (IPTi) is the administration of a therapeutic dose of an antimalarial drug at the time of selected routine vaccinations through the Expanded Programme on Immunization. In 2010, IPTi with sulphadoxine-pyramethamine (SP) was recommended by the WHO for use "in areas of moderate to high malaria transmission" and "where parasite resistance to SP is not high".

Data on the age patterns of clinical cases of *Plasmodium falciparum* malaria, hospital admission with malaria parasites and malaria-associated death were used to estimate the percentage of cases of these outcomes that would occur in children aged < 10 years under different transmission intensity and seasonality of malaria. A similar analysis of severe malaria syndromes was also undertaken. A stochastic mathematical model of IPTi was used to predict the number of cases likely to be averted by implementing IPTi.

These results were combined into an internet-based decision-support tool to help policy-makers assess whether IPTi would be effective for local malaria control.

The web-tool has been updated to enable variation in IPTi schedule and coverage, health systems costs, and local levels of SP drug-resistance. It is freely accessible at http://ipti.lshtm.ac.uk.

Modelling the protective efficacy of alternative delivery schedules for Intermittent Preventive Treatment (IPT) of malaria in infants and children.

***LSHTM Investigators**: Matt Cairns, Roly Gosling, Ilona Carneiro, Paul Milligan, Brian Greenwood & Daniel Chandramohan.*

***External Investigators/Collaborators:** Lucy Okell & Azra Ghani (Imperial College London, UK); Francis Anto & Victor Asoala (Navrongo Health Research Centre, Ghana); Seth Owusu-Agyei (Kintampo Health Research Centre, Ghana).*

***Funding Body:** Medical Research Council PhD studentship; Navrongo trial originally funded by the UK Department for International Development.*

Intermittent Preventive Treatment for malaria in infants (IPTi) using sulphadoxine-pyrimethamine delivered alongside routine infant vaccinations is a recommended WHO policy. However, in seasonal transmission settings, some IPTi courses will be given at a time of low malaria risk, and would not tackle the burden of malaria outside infancy.

We investigated potential alternatives to delivery alongside infant vaccinations for a range of transmission scenarios using a mathematical model. Targeting IPT at the peak in transmission rather than at vaccination contacts would substantially improve efficacy, even in situations where the malaria burden is not highly seasonal. Extending IPT to include older children results in major additional gains in protection; this will become even more important as transmission is reduced by other control efforts and the disease burden shifts to older children.

Seasonal malaria chemoprevention in children: where could it be implemented and what would be its impact?

***LSHTM Investigators**: Matt Cairns, Anne Wilson, Diadier Diallo, Paul Milligan & Brian Greenwood.*

***External Investigators/Collaborators:** Arantxa Roca-Feltrer (Malawi-Liverpool-Wellcome Trust Clinical Research Programme, Malawi); Tini Garske & Azra Ghani (Imperial College London, UK).*

***Funding Body:** The Bill & Melinda Gates Foundation & Medical Research Council, UK.*

Seasonal Malaria Chemoprevention (SMC, previously known as Intermittent Preventive Treatment in children, IPTc), is highly effective in areas with a short malaria transmission season. To assist policy decisions made by WHO on the areas where this intervention would be appropriate, we have reviewed malaria incidence data and defined a surrogate predictor of seasonality in incidence based on rainfall. Spatial rainfall, malaria endemicity and population data were then used to estimate the population and malaria burden in areas with highly seasonal malaria. We estimate that in areas suitable for SMC, there are 39 million children under five years of age, 33.7 million child malaria episodes and 152,000 childhood deaths from malaria each year. The majority of this burden occurs in the Sahelian or sub-Sahelian regions of Africa, where regimens currently available for SMC have been shown to be highly effective, safe and acceptable to communities. SMC has the potential to avert several million malaria cases and tens of thousands of childhood deaths each year if successfully delivered to the populations at risk.

Population biology and epidemiology of two newly recognized human malaria parasite species.

***LSHTM Investigators**: Mary Oguike, Debbie Nolder & Colin Sutherland.*

***External Investigators/Collaborators:** Mallika Imwong (Mahidol – Oxford Research Unit, Thailand); Abdoulaye Djimde (Malaria Research & Training Centre, Mali); Alyssa Barry (Walter & Eliza Hall Institute, Australia).*

***Funding Body:** Wellcome Trust.*

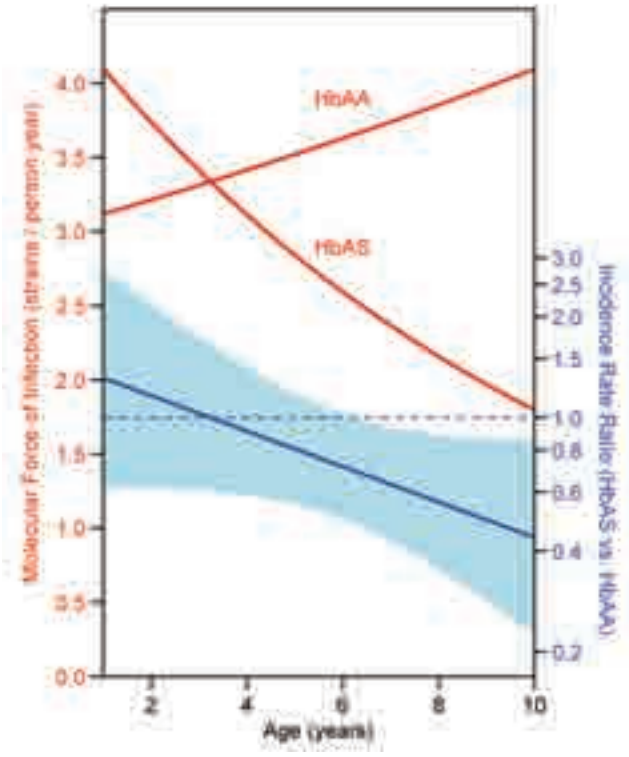
Recent evidence have shown that the human malaria parasite *Plasmodium ovale* exists as two non-recombining species, sympatric in many countries. New nested PCR assays targeting three different gene loci (*Potra, Pog3p* and *Porbp)* and real-time quantitative PCR assays for discriminating between *Plasmodium ovale curtisi* and *Plasmodium ovale wallikeri* have been developed and deployed in the field for species discrimination. Furthermore, we are investigating the possible barriers (biological, epidemiological and geographical) which keep these two species apart, when they occur together in much of tropical Africa and Asia. Recently, we have shown from analysis of samples and data from the Malaria Reference Laboatory archives that there is a significant difference in the latency period between *Plasmodium ovale curtisi* and *Plasmodium ovale wallikeri.* We are currently investigating the possible barriers to inter-species mating and recombination between the two species by looking at divergence of genes involved in fertilization. We have recently begun to evaluate both species by *in vitro* culture, and to test for sensitivity to a range of antimalarial drugs.

Evidence for both innate and acquired mechanisms of protection from Plasmodium falciparum in children with sickle cell trait.

***LSHTM Investigators**: Chris Drakeley.*

***External Investigators/Collaborators:** Lauren Gong, Philip Rosenthal, Alan Hubbard, Grant Dorsey & Bryan Greenhouse (University California San Francisco, USA); Catherine Maiteki-Sebuguzi (International Development Research Centre, Uganda).*

Sickle cell trait (HbAS) is known to be protective against *Plasmodium falciparum* malaria, but it is unclear when during the course of infection protection occurs and whether protection is innate or acquired. To address these questions, a cohort of 601 children aged 1-10 was enrolled in Kampala, Uganda, and followed for 18 months for symptomatic malaria and asymptomatic parasitemia. Genotyping was used to detect and follow individual parasite clones longitudinally within subjects. Children with HbAS were protected against establishment of parasitemia, as assessed by the molecular force of infection, at older but not younger ages (2 years old: incidence rate ratio [IRR] 1.16, 95%CI 0.62-2.19, p=0.6; 9 years old: IRR 0.50, 95%CI 0.28-0.87, p=0.01) suggesting an acquired mechanism of protection. Once parasitemic, children with HbAS were less likely to progress to symptomatic malaria, with protection again most pronounced at older ages (2 years old: RR 0.92, 95%CI 0.77-1.10, p=0.3; 9 years old: RR 0.68, 95%CI 0.51-0.91, p=0.008). Conversely, the youngest children were best protected against high parasite density (2 years old: relative density = 0.24, 95% CI 0.10-0.54, p=0.001; 9 years old: relative density = 0.59, 95%CI 0.30-1.19, p=0.14) suggesting an innate mechanism of protection against this endpoint.



CONFIA: causes of Non-malarial Fever in Afghanistan.

***LSHTM Investigators**: Amy Mikhail, Toby Leslie, Chris Whitty, Hugh Reyburn & Mark Rowland.*

***Funding Body:** The Bill & Melinda Gates Foundation through the ACT Consortium.*

In many areas of the world there has been a welcome reduction in malaria transmission. The result is that fewer cases who present with a fever have malaria. This makes diagnosis of both malaria and non-malaria cases important for patient care. It is often not known what the cause of the fever is when it is not malaria but when the symptoms in the patient resemble (and are often indistinguishable from) malaria.

The CONFIA project began enrolling patients in February 2012. It aims to identify as many of the non-malarial causes of fever as possible. These may be attributable to a range of viral, parasitic and bacterial diseases. The patients are enrolled in the study if they do not have malaria (their malaria negative status is confirmed with a malaria rapid diagnostic test). After taking a detailed history and physical examination, blood, respiratory and urine samples are taken from the patient and assessed using a range of laboratory techniques including culture, serological and molecular methods to screen for causative pathogens. The spectrum of diseases identified will be examined in light of their reported exposure factors and clinical data. Once there is a better understanding of non-malarial causes of fever, additional research can be undertaken to assist in improving treatment for fever when a malaria parasite test is negative and for providing information for control of the alternative causes of disease.

The project will be completed in December 2012 and results will be reported shortly thereafter.

Polyclinic in Afghanistan.

Evaluation of malaria diagnostic strategies using community health workers in South Central Asian where vivax is co-endemic with falciparum.

***LSHTM Investigators**: Toby Leslie, Amy Mikhail, Chris Whitty & Mark Rowland.*
***External Investigators/Collaborators:** HealthNet TPO; MERLIN & Health Protection and Research Organisation.*

***Funding Body:** The Bill & Melinda Gates Foundation through the ACT Consortium.*

In many low-resource settings where health services may be inaccessible, community health workers are relied upon to provide a range of services amongst which is the diagnosis and treatment of malaria. Until very recently this diagnosis was almost always based on clinical signs and symptoms of the patient. The clinical method of diagnosis based on symptoms alone, especially in areas with low malaria transmission, is notoriously inaccurate and results in up to 95% of patients being misdiagnosed with malaria when, in fact, the patient has some other cause of disease which may include severe bacterial diseases.

In partnership with three NGOs in Afghanistan, members of the Malaria Centre are undertaking a large-scale cluster randomised field trial to assess whether RDTs are appropriate tools for community health workers. The study is conducted amongst ~400 health workers who are administratively attached to 22 clinics in two provinces in Afghanistan. The primary outcome is the proportion of patients who are appropriately treated for their condition. The project is nearing its midpoint and results will be released in the coming year.

Malaria prevention in Kenyan school children.

***LSHTM Investigators**: Simon Brooker, Kate Halliday, Sian Clarke, Tom Drake, Lindsay Mangham, Kara Hanson, Caroline Jones & Elizabeth Allen.*

***External Investigators/Collaborators:** Kenya Medical Research Institute, Kenya; KEMRI-Wellcome Trust Research Programme, Kenya; University of Nairobi, Kenya; Kenya Ministry of Public Health and Sanitation, Kenya; Harvard University, USA; Duke University, USA.*

***Funding Body:** International Initiative for Impact Evaluation; World Bank & Wellcome Trust.*

Malaria can adversely affect schoolchildren, the full scale of which is yet to be investigated. An ongoing study is evaluating the impact of regular Intermittent Screening and Treatment (IST) for malaria on the health and educational achievement of schoolchildren living on the south coast of Kenya. Alongside the IST intervention, a training programme for teachers to improve literacy instruction has been introduced to investigate the interactions between health and education interventions. The evaluation uses a cluster-randomized design, with a total of 101 primary government schools randomly assigned to one of four groups (IST; education intervention; IST + education intervention; neither intervention). Baseline health and education surveys were conducted between January and March 2010. The first round of follow-up assessments was carried out between January and April 2011 and the second round between February and April 2012. The primary outcomes are educational achievement and anaemia, the hypothesised mediating variables through which education is affected. Secondary outcomes include malaria parasitaemia, school attendance and school performance. A nested process evaluation, using semi-structured interviews, focus group discussion and a stakeholder analysis is investigating the community acceptability, feasibility and cost-effectiveness of the interventions.

Malaria in school-aged children in Senegal: epidemiological risk, disease burden and strategies for control.

***LSHTM Investigators**: Sian Clarke, Daniel Chandramohan, Badara Cisse, Paul Milligan, Simon Brooker & Chris Drakeley.*

***External Investigators/Collaborators:** Jean-Francois Trape, Cheikh Sokhna & Alioune Badara Ly (Institut de recherché pour le developpement, Senegal); Oumar Gaye, Jean-Louis Ndiaye (Université Cheikh Anta Diop, Senegal); Malick Sembene, Aliou Dia & Khady Diallo (Ministry of Education, Senegal); Fatou Ba Fall (National Malaria Control Programme, Senegal); Matthew Jukes (Harvard Graduate School of Education, USA).*

***Funding Body:** Wellcome Trust.*

The risk of malaria is greatest in early childhood and most research and control has justifiably focussed on younger children. Less is known about the impact of malaria in older school-aged children. A recent intervention trial, in an area of intense perennial transmission in Kenya, showed that preventive treatment in schools, given once each term, can reduce malaria-related anaemia and increase sustained attention in class – suggesting that malaria control in schoolchildren could yield educational, as well as health benefits.

This study aims to characterise the epidemiological impact of malaria in school-aged children in a setting of marked seasonal transmission in the West African Sahel. The study compares the burden of malaria in children aged 6 months - 14 years in three different epidemiological settings within in Senegal: following three cohorts of 2400 children exposed to perennial transmission, intense seasonal transmission and low-moderate seasonal transmission over a 12-month period. The incidence of clinical attacks, prevalence of asymptomatic infection, anaemia and cognitive function is examined in two age groups (children below school age, and children enrolled in primary school) in each transmission setting.

The study is intended to inform the development of preventive interventions in schools suited to areas of seasonal transmission.

Stability of hotspots after implementation of community wide vector control with indoor residual spraying.

***LSHTM Investigators**: Simon Hemelaar, Teun Bousema, Nahla Gadalla, Brian Greenwood, Chris Drakeley, Daniel Chandramohan & Colin Sutherland.*

***External Investigators/Collaborators:** Jacklin Mosha (Kilimanjaro Christian Medical College, Tanzania); Ramadhan Hashim (National Institute for Medical Research, Tanzania); Roly Gosling (University of California, USA).*

***Funding Body:** The Bill & Melinda Gates Foundation.*

Malaria tends to be clustered in hotspots of high transmission intensity. The heterogeneity of malaria creates opportunities for targeted interventions, but it is unclear if hotspots remain stable after implementation. We aimed to establish the stability of hotspots after intervention with Indoor Residual Spraying (IRS).

Two surveys were conducted in the Mwanza region, Tanzania, before and after implementation of IRS. In total, 3031 people were included in both surveys. Parasite carriage was determined using sensitive nested PCR. Responses against malaria specific antibodies, *AMA*-1 and *MSP-1*, were used to determine the stable spatial patterns in transmission intensity. In the first survey, before implementation of IRS, parasite prevalence was 31.1% but varied between villages, from 24.9% to 60%. Two hotspots ($p \leq 0.01$) and two coldspots ($p \leq 0.01$) were detected. The current ongoing work aims to determine the stability of these hotspots and coldspots over time, 12 months after the initial survey and IRS implementation. We describe the persistence of hotspots after community-wide implementation of vector control. The success of any intervention greatly depends on its ability to reduce malaria transmission in hotspots.

Identifying and characterising foci of malaria transmission in potential malaria elimination settings in rural Senegal.

***LSHTM Investigators**: Badara Cissé, Catherine Pitt, Matt Cairns, Colin Sutherland, John Cox, Immo Kleinshmidt, Chris Drakeley, Brian Greenwood & Paul Milligan.*

***External Investigators/Collaborators:** Lansana Konaté, Babacar Faye, Ousmane Faye & Oumar Gaye (University Cheikh Anta Diop, Senegal); El hadj Ba, Jules-François Gomis, Cheikh Sokhna, Jean-François Trape (Institut de Recherche pour le Développement, Senegal).*

***Funding Body:** Bill & Melinda Gates Foundation through the Malaria Capacity Development Consortium.*

Sparse distribution of malaria morbidity has become a consistent feature of the disease, particularly in the new context of malaria decline. Foci of residual transmission (hot spots) are particularly relevant to malaria control programme in countries such as Senegal with low transmission intensity, since their presence makes it very difficult to eliminate the disease.

Based on the 2008 and 2009 general changes on the epidemiological profile of the disease and in particular on the very low incidence rates observed all over the sub-region, we designed this project to identify and characterize the foci of residual transmission and to pilot methods for surveillance that could be used as part of an elimination programme. Scientific research questions were therefore the following:

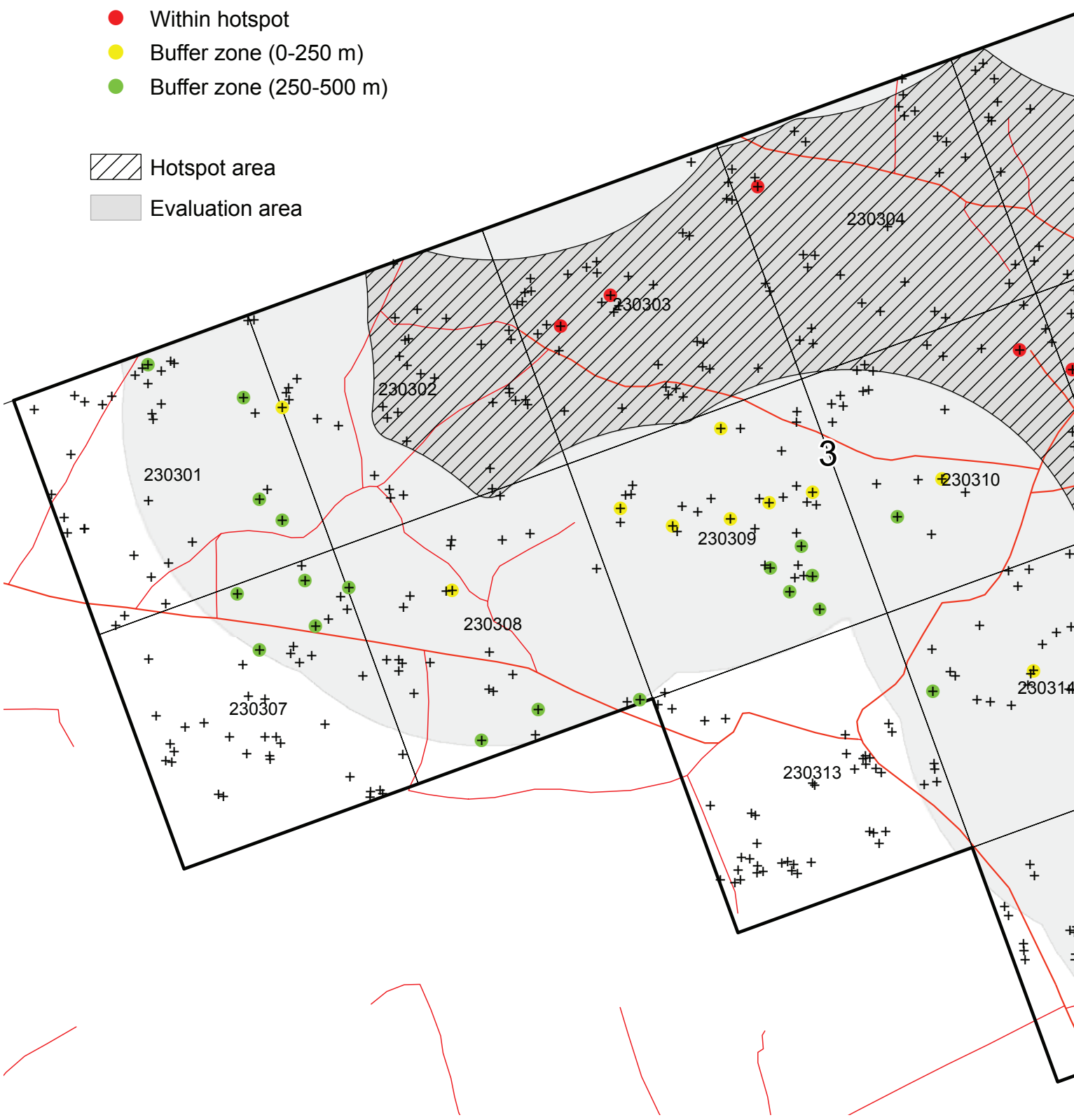
- Has transmission stopped in some areas?
- Evaluation of the validity of health facilities records
- Use of serology to determine if transmission of malaria has stopped in defined area.
- Why is transmission persisting in some places?

Several factors could explain the patchy distribution of malaria (use of preventive measures, housing, environmental or climatic factors.

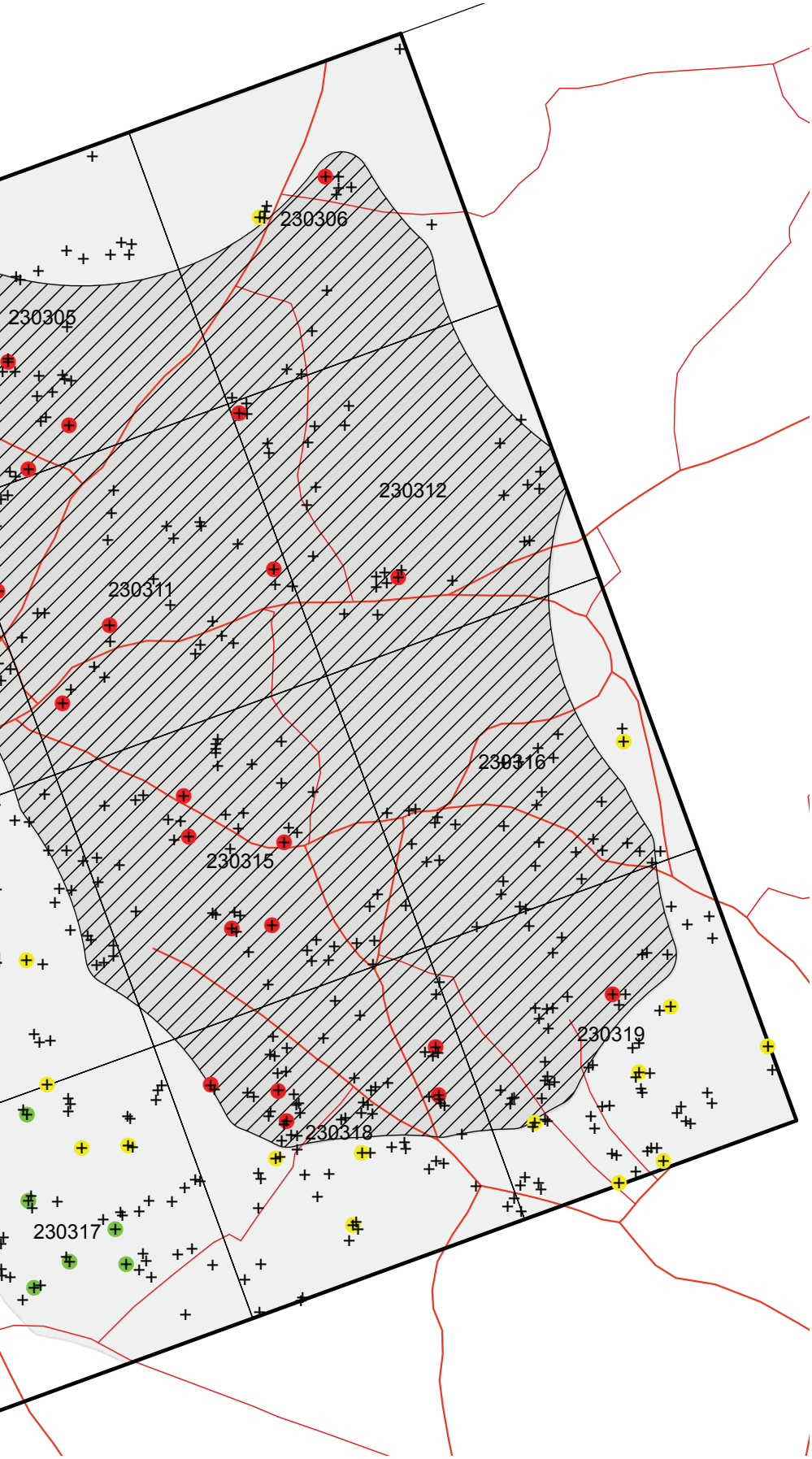
Several activities were conducted to answer to the research questions including:

- A collection and analyses and validity assessment of 2008-2011 health facilities records.
- A case-control study with a community survey around all cases and controls intervention coverage in the vicinity of where the cases and the controls live.
- Cross sectional surveys to assess level of malaria transmission from serology.
- An environmental evaluation in and in areas with low transmission.

The entomological evaluation including nets assays in hotspots and non hotspots areas will be carried out in 2012.



Detail of hotspot intervention area in highland Kenya



Reducing the burden of malaria by targeting hotspots of malaria transmission (REDHOT).

***LSHTM Investigators**: Teun Bousema, Jonathan Cox, Jennifer Stevenson, Ulrike Fillinger & Chris Drakeley.*

***External Investigators/Collaborators:** Nabie Bayoh, Meghna Desai, Simon Kariuki, Kayla Laserson, John Vulule (Kenya Medical Research Institute, Kenya & Centers for Disease Control and Prevention, USA); Robert Sauerwein (Radboud University Nijmegen Medical Centre, the Netherlands); Willem Takken (Wageningen University, the Netherlands).*

***Funding Body:** The Bill & Melinda Gates Foundation through the Malaria Transmission Consortium and Grand Challenge.*

In the Rachuonyo district in the Kenyan highlands, the burden of malaria is not equally distributed with community parasite prevalences ranging from 0% to >70%. The persistence of malaria in small geographical areas despite high coverage with Insecticide Treated Nets (ITNs) and Indoor Residual Spraying (IRS) may be partially explained by outdoor biting and resting of malaria vectors and suggests that a revision of malaria control strategies may be needed to successfully control and eliminate malaria.

We identified 10 hotspots of intense malaria transmission by serological markers of malaria exposure. These hotspots will be randomly assigned to serve as intervention or control clusters. In the intervention clusters, four malaria interventions will be targeted to the hotspots: local upscaling of IRS and ITNs, larviciding and a focal screening and treatment campaign. In control clusters, IRS and ITN distribution will continue as planned by the Kenyan division of malaria control.

The impact of the targeted interventions on overall transmission intensity will be assessed in the context of currently ongoing malaria control activities in a 2-year study.

Clinical development and field evaluation of a malaria transmission blocking vaccine (REDMAL).

***LSHTM Investigators**: Teun Bousema, Sophie Jones, Michael Bretscher & Chris Drakeley.*

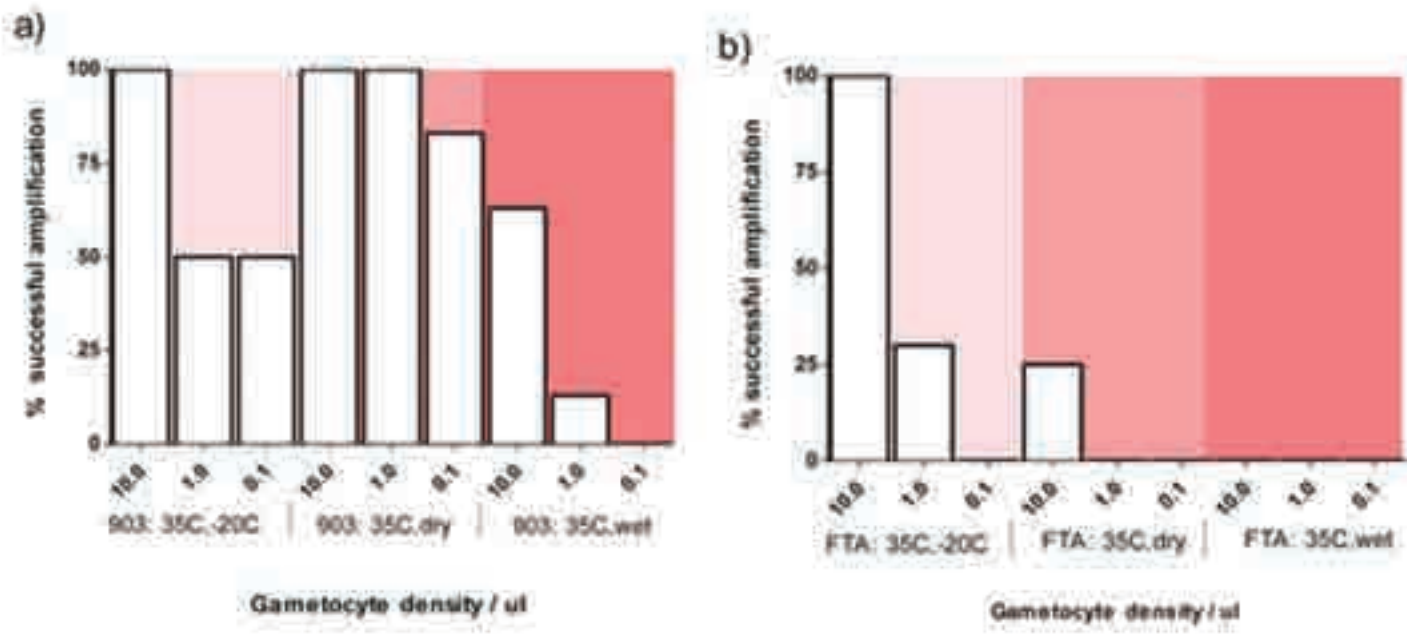
***External Investigators/Collaborators:** Robert Sauerwein, Will Roeffen (Radboud University Nijmegen Medical Centre, the Netherlands); Michael Theisen (Statens Serum Institute, Denmark); Sanjay Singh (Gennova Biopharmaceuticals, India); Jaffu Chilongola (Kilimanjaro Christian Medical Centre, Tanzania); André Lin Ouédraogo (Centre National de Recherche et de Formation sur le Paludisme, Burkino Faso); Dawit Ejigu (African Malaria network Trust, Tanzania).*

***Funding Body:** European Committee, 7th framework programme.*

REDMAL aims to develop a transmission blocking vaccine for malaria. Transmission-blocking vaccines arrest the development of the sexual stage of the malaria parasite. This interruption of the life cycle of the parasite inhibits the generation of infectious mosquitoes leading to reduction of the spread of malaria. This is indispensible for sustained control, elimination and eventually eradication of malaria.

The workpackage coordinated by LSHTM involves the development of field assays for trials with malaria transmission blocking vaccines and the preparation of field sites. One of the first components of this work involved testing different strategies for the detection of low density gametocyte carriage. Different filter paper matrices and different gametocyte detection tools were compared. In the figure below, 903 protein saver cards (a) and FTA cards (b) are compared for storage of gametocyte mRNA under tropical conditions. Cultured gametocytes were blotted at different concentrations (10, 1 or 0.1 gametocytes/µL); filter papers were stored for one week at 35°C and subsequently for three months at 35°C or -20°C. Filter papers were stored with dessicant or in wet conditions. Gametocyte detection by *Pfs25 QT-NASBA* indicates that RNA degradation occurs at higher temperatures and when filter papers are not properly dried but that degradation depends on the filter paper used. *Pfs25* mRNA can be reliably extracted from 903 Protein Saver Cards several months after storage.

Submicroscopic gametocyte carriage is now determined in relation to sexual stage specific immune responses at three field sites in West and East-Africa.



Gametocyte detection using two filter paper types stored under tropical conditions.

Assessment of the Infectious Reservoir of Malaria (AFIRM).

***LSHTM Investigators**: Teun Bousema & Chris Drakeley.*

***External Investigators/Collaborators:** Robert Sauerwein (Radboud University Nijmegen Medical Centre, the Netherlands); André Lin Ouédraogo, Sodiomon Sirima (Centre National de Recherche et de Formation sur le Paludisme, Burkina Faso); Kevin Marsh & Charles Mbogo (Kenya Medical Research Institute, Kenya); Richard Mukabana (ICIPE, Kenya); Tom Churcher (Imperial College, London, UK).*

***Funding Body:** The Bill & Melinda Gates Foundation.*

The AFIRM project will establish a standardized framework for assessing population infectivity to mosquitoes and thus the infectious reservoir in different malaria endemic settings. This will enable identification of target populations and optimal timings for control efforts including malaria Transmission Blocking Vaccines (MTBV). Data generated by the project will fill crucial gaps in mathematical models of malaria transmission and will allow examination of correlates between routinely collected clinical, parasitological and entomological metrics by quantifying the processes that link these metrics.

As a first step the project is improving and validating currently available assays in order to develop a quantitative measure of infectivity to mosquitoes that is suitable for large scale screening of naturally infected individuals. Molecular and serological approaches will be validated in laboratory settings and subsequently implemented in field trials that aim to determine the human infectious reservoir for malaria in settings in Burkina Faso and Kenya that are representative of the different patterns of malaria transmission in Africa. Longitudinal studies will be conducted to determine the relative importance of symptomatically and asymptomatically infected individuals in the context of natural exposure to mosquitoes.

Epidemiology of border malaria in Namibia.

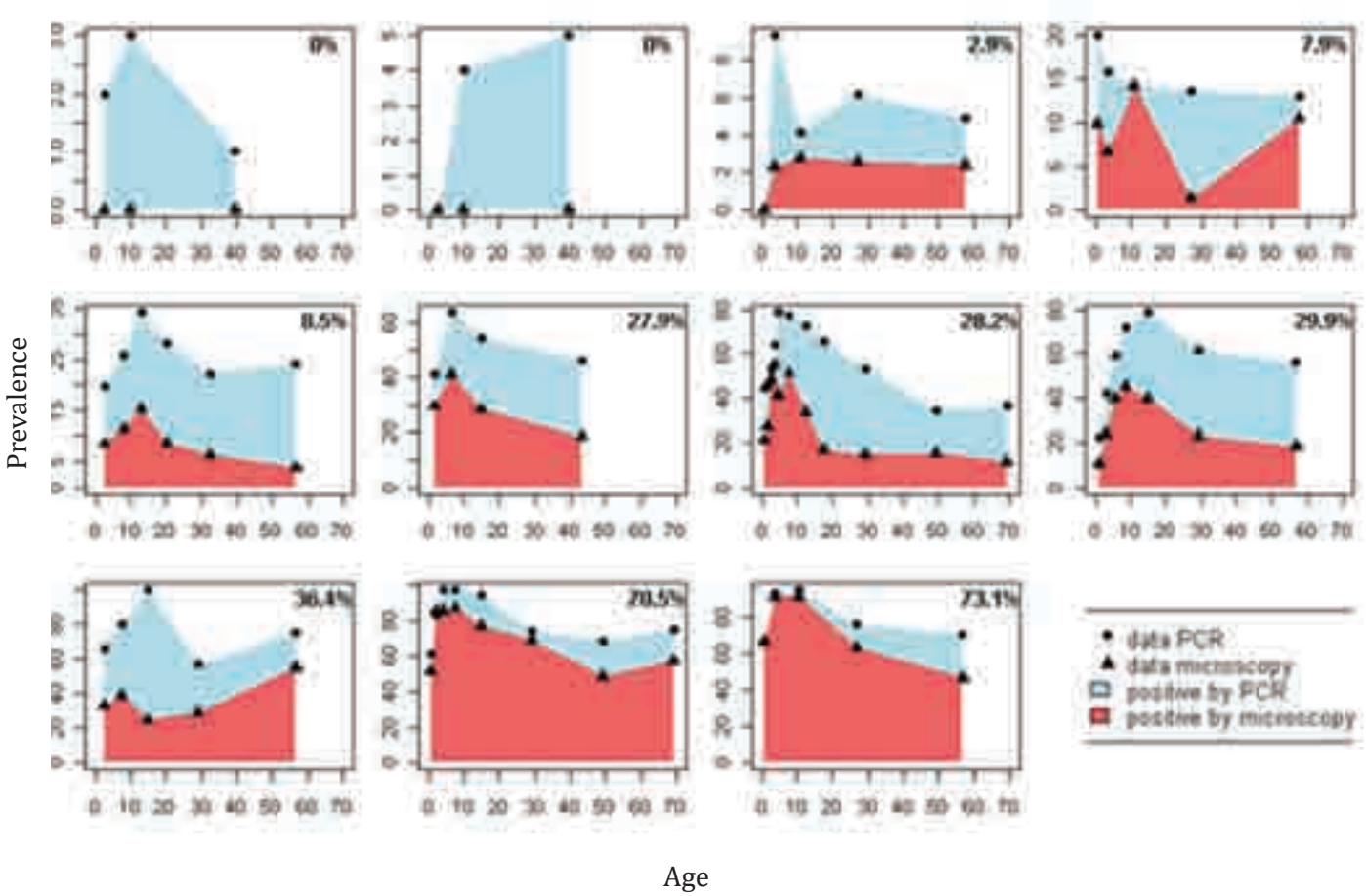
***LSHTM Investigators**: Immo Kleinschmidt.*

***External Investigators/Collaborators:** Roly Gosling & Hugh Sturrock (University of California, USA), Daves Mumbengegwi (University of Namibia, Namibia), Stark Katokel (National Malaria Program, Namibia).*

***Funding Body:** The Bill & Melinda Gates Foundation.*

Malaria incidence has declined to low levels in Namibia, and the country has set 2020 as the target for malaria elimination. Many of the residual cases occur in the border regions with Angola. It is possible that malaria transmission is associated with cross border movements of people between Angola and Namibia. This study will document the epidemiology of malaria in northern Namibia and strengthen the malaria surveillance system in the border district of Engela, in Ohangwena region. A case control study will be carried out to determine risk factors for malaria, including cross-border travel, intervention coverage and compliance, and local factors that may be linked to transmission of malaria. By mapping cases and controls, and testing for sub-patent infections and antibody seroconversions in the neighbourhood of each case and each control, the study will investigate the existence of any hotspots of transmission. It is expected that the results of the study will considerably aid the National Malaria Control Program of Namibia to target and optimise its strategies for malaria elimination.

Children on their way to school in The Gambia.



Molecular detection of malaria: what lies beneath the microscopy detection threshold and is it relevant for control?

***LSHTM Investigators**: Chris Drakeley & Teun Bousema.*

***External Investigators/Collaborators:** Lucy Okell, Jamie Griffin & Azra Ghani (Imperial College London, UK); André Lin Ouédraogo (CNFRP, Burkina Faso).*

***Funding Body:** The Bill & Melinda Gates Foundation; Medical Research Council, UK & Wellcome Trust.*

Malaria prevalence in population surveys has traditionally been assessed by microscopy however there are increasing numbers of surveys which describe estimates of parasite carriage by molecular methods. PCR estimates are almost invariably higher than those by microscopy and raise important questions for epidemiology, basic biology and control of the parasite. This study quantifies the relationship between microscopy and molecular parasite prevalence estimates and shows that overall 54.1% (95% CI 50.3-58.2%) more individuals are infected when assessed by PCR compared with microscopy. We further described the prevalence of submicroscopic parasite carriers and assess their contribution to onward transmission to mosquitoes according to level of endemicity. We have shown that these low density submicroscopic infections are common in adults and in low endemic settings where they can form as much as half of the human infectious reservoir.

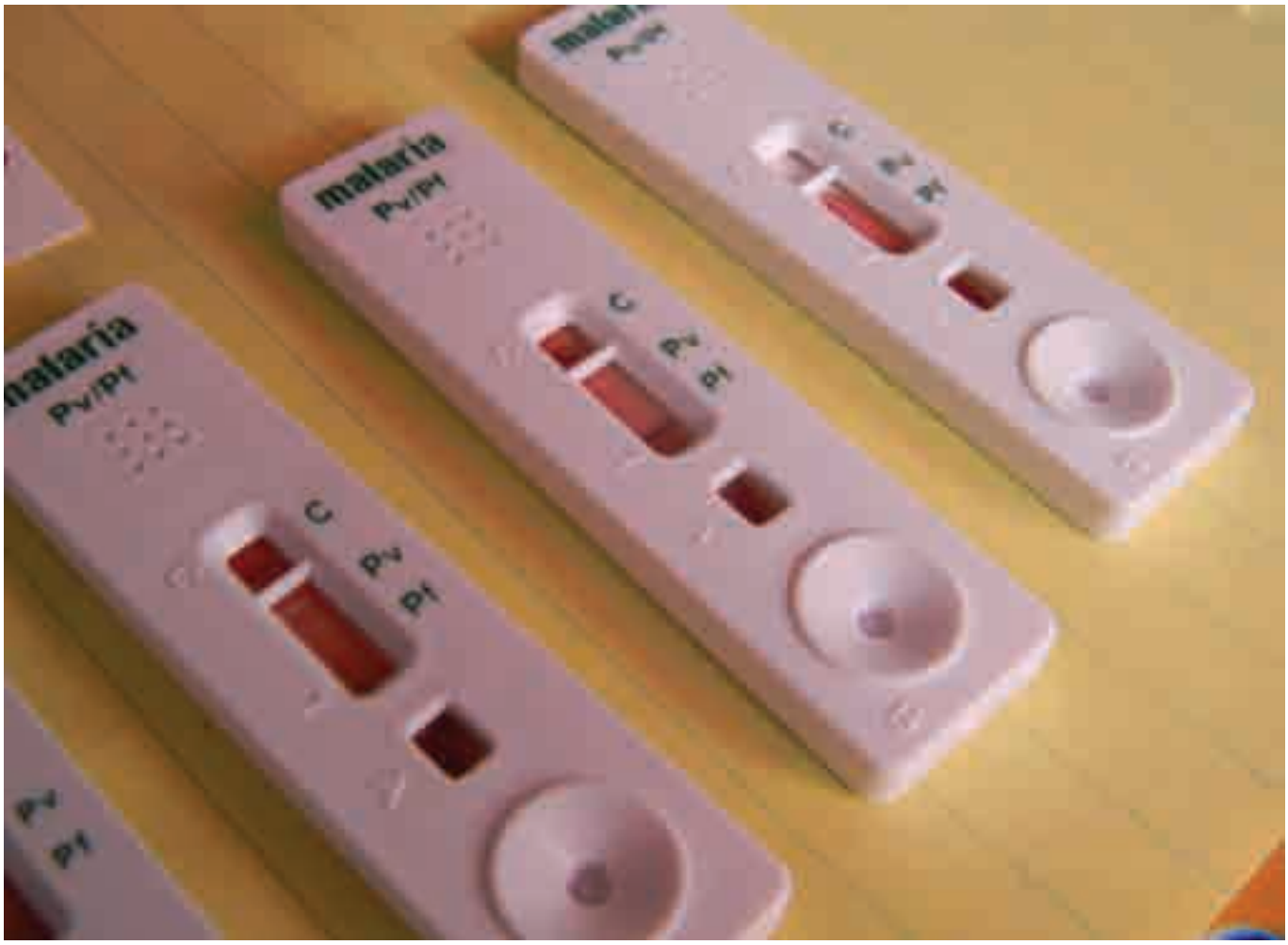
Identifying areas of ongoing malaria transmission in Southern Mindanao, the Philippines.

***LSHTM Investigators**: Mary Grace Dacuma, Rachel Hallett & Colin Sutherland.*

***External Investigators/Collaborators:** Antonio Yasaña (Provincial Health Office, Philippines); Federico Yadao (Malaria Control Office, Philippines); Ernesto Bona (Department of Health Region XII, Philippines); Walter Notario; (Pilipinas Shell Foundation, Philippines); Judeline Dimalibot (University of the Philippines Los Baños, Philippines).*

***Funding Body:** The Ford Foundation; The Chadwick Trust; University of the Philippines Los Baños; Philippine Council for Health Research and Development.*

Intensive malaria control efforts have reduced parasite prevalence to very low levels in parts of the Philippines, but pockets of transmission remain. To realise the goal of malaria elimination in this region, it is important to determine where parasites still circulate. In this ongoing PhD study, a cross sectional survey was conducted in nine villages in Sarangani, Southern Mindanao and blood samples from participants were tested by Rapid Diagnostic Test (RDT) and Polymerase Chain Reaction (PCR). RDTs significantly underestimated the prevalence of parasites, compared to PCR, particularly in infections with *Plasmodium vivax*. This indicates that sensitive molecular methods are more suited to detecting remaining low levels of parasite infection. A further use of PCR has been to show that the simian *Plasmodium knowlesi* parasite, recently shown to cause human disease in Malaysian Borneo, is not present in this study population. Remaining work will be to assess the participants' levels of antimalaria antibodies in a bid to further understand the precise location and risk factors for ongoing malaria infection in the Southern Mindanao region. It is hoped that results will enable local malaria control programmes to focus efforts and resources appropriately as they move towards elimination.



Rapid diagnostic test for detection of Plasmodium falciparum and Plasmodium vivax infections during a cross sectional survey in selected villages of Southern Mindanao, the Philippines.

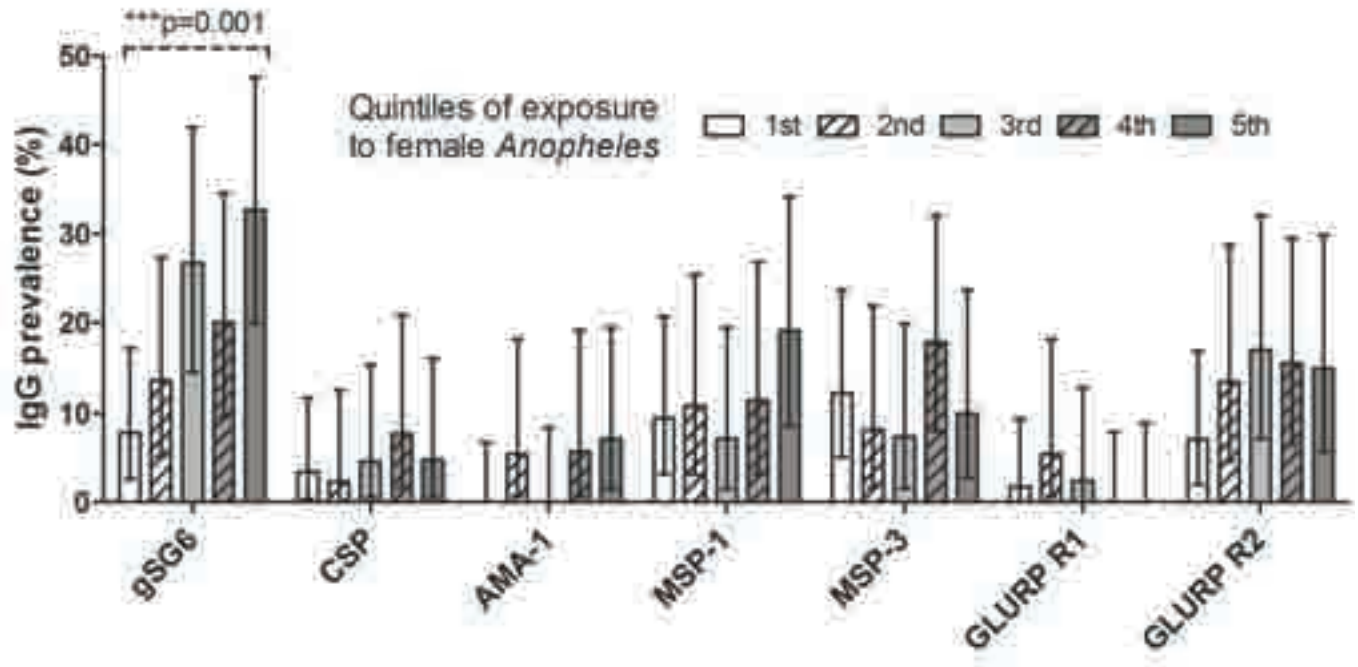
IgG responses to the recombinant Anopheles gambiae salivary antigen gSG6 detect small-scale spatial variation associated with malaria vectors and predict risk of clinical disease.

***LSHTM Investigators**: Will Stone, Teun Bousema, Sophie Jones, Ilona Carneiro, Daniel Chandramohan & Chris Drakeley.*

***External Investigators/Collaborators:** Samwel Gesase & Ramadani Hashim (National Institute for Medical Research, Tanzania); Roly Gosling (University of California, USA); Thor Theander (Centre for Medical Parasitology, Denmark); Bruno Arca (La Saienza, Italy).*

***Funding Body:** Wellcome Trust & the IPTi Consortium.*

Accurate assessment of exposure to malaria vectors is essential to our understanding of spatial and temporal variations in disease risk and facilitates the reliable targeting and evaluation of control efforts. Recently, an immunogenic *Anopheles gambiae* salivary protein (gSG6) was identified and proposed as the basis of an immuno-assay determining exposure to Afrotropical malaria vectors. In the present study, IgG responses to gSG6 and 6 malaria antigens (CSP, AMA-1, MSP-1, MSP-3, GLURP R1, and GLURP R2) were measured among children from Korogwe district, Tanzania; an area of moderate and heterogeneous malaria transmission. Anti-gSG6 responses were positively associated with geographical variations in *Anopheles* exposure, in accordance with the distribution of anti-malarial responses previously observed in the same area. Additionally, at the individual level, IgG responses to gSG6 showed a strong positive association with mosquito exposure. IgG level for all antigens except AMA-1 was associated with the frequency of malaria episodes following sampling, and strikingly - gSG6 seropositivity was a strong positive indicator of subsequent malaria incidence, comparable to malaria antigens MSP-1 and GLURP R2. Our results indicate that the gSG6 assay is sensitive to micro-epidemiological variations in exposure to Anopheles mosquitoes, and may provide a correlate of malaria risk that is unrelated to immune protection. Overall, these findings strengthen the case for the application of the gSG6 assay in epidemiologic studies and in the evaluation and planning of targeted and preventative anti-malaria interventions.



Relationship between mosquito exposure and antibody responses to gSG6 and malaria antigens.

Bioko Island malaria control project, phase II.

***LSHTM Investigators**: Immo Kleinschmidt, Chris Drakeley, Colin Sutherland, Andrea Rehman, John Bradley & Andrea Mann.*

***External Investigators/Collaborators:** Christopher Schwabe (Medical Care Development International); Michel Slotman (Texas A&M University, USA); Janet Hemingway (Liverpool School of tropical Medicine, UK); John Vontas (Innovative Vector Control Consortium).*

***Funding Body:** Marathon Oil Co.*

The Bioko Island Malaria Control Project, in collaboration with the government of Equatorial Guinea introduced an integrated malaria control programme in 2004 consisting of delivery of Indoor Residual Spraying (IRS) and Long lasting Insecticidal Nets (LLIN) to all households, malaria case management consisting of Artemisinin-based Combination Therapy and definitive diagnosis, Intermittent Preventive Treatment for pregnant women, training of health workers and information and education campaigns. Major reductions in malaria transmission were documented during the first five year phase of the project (2004-2008). Progress and impact are being extensively monitored through a surveillance system consisting of (1) annual household surveys collecting a number of biomarkers, and information on child mortality, illness episodes, household wealth and health seeking behaviour; (2) patient information systems; (3) entomological surveillance; (4) serological surveys; and (5) monitoring of drug resistance associated mutations in *Plasmodium falciparum.*

This project serves to gain experience in critical aspects of malaria control and surveillance in control and pre-elimination phases.

In a programmatic setting, the following questions are being addressed: the causes of the heterogeneity of the impact of the interventions on the island; the evaluation of insecticide resistance management; evaluation of the effectiveness of combining interventions; developing and evaluating new malaria control monitoring tools (serological conversion, ITN and IRS test kits, computerised microscopy for parasitology); investigating the operational impact of the kdr gene in *Anopholes gambiae* on the effectiveness of pyrethroid based vector control; documentation of changes in malaria epidemiology; assessment of practical limitations of IRS and LLINs.

PRISM: Program for Resistance, Immunology, Surveillance and Modeling of Malaria in Uganda.

***LSHTM Investigators**: Sarah Staedke & Chris Drakeley.*

***External Investigators/Collaborators:** Grant Dorsey & Philip Rosenthal, Edwin Charlebois & Bryan Greenhouse (University of California, USA); Moses Kamya, Harriet Mayanja-Kizza, Samuel Nsobya & Fred Wabwire-Mangen (Makerere University, Uganda); David Smith & Andy Tatem (University of Florida, USA); Martin Donnell (Liverpool School of Tropical Medicine, UK); Steve Lindsay (Durham University, UK).*

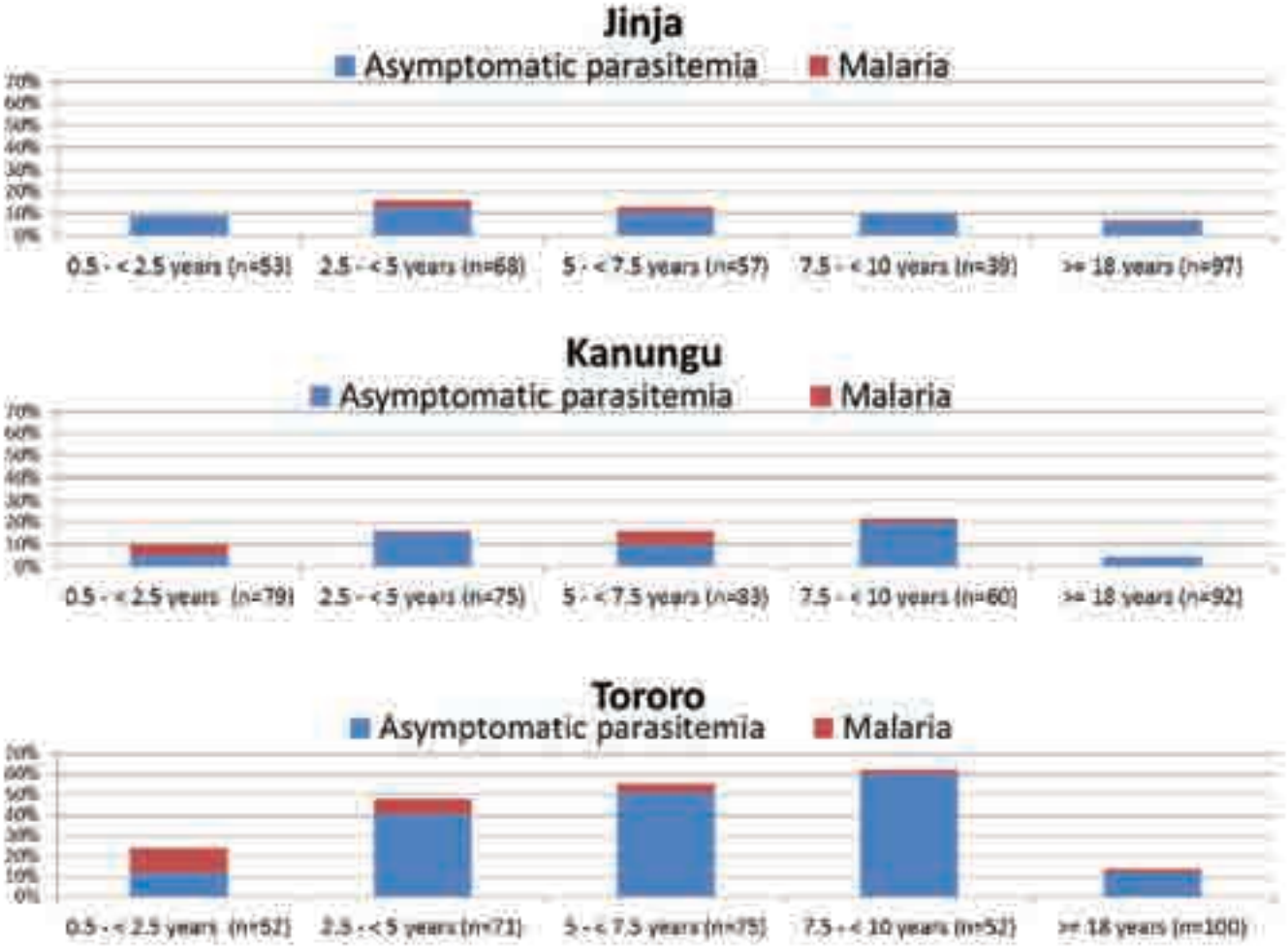
***Funding Body:** US National Institutes of Health.*

An International Centre of Excellence in Malaria Research was established in Uganda in 2010, focusing on surveillance, immunology and parasite and insecticide resistance. This programme aims to address the complexity of interactions between the mosquito vector, malaria parasite, and human host, and combine standard malaria surveillance techniques and metrics with cutting-edge methods designed to improve surveillance.

The surveillance project will identify optimal strategies for malaria surveillance, estimate the impact of key control interventions and conduct an economic evaluation of control interventions in different epidemiological settings. The immunology project will characterize the individual-level relationships between *Plasmodium falciparum* exposure, the immune response and protection from disease and develop and validate immunologic assays for estimating the population-level dynamics of exposure to *Plasmodium falciparum*. The resistance project will compare the prevalence of molecular markers of antimalarial drugs and anopheline insecticide resistance and search for novel mediators of antimalarial and insecticide resistance using transcriptome and high throughput sequencing techniques.

Comprehensive malaria surveillance, including outpatient and inpatient surveillance, entomology studies, cohort studies and cross-sectional surveys of communities and primary schools, was implemented in three sites in Uganda in 2011. Samples collected in the field are being utilized by the immunology and resistance projects. In 2013, the surveillance field activities will be streamlined and will continue through 2017.

Cross-sectional parasitemia data at enrollment



Prevalence of asymptomatic parasitemia and clinical malaria by age, in cross-sectional surveys in Uganda.

PRISM Cohort study team, Jinja, Uganda.