
Malaria centre
Malaria Centre
Report
2017-2019
LONDON
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MEDICINE

Contents

	Executive Summary	4
	New Malaria Labs	6
	Worldwide Locations of Research	8
	Malaria Research in The Gambia	10
	Parasite Biology & Drug Discovery	13
	Human Pathology and Disease Response	29
	Vector Biology	38
	Epidemiology and Transmission	45
	Vector Control	52
	Chemoprevention	61
	Clinical Research, Drug Efficacy & Resistance	69
	Malaria Elimination	77
	Surveillance	83
	Social, Economic and Policy Research	92

Executive Summary

In 2018 the LSHTM Malaria Centre celebrated its 20th anniversary, and this report describing the Centre's activities over the past few years demonstrates our global impact as a hub for malaria research. The Malaria Centre has changed over time, as our multi-disciplinary nature permits us to adapt to evidence needs, respond to findings that require a change of direction and take advantage of new opportunities in collaboration and funding. As such our work at LSHTM reflects changes in malaria policy over the last two decades:

- In the early years of the Malaria Centre, we were performing controlled trials to demonstrate the advantages of insecticide-treated nets (ITN) over untreated nets; with ITN now distributed in vast numbers across the malaria endemic world, we are working on demonstrating efficacy of a new generation of nets.
- In the noughties, our drug trials were demonstrating the benefits of artemisinin combination therapy (ACT), over chloroquine and sulfadoxine-pyrimethamine, for treating African children with malaria; with ACT now standard policy in virtually all malaria endemic countries. We are working hard towards the development and evaluation of the next generation of antimalarial drugs as there is some threat to artemisinin efficacy in SE Asia, which may spread to other regions.
- Malaria Centre staff and PhD students performed the first trials of intermittent preventive treatment in pregnant women (IPTp), infants (IPTi) and children under 5 in seasonal areas (SMC). Now that all three strategies are accepted and promoted by the WHO, we are contributing to rolling out seasonal malaria chemoprevention (SMC) at implementation scale across sub-Saharan African, and monitoring the impact of the intervention on the development of drug resistance in malaria parasite populations.

As a Centre our research portfolio is very broad, but there focus on three key areas over the time period captured in the Report. Firstly, in response to evidence of increasingly widespread insecticide resistance in malaria vector populations, there are a number of projects on aspects of vector control, including early testing of new insecticidal compounds. Secondly, as we are in the second decade of ACT use on a global scale, and there is evidence of falling efficacy, we have a group of studies aimed at describing and monitoring determinants of resistance to artemisinin and its partner drugs, and others supporting the drug development pipeline at the discovery, pre-clinical and clinical testing stages. Thirdly, something unheard back in 1998, malaria elimination is now high on the agenda of the Malaria Centre, and projects in this area are a good example of how we have broadened our geographical focus to low transmission settings in Africa, Asia and the Americas, including Haiti.

Good malaria research requires good infrastructure, and in December 2018 Malaria Centre members moved into a dedicated suite of brand new, well equipped malaria laboratories, the result of substantial investment from LSHTM, and the generosity of donors including the Wellcome Trust and the Wolfson Foundation. Inside the report, you will find a feature article on the new labs.

Another important development is the welcoming of the MRC The Gambia, and the MRC Uganda, under LSHTM. Welcome to all our new Malaria Centre members from both of these sites! We provide a feature summarising current malaria activities in The Gambia, and also expect to be setting out new proposals for malaria-related work with our new partners in Uganda. These welcome developments provide us with great opportunities to further enhance the Centre's research presence in these two important malaria-endemic areas – the West African Sahel and the Great Lakes Region – where we already have valued partners and collaborators.

International collaboration is the key to success as we seek to contribute as the globally-recognised authoritative voice on malaria research – the projects summarised in this report illustrate perfectly the value Centre members place on these relationships with important partners and around the world.

It is this authoritative voice that engages in conversation with Malaria Control Programmes across Asia, Africa and the Americas, through crucial partnerships such as the LINK project and Access-SMC (see abstracts). This brings with it the responsibility for nurturing the next generation of endemic country and UK researchers, through joint research projects, PhD training, our internal and external Masters programmes, exchange visits and our support for African Post-Docs through the MARCAD programme. This commitment to capacity strengthening is implicit in everything we do.

However, after unprecedented reductions in malaria mortality since the millennium, we now see a plateau worldwide. Malaria is still one of the world's deadliest diseases and presents a sustained public health threat, particularly for the most vulnerable populations. Urgency must continue over the next 20 years to encourage further scientific advancements and make malaria elimination realistic on a global scale. We do hope you enjoy reading this report and look forward to the developments this research will bring.

Sian Clarke

Colin Sutherland

(Co-Directors of the LSHTM Malaria Centre)

Hannah Gladstone
(Malaria Centre Coordinator)

New Malaria Labs

LSHTM has recently embarked upon a £20 million refurbishment programme to develop new world-class research facilities. This included the commissioning of a new malaria laboratory suite on the third floor of our Keppel Street site. This state-of-the art facility is comprised of a large open-plan molecular biology laboratory with bench space for 32 researchers and a shared malaria parasite culture suite with 8 microbiological safety hoods, dedicated autoclaves and full Containment Level 3. The new lab brings together more than 10 different independent research groups previously spread across six separate laboratories. This has resulted in significant gains in usable space and means equipment can be far more efficiently shared.

The researchers working in this space represents the majority of laboratory-focused human *Plasmodium research within the LSHTM, and cut across 3 key themes:*

- serological and molecular epidemiology (Beshir, Sutherland, Tetteh groups),
- *Plasmodium population genetics (Campino, Clark, Conway, Sutherland groups)*
- *in vitro studies of cultured human malaria parasites (Baker, Conway, Delves, Moon, van Ooij, van Schalkwyk, Sutherland groups)*

This selection creates significant synergies, both in terms of encouraging collaboration and in ensuring access to multi-user equipment. Molecular biology lab researchers share:

- facilities for DNA and protein production and electrophoretic analysis
- bacterial cloning tools
- thermocyclers for PCR and qPCR for analysis of parasite samples from the field or transgenic parasites generated in the culture facilities

The malaria culture suite is a containment facility providing all the equipment required to efficiently culture and genetically modify both laboratory-adapted-strains and clinical isolates of *Plasmodium falciparum* and *P. knowlesi*, and short-term culture of clinical isolates of the other human infective species – *P. vivax*, *P. ovale* spp., *P. malariae*. Malaria Centre staff and students working in this facility are evaluating novel vaccine and drug candidates as well as establishing the biological role of individual parasite proteins through gene knockout and fluorescent tagging experiments.

> ***Shiny and new, I couldn't resist sneaking in to the new labs to run some PCR experiments myself over the Christmas break***
>
> Colin Sutherland, Co-Director of the Malaria Centre

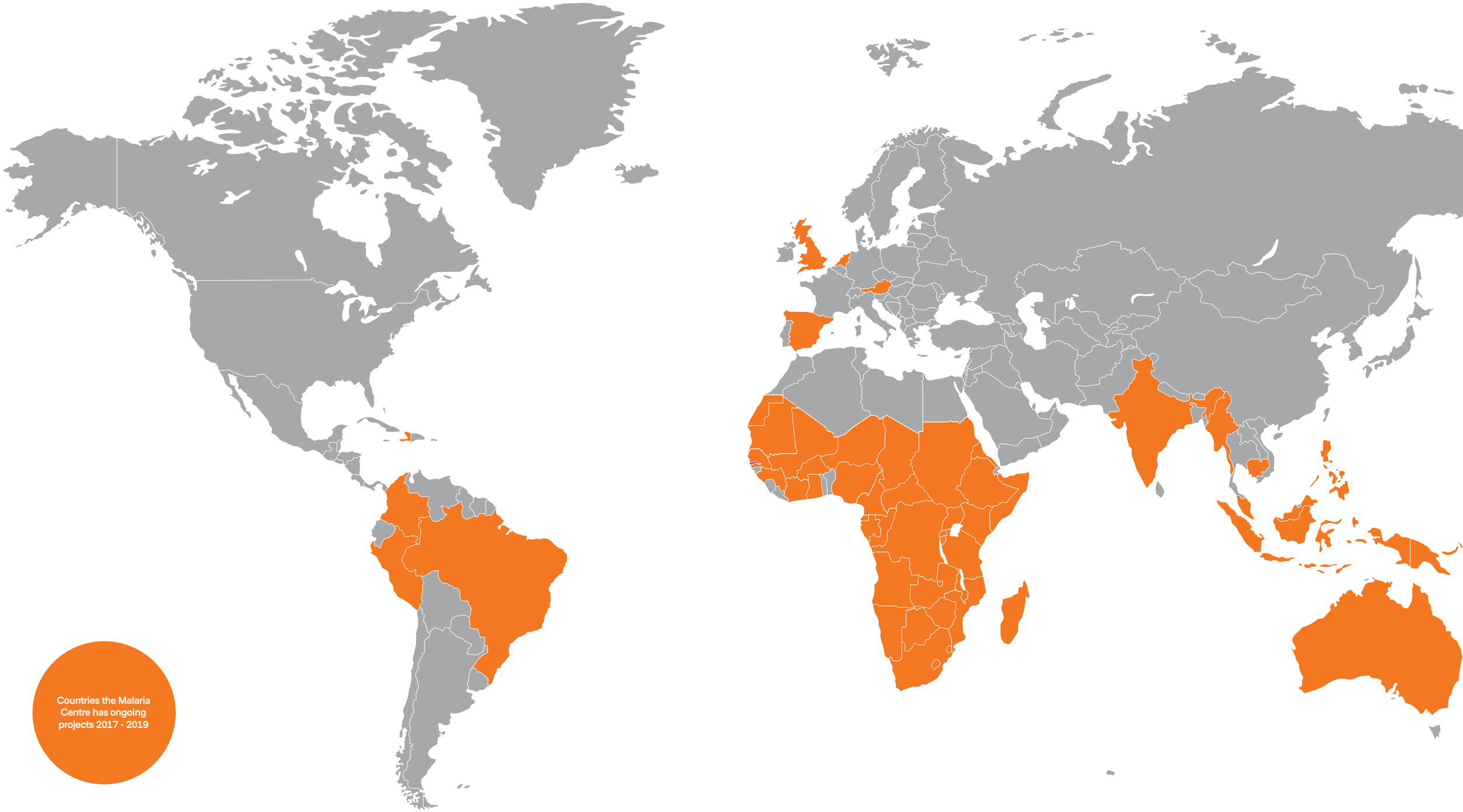
With support from the Wolfson Foundation totalling £500,000 to LSHTM through ITD and the Malaria Centre, the labs are being fitted out with new dedicated equipment. For example, a new Zeiss LSM880 confocal is designed to enable high frequency, super resolution confocal imaging of live cells – perfect for capturing dynamic processes such as red blood cell invasion by malaria parasites. Other equipment includes a robot, known as "Plasmotron", donated by the Wellcome Trust Sanger Institute in collaboration with the Sanger Institute Technology Transfer Office, Moon (LSHTM) and Rayner (Sanger) Groups. Once established it will enable all culture room users to work at higher throughput than ever before and relieve significant work flow bottlenecks - paving the way for genome wide experimental genetics approaches, and faster testing of both drug and vaccine candidates.

Users are unanimous that the labs are a beautiful place to work and a substantial upgrade on anything we have had before. There is a palpable excitement about how we can use this momentum to build stronger collaborations and work in new ways.

> ***Moving is hard and messy work and incredibly time consuming, but absolutely worth it. The new labs are bright and feel really spacious, and I think they work really well in bringing labs together with shared equipment and resources.***
>
> Elizabeth McCarthy, lab manager for the new facility

> ***The highly collaborative setup we have is enabling a lot of exchange of ideas and viewpoints. In the long term, it will definitely improve our scientific output and combined effort to combat malaria parasites***
>
> Avi Patel, postdoc in the Baker group

Worldwide locations of research



Countries the Malaria Centre has ongoing projects 2017 - 2019

Malaria Research in The Gambia

MRC Unit The Gambia at the London School of Hygiene & Tropical Medicine (MRCG at LSHTM) is delighted to bring its staff and students into the Malaria Centre family now that we are part of LSHTM. We conduct a wide scope of malaria research, throughout West Africa, broadly categorised into the following six areas.

1. Studies of Epidemiological trends and transmission dynamics

I. Malaria Programme Grant:

Our aim is to understand the determinants of malaria heterogeneity and the spatial and temporal spread of malaria infections. Our studies focus on:

- the human reservoir of infection and its transmission potential
- asymptomatic parasite carriage and identify the related risk factors
- heterogeneity in physical and behavioural insecticide resistance in vector populations

II. Infant malaria study:

This study examines the burden of malaria in young infants, to inform treatment policy decisions for this age group.

- We aim to better characterise the of the prevalence of malaria among children<6 months of age in three countries with varying malaria transmission intensity - The Gambia, Benin and Guinea

2. Entomological studies:

I. Monitoring insecticide resistance profile in Anopheles populations in The Gambia

II. Leveraging our experimental mosquito feeding capacity and expertise to standardise Standard Membrane Feeding Assay (SMFA) and Direct Membrane Feeding Assay (DMFA) outputs from different labs in the region

III. Impact of insecticide resistance on malaria vector longevity and transmission potential in the wild

3. Interventional studies targeting malaria prevention, treatment and elimination

I. MASSIV study: Mass drug administration of ivermectin and dihydroartemisinin-piperaquine as an additional intervention for malaria elimination.
This cluster-randomized controlled trial will test MDA with IVM and DP as a means to interrupt malaria transmission in medium to low transmission settings by:
- decreasing vector survival
- reducing the human infection reservoir

A total of 32 villages are randomised to either MDA with IVM or standard malaria treatment as implemented by the National Malaria Control Program. Main outcome measures are prevalence of malaria infection in intervention villages compared to controls. The proportion of female *Anopheles* carrying eggs 1-2 weeks after intervention.

II. RHOST Study: Reactive household-based self-administered treatment against residual malaria transmission: a cluster randomised trial with a community- driven intervention that involves reactive treatment of household contacts of an index clinical malaria case.

This study aims to answer the following questions;

- Would treating household members of clinical malaria cases reduce residual parasite carriage where this is already low?
- Can malaria patients (or their parents) administer antimalarial treatment provided at health facilities to their household members?
- Is there a socially acceptable and sustainable way of implementing this intervention and embedding it within local communities?
- What is the impact on the existing health system?
- What is the cost-effectiveness of this intervention in reducing the malaria burden?

III. ROOPfs Study: Assessment of improved housing to further reduce the burden of clinical malaria where coverage of LLIN is high.

Specific objectives are to determine whether improved housing:

- reduces the rate of parasite infection, parasite density and anaemia in children
- reduces vector density inside houses when compared with LLIN alone
- is acceptable and durable to adults in the study population
- is cost effective by standard economic measures
- can potentially be scaled-up effectively

4. Evaluation of malaria diagnostics

I. MalariSense Study: Feasibility of vapor nanobubble technology for malaria diagnostics. A highly innovative approach based on non-invasive detection of vapor nanobubbles produced through the skin by the excitation of malaria-specific hemozoin in infected people.

II. Alere Field Evaluation: Comparing a hypersensitive RDT against standard PCR techniques

III. Sysmex Project: Observational study to evaluate and optimize this blue laser-based technology for detection of malaria infection: the primary objective is to compare the sensitivity and specificity of the Sysmex XN-31 prototype (XN-30) to microscopy and RDT in suspected clinical malaria cases and in asymptomatic, malaria-infected carriers.

5. Antimalarial drug resistance and drug development research

I. CKAF: an early phase 2 randomised controlled multi-centre trial of investigational drug KAF156 in combination with a new dispersible formulation of lumefantrine, compared to artemether-lumefantrine for the treatment of uncomplicated malaria.

6. Anthropological and Social sciences research

I. Designing an innovative malaria elimination strategy based on reactive case detection and community participation: a mixed-methods study

II. Understanding MDA coverage, potential bottlenecks, adherence and acceptability, using qualitative ethnographic and quantitative surveys.

New areas of Research

Malaria researchers at MRCG at LSHTM are also developing new research areas. These include the **Controlled Human Malaria Infection Study** - a single centre, open-label, clinical trial that assessed the effect of pre-exposure to *Plasmodium falciparum* on parasite kinetics, clinical symptoms and immunity after controlled human malaria infection by *PfSPZ Challenge* in adult Gambian volunteers.

Parasite Biology & Drug Discovery

Research of the Malaria Centre in the areas of Malaria Parasite Biology and Drug Discovery involves a number of disciplines ranging from population genetics and genomics, to cell biology and immunology, through to design of small molecule inhibitors.

The ability to obtain whole genome sequences of malaria parasite isolates in large numbers represents a breakthrough that has allowed us to address key questions about malaria which were just not possible, until recently. Large-scale genome sequencing of malaria parasites of all species ongoing at LSHTM is illuminating all aspects of parasite biology, including parasite genome and population structure and dynamics, surveillance of drug resistance and species-specific mechanisms of immune evasion.

Most of the over 400,000 annual deaths from malaria are caused by infection with *P. falciparum*, but we now know that severe disease and death can also be caused by *P. knowlesi*. This is largely a parasite of primates, but since 2004 evidence has accumulated that humans living in proximity to monkeys in Malaysia, Indonesia and neighbouring countries can become infected with *P. knowlesi*. Development of a highly efficient reverse genetics technology for *P. knowlesi in vitro* at LSHTM has transformed our ability to study this important pathogen and gives hope that new control measures will follow.

The increasing incidence of both prolonged treatment time and treatment failure with artemisinin-based drug combinations in parts of Southeast Asia over the last 12 years is linked to the presence of mutant forms of the Kelch 13 protein. So far, these mutants have not been detected in Africa, where alternative mechanisms may be in play. Exciting findings at LSHTM, deploying new gene editing techniques for *P. falciparum,* have identified the AP-2µ adaptor protein as a strong candidate resistance marker linked to artemisinin susceptibility.

These newly recognised threats, together with previous experience, indicate that the emergence of drug resistant parasites is possible whenever a new drug is rolled out, and emphasise the need for ongoing drug development. Novel compounds that can kill malaria parasites need to be identified, either by screening large chemical libraries, or through a rational approach in which inhibitors are designed to block the activity of functions essential for parasite development.

With this rational design objective, conditional reverse genetics is being deployed at LSHTM to target *P. falciparum* merozoite egress and invasion at the molecular level, with a view to developing new antimalarial drugs. In our new malaria labs, we are attempting to block a key signalling molecule (the cGMP-dependent protein kinase, PKG) with small molecule inhibitors, some of which are identified by high throughput screening in partnership with the Tres Cantos Open lab Foundation.

Serology is a powerful approach when coupled with recent advances in recombinant protein technology, and LSHTM researchers are deploying both to study malaria epidemiology and the human immune response to infection. Recombinant protein microarrays covering an ever-increasing repertoire of parasite proteins is generating new epidemiological understanding in a number of settings worldwide, for multiple parasite species, by interrogating antibody responses in a large sample of malaria exposed people to more than a hundred recombinant *Plasmodium* proteins. This approach has also led to development of serological tools for measurement of *P. vivax* and *P. knowlesi* exposure in the field.

Schizont transcriptome variation among clinical isolates and laboratory-adapted clones of the malaria parasite *Plasmodium falciparum*

Location of study: LSHTM

LSHTM Investigators: Sarah J Tarr, Ofelia Díaz-Ingelmo, Lindsay B Stewart, Suzanne E Hocking, Lee Murray, Craig W Duffy, David J Conway

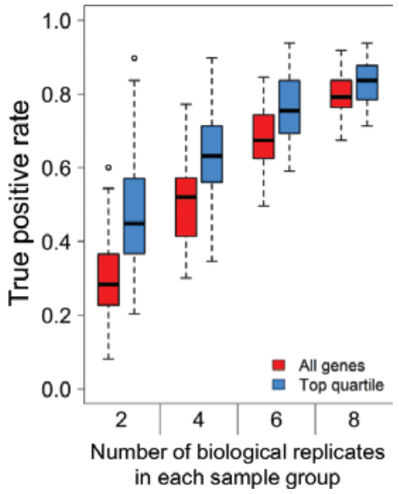
External collaborators: Thomas D Otto, Lia Chappell, Julian C Rayner (Wellcome Sanger Institute, UK), Gordon A Awandare (WACCBIP, University of Ghana)

Funding Body: European Research Council (ERC)

Malaria parasites are genetically polymorphic and phenotypically plastic. We investigated variation in the major human parasite Plasmodium falciparum, generating RNA-seq data from a panel of clinical isolates and from long-term laboratory-adapted clones, with a goal of robustly identifying differentially expressed genes.

Analysis of multiple biological sample replicates greatly improves identification of genes variably expressed between different cultured parasite lines. Here, six independent replicates of each parasite line allowed identification of most differences detected with larger numbers. For highly expressed genes, focusing on the top quartile at schizont stages, there was more power to detect differences. Variable expression was extremely strongly, but not exclusively, associated with genes targeted by Heterochromatin Protein 1. Clinical isolates recently established in culture show differences from long-term adapted clones in transcript levels of particular genes, and are suitable for analyses requiring biological replicates to understand parasite phenotypes and variable expression likely to be relevant in nature. Genes more highly expressed in the laboratory-adapted clones include those encoding an AP2 transcription factor (PF3D7_0420300), a ubiquitin-binding protein and two putative methyl transferases.

In contrast, higher expression in clinical isolates was seen for the merozoite surface protein gene dblmsp2, proposed to be a marker of schizonts forming merozoites committed to sexual differentiation.



Increasing numbers of sample replicates improves identification of schizont-stage genes varying in expression between P. falciparum lines. Proportions of genes captured as differentially expressed between two different parasite clones, by taking 100 random sub-samples of two, four, six and eight replicates of each (out of ten initially analysed replicates that identified 123 genes with significant differences between the clones).

Plasmodium falciparum multiplication rates in clinical isolates correlate with parasitaemia levels in patients and increase over time in culture

Location of study: LSHTM, and Ghana

LSHTM Investigators: Lindsay B. Stewart, Ofelia Diaz-Ingelmo, David J. Conway

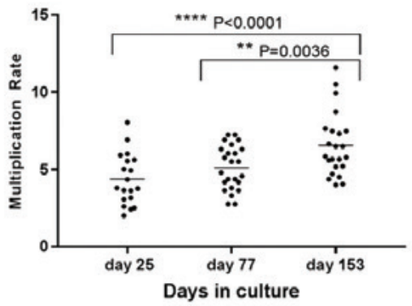
External collaborators: Gordon A. Awandare (WACCBIP, University of Ghana)

Funding Body: European Research Council (ERC)

Marked variation in multiplication rates of the malaria parasite Plasmodium falciparum in culture occurs, with parasites in clinical isolates having lower rates than parasite lines previously cultured for years. To investigate the spectrum of variation in an endemic area and the effects of culture adaptation, new isolates were studied from malaria patients in Ghana.

Twenty five new isolates were tested repeatedly over five months of continuous culture, using a standardised assay with biological replicates measured over multiple cycles. At the first point of testing after 25 days of culture, parasite multiplication rates had a mean of 4.4 fold per 48 hours (range from 2.0 to 8.0 fold for different isolates). The multiplication rates of most isolates increased over time, showing a mean of 5.1 fold after 77 days, and 6.6 fold after 153 days of culture. The multiplication rates correlated positively with parasitaemia levels in patients measured at clinical presentation.

Marked variation in parasite multiplication rates in clinical isolates persists for at least five months of culture, and although rates increase over time they remain significantly correlated with patient parasitaemia measured at clinical presentation. This persistent measurable variation is likely to reflect intrinsic differences in parasite multiplication *in vivo*.



The distribution of parasite multiplication rates in a panel of Ghanaian isolates tested at three different times over five months of continuous culture.

Multi-population genomic analysis of malaria parasites indicates local selection and differentiation at the *gdv1* locus regulating sexual development

Location of study: LSHTM UK, Gambia, Guinea, Senegal, Mali, Mauritania and Ghana

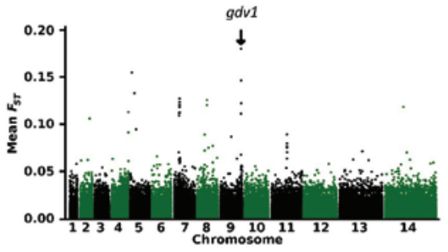
LSHTM Investigators: Craig W. Duffy, Alfred Amambua-Ngwa, Sarah J. Tarr, Lee Murray, Lindsay B. Stewart, Umberto D'Alessandro, David J. Conway

External collaborators: Ambroise D. Ahouidi (UCAD, Senegal), Mahamadou Diakite (University of Bamako, Mali), Gordon A. Awandare (WACCBIP, University of Ghana), Hampate Ba (INRSP, Mauritania), Thomas D. Otto, Dominic P. Kwiatkowski (Wellcome Sanger Institute)

Funding Body: European Research Council (ERC), Medical Research Council (MRC) and Royal Society

Parasites infect hosts in widely varying environments, encountering diverse challenges for adaptation. Identifying the genes under local selection in different environments can help reveal potential targets for interventions.

To identify malaria parasite genes under locally divergent selection across a large endemic region with a wide spectrum of transmission intensity, genome sequences were obtained from 284 clinical *Plasmodium falciparum* infections from four newly sampled locations in Senegal, The Gambia, Mali and Guinea. Combining these with previous data from seven other sites in West Africa enabled a multi-population analysis to identify discrete loci under varying local selection. A genome-wide scan showed the most exceptional geographical divergence to be at the early gametocyte gene locus gdv1 which is essential for parasite sexual development and transmission.



Scan for allele frequency divergence among 11 populations sampled across West Africa. Mean inter-population FST scores for all pairwise site by site comparisons are plotted for each single nucleotide polymorphism across the genome. The arrow shows the locus with highest inter-population divergence.

We identified a major structural dimorphism with alternative 1.5 kb and 1.0 kb sequence deletions at different positions of the 3'-intergenic region, in tight linkage disequilibrium with the most highly differentiated single nucleotide polymorphism, one of the alleles being very frequent in Senegal and The Gambia but rare in the other locations. Long non-coding RNA transcripts were previously shown to include the entire antisense of the *gdv1* coding sequence and the portion of the intergenic region with allelic deletions, suggesting adaptive regulation of parasite sexual development and transmission in response to local conditions.

A malaria parasite subtilisin propeptide-like protein is a potent inhibitor of the egress protease SUB1

Location of study: LSHTM

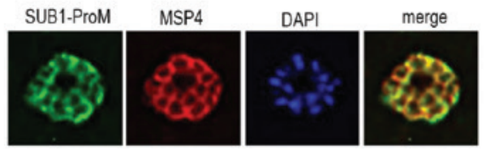
LSHTM Investigators: Sarah J Tarr, David J Conway, and Michael J Blackman

External collaborators: Chrislaine Withers-Martinez (Francis Crick Institute, UK)

Funding Body: ERC and MRC

Subtilisin-like serine peptidases (subtilases) play important roles in the life cycle of many organisms, including malaria parasites. As with other peptidases, subtilase proteolytic activity has to be tightly regulated in order to prevent uncontrolled protein degradation. Maturation of most subtilases requires an N-terminal propeptide that facilitates folding of the catalytic domain.

We have identified a stand-alone malaria parasite propeptide-like protein, called SUB1-ProM, encoded by a conserved gene that lies in a highly syntenic locus adjacent to three of the four subtilase genes in the Plasmodium genome. Homology modelling showed that the SUB1-ProM core structure is most similar to the x-ray crystal structure of the propeptide of SUB1, an essential parasite subtilase that is discharged into the parasitophorous vacuole in which the parasite replicates to trigger parasite egress from infected host cells. Recombinant *Plasmodium falciparum* SUB1-ProM was found to be a fast-binding, potent inhibitor of *P. falciparum* SUB1, but not of the only other essential blood-stage parasite subtilase, SUB2, or of other proteases examined. Mass-spectrometry and immunofluorescence showed that SUB1-ProM is expressed in the parasitophorous vacuole of blood stage P. falciparum, where it may act as an endogenous inhibitor to regulate SUB1 activity in the parasite.



Parasitophorous vacuole-like location of SUB1-ProM protein compared with the merozoite surface protein MSP4 by schizont immunofluorescence with specific antibodies, and nuclei stained with DAPI.

High throughput screening of the Tres Cantos Screening Collection for inhibitors of the cGMP-dependent protein kinase (PKG)

Location of study: LSHTM and GSK Tres Cantos Medicines Development Campus, Spain

LSHTM Investigators: David Baker and Maria Penzo

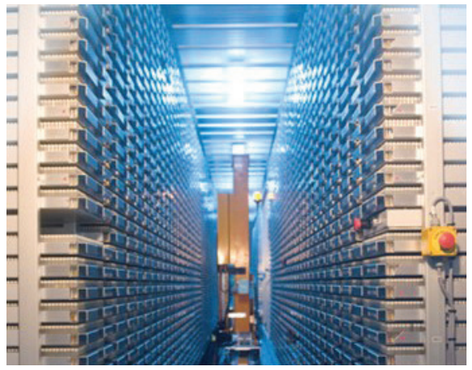
External collaborators: Laura de las Heras-Dueña, Lydia Mata Cantero, Beatriz Diaz-Hernandez, Maria-Jesus Vazquez-Muñiz and Elena Fernandez-Alvaro,(Diseases of the Developing World, GlaxoSmithKline, Spain); Sonja Ghidelli-Disse, (Cellzome, Germany)

Funding Body: Tres Cantos Open Lab Foundation and the European Commission FP7 Marie Curie co-funding program.

The emergence of drug resistant malaria parasites threatens the effectiveness of current drugs. We must therefore aim to develop new antimalarial drugs to tackle resistance. We have carried out high throughput screening of a GSK library of 1.7 million compounds to find new inhibitors of the cGMP-dependent protein kinase (PKG).

We and others have shown that the malaria parasite cGMP-dependent protein kinase (PKG) is essential for all stages of the complex malaria parasite life cycle. A previous medicinal chemistry partnership between LSHTM and LifeArc (formerly MRC Technology) developed highly potent imidazopyridine PKG inhibitors. These inhibitors cleared blood stage parasitaemia in an *in vivo Plasmodium falciparum* model and blocked transmission of gametocytes to mosquitoes using standard membrane feeding assays. To provide additional chemistry start points for targeting PKG, we undertook high throughput screening of the Tres Cantos Screening Collection in partnership with GSK scientists in a project funded by the Tres Cantos Open Lab Foundation.

A number of new inhibitor scaffolds were identified that had potent activity against blood stage and sexual stage parasites which also had some attractive physico-chemical properties. A thiazole scaffold identified showed a faster speed of kill than the imidazopyridines. Evidence suggests that this desirable property might be conferred by targeting another parasite kinase in combination with PKG. In partnership with Cellzome, using their Kinobeads Profiling technology, we have identified a small number of candidate protein kinases that might mediate the faster speed of kill. The compounds we have identified represent promising leads for progression.



To be able to screen almost 2 million chemical compounds in a short space of time, a highly efficient and automated high throughput screening system such as this is required. Image provided by GSK.

Functional analysis of the *Plasmodium falciparum* phosphodiesterase β; a master regulator of erythrocyte invasion

Location of study: LSHTM and the Francis Crick Institute

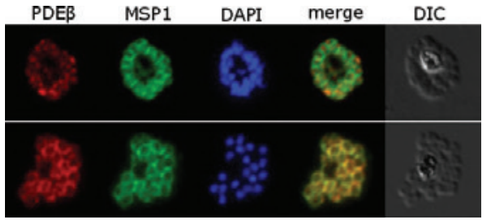
LSHTM Investigators: David Baker, Christian Flueck, Laura Drought, Avnish Patel, Eloise Walker, Stephanie Nofal and Mike Blackman

External collaborators: Abigail Perrin, Ambrosius Snijders and Andrew Jones, (Francis Crick Institute)

Funding Body: Wellcome Trust (Senior Investigator Award to David Baker and Mike Blackman)

To develop new effective antimalarial drugs, we need a better understanding of malaria parasite biology at the molecular level. We have determined the role of a central player in the cyclic nucleotide signalling pathway in parasite invasion of red blood cells and have shown it is a promising drug target.

Cyclic nucleotides (cAMP and cGMP) are important intracellular messengers that are synthesised and degraded by cyclase and phosphodiesterase enzymes respectively. On reaching a concentration threshold, they activate cyclic nucleotide-dependent protein kinases. We have used conditional gene knockout to demonstrate that phosphodiesterase β (PDEβ) is essential for *Plasmodium falciparum* merozoite invasion. We have shown that the enzyme can degrade both cAMP and cGMP making it a pivotal regulator of the pathway. PDEβ is first expressed at an apical location in mature schizonts, but relocates to the plasma membrane of the individual merozoites just prior to egress. We have shown that the invasion phenotype is due to elevated cAMP levels and the resulting hyper-activation of the cAMP-dependent protein kinase (PKA). Cellular cGMP levels remain normal in the absence of PDEβ as PDEα (that hydrolyses cGMP) remains in-tact. Our results show that no other parasite PDE can hydrolyse cAMP in the absence of PDEβ making it a promising drug target. Several human disorders can be treated safely with drugs that are PDE inhibitors. Phosphoproteomic analysis has identified schizont proteins that exhibit increased phosphorylation in the PDEβ knockout, some of which are likely important downstream players in cAMP signalling and substrates for PKA.



Immunofluorescence analysis of PDEβ (by Christian Flueck) shows that in mature P. falciparum schizonts, the enzyme has a punctate pattern resembling that of micronemal proteins. However, just prior to merozoite egress, PDEβ relocates to a peripheral position, likely the merozoite plasma membrane, since it co-localises with merozoite surface protein 1 (MSP1).

Identification of a potent series of inhibitors of the malaria parasite cGMP-dependent protein kinase that clear blood stage infection in vivo and block transmission

Location of study: LSHTM and LifeArc (formerly MRC Technology)

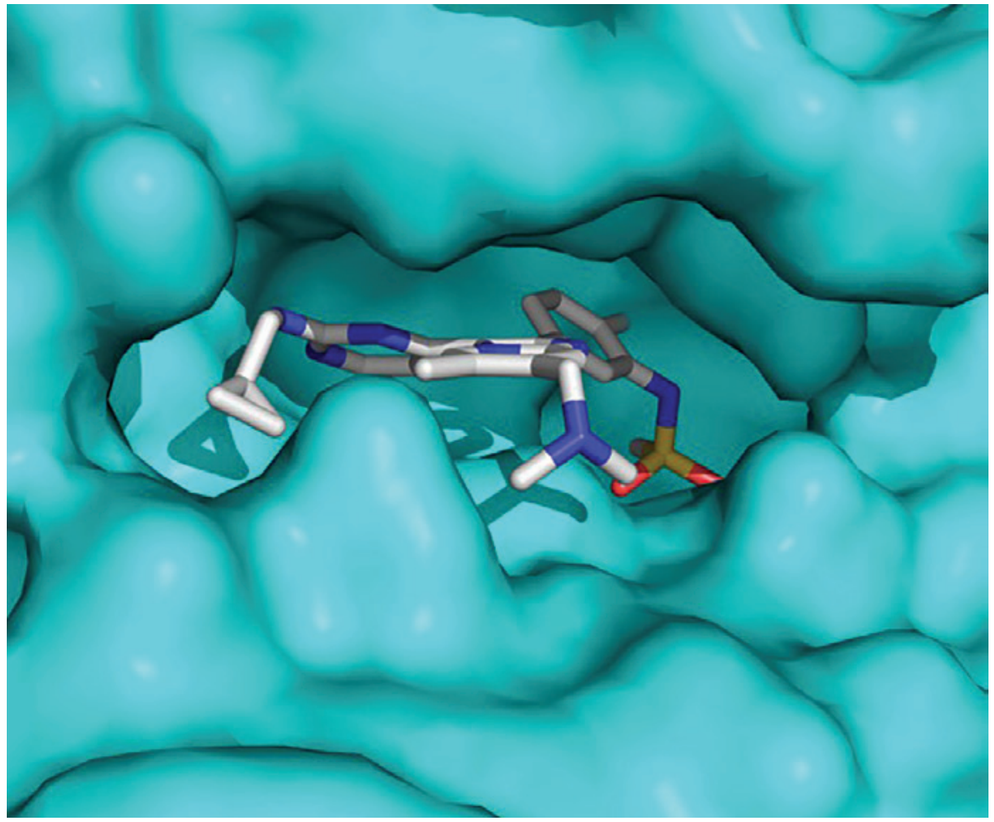
LSHTM Investigators: David Baker, Lindsay Stewart, Win Gutteridge, Simon Croft, Hans Dessens, Teun Bousema and Eloise Walker.

External collaborators: Simon Osborne, Katy Kettleborough, Andy Merritt, Jon Large, Kristian Birchall, Keith Ansell and colleagues, (LifeArc, UK); Javier Gamo, Laura Sanz and colleagues, (GSK, Spain); Ray Hui and colleagues, (Structural Genomics Consortium, University of Toronto, Canada); Koen Dechering, (TropIQ Health Sciences, The Netherlands)

Funding Body: MRC Developmental Pathway Funding Scheme

Due to the emergence of drug resistant malaria parasites new antimalarial drugs must be developed urgently. We have generated a highly potent series of inhibitors of the cGMP-dependent protein kinase (PKG) that clear blood stage infection in an *in vivo P. falciparum* model and block transmission of gametocytes to mosquitoes.

The malaria parasite cGMP-dependent protein kinase (PKG) is essential for all the key stages of the complex malaria parasite life cycle and so a drug that targets this enzyme has the potential to interrupt the malaria life cycle at multiple stages. Through partnership with LifeArc (formerly MRC Technology), we carried out a medicinal chemistry programme and developed a highly potent imidazopyridine series of PKG inhibitors. The best compound (ML10) blocks blood stage Plasmodium falciparum development with an EC50 value of ~2 nM. Importantly, the series also has potent activity against sexual stages and blocks transmission to mosquitoes. Using a chemical genetic approach we showed that ML10 is highly selective for the malaria parasite PKG. ML10 cleared blood stage parasitaemia in a GSK *in vivo Plasmodium falciparum* model providing proof of concept for PKG as an antimalarial drug target. Finally, through partnership with scientists at the Structural Genomics Consortium, we were able to determine the co-crystal structure of PKG bound to ML10 revealing intimate molecular contacts that explain the high levels of potency and selectivity we have observed. The structural data will greatly assist future medicinal chemistry efforts to optimise the series.



The image (from Ray Hui and colleagues at SGC, Toronto) depicts the intimate molecular interactions of malaria parasite PKG with the highly potent inhibitor ML10

Identification of a protease cascade required for malaria parasite escape (egress) from the human red blood cell

Location of study: The Francis Crick Institute and LSHTM

LSHTM Investigators: Michael J Blackman, David A Baker

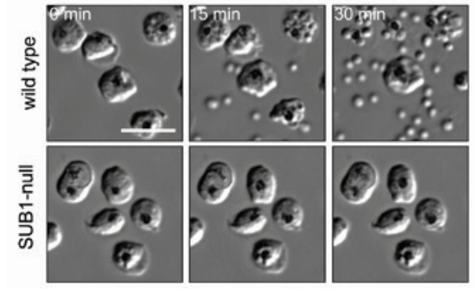
External collaborators: James A Thomas, Michele SY Tan, Aaron Borg, Fiona Hackett and Ambrosius Snijders (Francis Crick Institute, London). Claudine Bisson, Trishant Umrekar, Victoria Hale and Helen Saibil (Birkbeck College, London). Gema Vizcay-Barrena and Roland Fleck (Kings College London).

Funding Body: Francis Crick Institute and Wellcome Trust (Senior Investigator Award to David Baker and Mike Blackman)

The aim of this study was to understand how malaria parasites destroy and escape from red blood cells. It has identified a set of enzymes that function in a 'cascade' (a series of sequential, inter-dependent steps) to achieve this. These enzymes are potential targets for new types of antimalarial drugs.

All the clinical manifestations of malaria are a result of proliferation of the malaria parasite in host red blood cells. At the end of each round of intracellular replication, the host red cells are actively ruptured to release invasive merozoites, a process called egress. Using a combination of conditional mutagenesis and biochemical approaches, we have shown that egress is controlled by a series of parasite enzymes that act in a tightly-controlled sequence.

The process is triggered by a cyclic nucleotide-dependent protein kinase called PKG, which induces the rapid activation of a parasite serine protease called SUB1. This is turn cleaves and activates a second parasite protease called SERA6, which is responsible for red cell membrane rupture, the final step in egress. In the absence of any one of these three enzymes, egress is prevented. Current work is focused on (1) the precise molecular mechanisms by which these enzymes work and (2) the identification of drug-like small molecule inhibitors that might be used individually or in combinations to block the pathway and prevent parasite replication. This could lead to a new generation of protease inhibitor-based antimalarial drugs.



Disruption of the Plasmodium falciparum SUB1 gene prevents egress.

Parasite actinomyosin-based motility is not required for malaria parasite escape (egress) from the human red blood cell

Location of study: The Francis Crick Institute and LSHTM

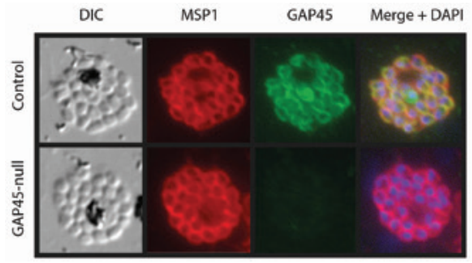
LSHTM Investigators: Michael J Blackman, David A Baker

External collaborators: Abigail Perrin, Christine Collins, Matthew Russell and Lucy Collinson (Francis Crick Institute, London).

Funding Body: Francis Crick Institute and Wellcome Trust (Senior Investigator Award to David Baker and Mike Blackman)

This study was designed to provide insights into the function of a malarial actinomyosin-based molecular 'motor', showing that although the motor is essential for invasion of red blood cells, it is dispensable for escape (egress) from the cell. These findings emphasise the critical role of proteolytic enzymes in egress.

Malaria parasites invade within circulating red blood cells, replicating within them to produce a new generation of daughter merozoites. These are eventually released from the cell in a lytic process called egress, and rapidly invade fresh red cells. Invasion is thought to involve the activity of a specialised actinomyosin-based 'motor' that the merozoite uses to pull itself into the invaded cell. In this work we addressed whether this motor has a role in egress. Using conditional mutagenesis, we showed that parasites lacking a key component of the motor (a protein called GAP45) undergo efficient egress but have a fatal defect in invasion. This finding conclusively uncouples these two crucial steps in parasite proliferation, in contrast to some related parasites such as the food-borne pathogen Toxoplasma gondii, which uses active motility to egress from infected cells. Furthermore, our findings places additional emphasis on a kinase-triggered proteolytic cascade which our collaborative programme has implicated in malarial egress. Collectively, this study has shed substantial new insights into malarial egress and enabled us to rationally prioritise future research directions.



Tightly-regulated conditional ablation of the Plasmodium falciparum GAP45 gene.

Unravelling the intracellular interactions and signalling of Apical membrane antigen-1 (AMA1) in malaria parasites

Location of study: LSHTM

LSHTM Investigators: Dr James A. Thomas, Dr Robert W. Moon

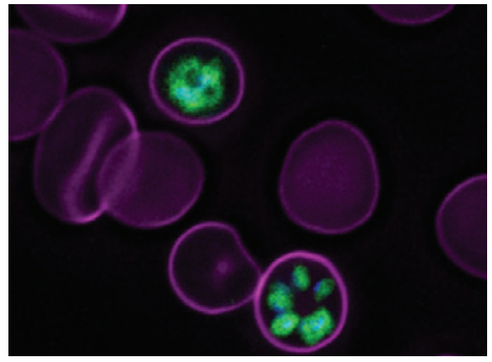
External collaborators: Dr Moritz Treeck, (Francis Crick Institute), London, UK, Prof Helen Saibil, (Birkbeck College), London, UK

Funding Body: The Wellcome Trust

The symptoms of malaria result from invasion of red blood cells by malaria parasites. This project aims to understand some of the crucial genes that mediate this process so drugs or vaccines might be developed. This project is principally funded by a Sir Henry Wellcome Fellowship awarded to James Thomas.

Apical membrane antigen-1 (AMA1) is a type-I integral membrane protein of malaria parasites, required for host cell invasion. Invasion requires phosphorylation of the AMA1 cytoplasmic domain (CD), suggesting a signalling role. Exactly what process(es) the AMA1 CD regulates, and how it does so, are unknown.

This project will examine the role of the AMA1 CD in regulating cellular events during invasion, using the recently culture-adapted malarial species Plasmodium knowlesi. A conditional mutagenesis system (DiCre) will be used to switch expression of wild-type AMA1 to mutant alleles (e.g. non-phosphorylated forms) to examine the role of the CD in invasion. The impact of mutagenesis on discharge of secretory organelles containing invasion ligands will be examined using immunofluorescence and electron microscopy.



Green fluorescent Plasmodium knowlesi (a causative agent of malaria) residing within human red blood cells (purple). Image courtesy of Melissa Hart.

Proteins that interact with the AMA1 CD will be identified using directed biotinylation by fusion of a promiscuously biotinylating enzyme to AMA1 CD, followed by pulldown and mass spectrometry. The role of interacting proteins in invasion will then be explored using DiCre-mediated knockout. A high-throughput Cas9 mutagenesis screen of the AMA1 CD will also be performed to identify crucial residues involved in mediating interaction of partner proteins with the AMA1 CD. This project aims to elucidate the long-hypothesised signalling role of AMA1.

Dissecting the Red Blood Cell Invasion Pathways of the Malaria Parasite *Plasmodium knowlesi*

Location of study: LSHTM

LSHTM Investigators: Robert W. Moon, Franziska Mohring, Melissa Hart, James Charleston, Colin Sutherland, Susana Campino

External collaborators: Simon Draper (Jenner Institute, UK), Helen Saibil (Birkbeck, UK), Mike Blackman (Francis Crick Institute, UK), Tony Holder (Francis Crick Institute, UK), Arnab Pain (King Abdullah University of Science and Technology, Saudi Arabia), Jake Baum (Imperial College London, UK, Neil Almond (National Institute of Biological Standards and Control, UK), Eun Taek Han (Kwandon National University, Korea)

Funding Body: Medical Research Council and Department for International Development through an MRC Career Development Award to Rob Moon., Bloomsbury Research Studentship to Melissa Hart, BBSRC LIDO Studentship to James Charleston

Malaria is caused by single-celled parasites called *Plasmodium*. Symptoms of malaria arise from the parasite destroying red blood cells (RBCs) by entering them, multiplying within and bursting out again in a continuous cycle. The parasites produce a range of adhesive proteins enabling them to bind to the surface of RBCs and invade them. These parasite proteins can determine disease severity and which hosts are susceptible, as well as presenting important targets for vaccine design.

This MRC Career Development award Fellowship, jointly funded by the MRC and DfID, investigates the role of these adhesive proteins during RBC invasion using a malaria parasite known as *Plasmodium knowlesi.* This naturally infects macaques in SE Asia, and is now known to cause of severe and fatal human infections. We have adapted CRISPR Cas9 genome editing techniques to P. knowlesi and used this to systematically tag and knock out each of the genes with putative roles in invasion, determining that two, NBPXa and DBPa are required for invasion of human red blood cells. Using live cell imaging and electron tomography we will now determine their precise role during invasion. We have also successfully developed new tools to allow us to use *P. knowlesi* to aid vaccine development for other important malaria parasites like *P. vivax*, which remain difficult to study within the lab.

A Long Tailed Macaque, the natural host for P. knowlesi in Malaysia.

Transmission biology of the human malaria parasites *Plasmodium ovale curtisi* and *P. ovale wallikeri*

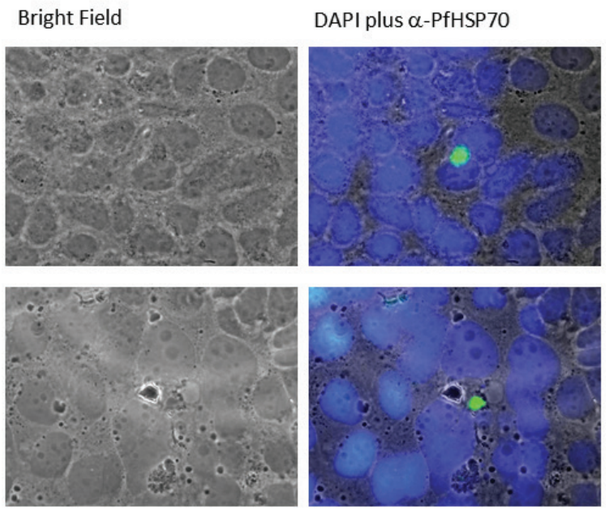
Location of study: LSHTM

LSHTM Investigators: **M**ary Oguike, Mojca Kristan, Sam Thorburn, Julius Hafalla, Colin Sutherland

Funding Body: MRC

Ovale malaria is caused by two closely related parasite species, *Plasmodium ovale curtisi* and *Plasmodium ovale wallikeri*. but is little studied. As a first step towards understanding the liver-stage of these parasites, we blood-fed *Anopheles coluzzi* mosquitoes with both species, obtained sporozoites and successfully established hepatic infections in cultured human liver cells.

Recent evidence suggests that *Plasmodium ovale curtisi* and *Plasmodium ovale wallikeri* are very closely related malaria parasites yet genetically distinct. There is also a clear phenotypic difference between them related to the duration of pre-erythrocytic latency. This represents an ideal system in which to identify candidate genes linked to hypnozoisis. Full genome data has provided new insights into the recombination barrier between them and confirms that these two parasites should be considered different species. We are seeking to identify any phenotypic differences between the two species in the mosquito and intra-hepatic stages, and so set out to demonstrate proof-of-principle mosquito transmission and hepatocyte infection with patient-derived parasites. We successfully obtained intra-hepatic parasites at low frequency, and also pioneered the use of Loop-Activated Amplification (LAMP) to track infection status of blood-fed mosquitoes for the duration of each experiment.



P. ovale curtisi sporozoite-exposed HuH7 hepatocytes from experiment 3 were observed by confocal microscopy on day 3. Host and parasite DNA was stained with DAPI (blue). P. ovale curtisi EEF were immunostained with FITC-conjugated monoclonal anti-P.f HSP70 antibodies (green).

A forward genetic screen reveals genes involved in *Plasmodium falciparum* alternative erythrocyte invasion pathways

Location of study: LSHTM and Sanger Institute

LSHTM Investigators: Susana Campino, Taane Clark

External collaborators: Julian Rayner (Wellcome Trust Sanger Institute)

Invasion of human erythrocytes is an essential step for malaria parasite survival and pathogenesis. Erythrocyte invasion in *Plasmodium falciparum* is an extremely variable phenotype and not very well understood. We tested the invasion pathways in closely related parasites and compared their genome sequences to identify genes responsible for erythrocytes invasion.

We used a forward genetic approach to identify genes responsible for variable erythrocyte invasion by phenotyping the parents and progeny of previously generated experimental genetic crosses.

Linkage analysis using whole genome sequencing data revealed a single major locus was responsible for the majority of phenotypic variation in two invasion pathways. This locus contained the *PfRh2b* and *PfRh2a* genes, members of one of the major invasion ligand gene families, but not widely thought to play such a dominant role in specifying invasion phenotypes. Variation in invasion pathways was linked to significant differences in *PfRh2a* and *PfRh2b* expression between parasite lines, and their role in the invasion process was confirmed by CRISPR-Cas9-mediated genome editing. Expansion of the analysis to a large set of clinical *P. falciparum* isolates revealed widespread copy number variation and indels at this locus, suggesting that genetic variation at this locus is a major cause of variation in invasion pathways in the endemic setting. This work has implications for blood-stage vaccine development and will help inform the design and location of future large-scale studies of invasion in clinical isolates.

Global genetic diversity of var2csa in *Plasmodium falciparum* with implications for malaria in pregnancy and vaccine development

Location of study: LSHTM

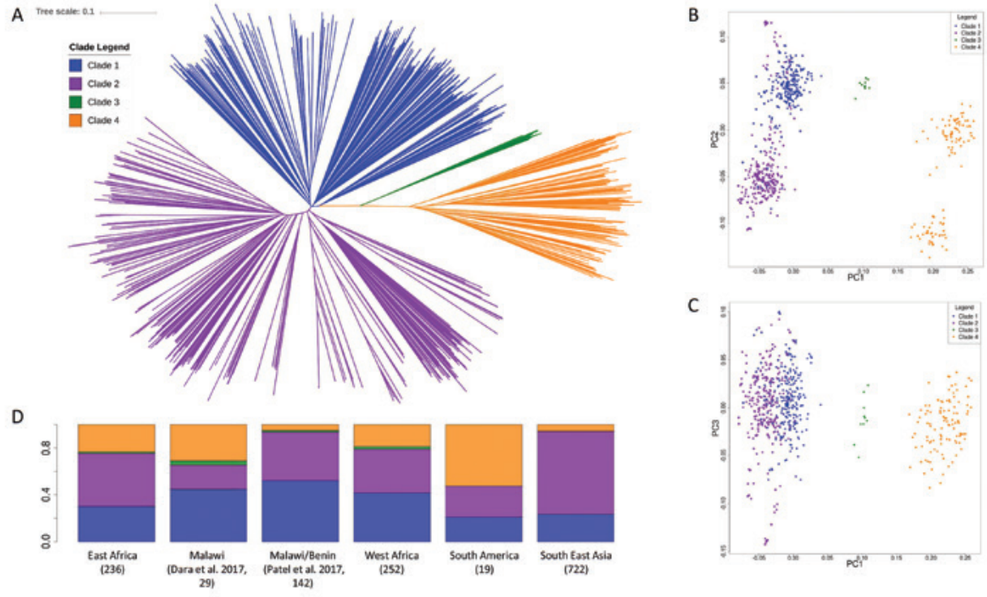
LSHTM Investigators: Ernest Diez Benavente, Roper C, Marinho CRF, Sutherland CJ, Hibberd ML, David Baker, Taane Clark, Susana Campino

External collaborators: Claudio Marinho (Univ. Sao Paulo, Brazil)

Funding Body: MRC

Malaria infection during pregnancy, caused by *Plasmodium falciparum* parasites, is a major public health burden in tropical areas of Africa and South East Asia, being responsible for substantial maternal and infant morbidity and mortality. The interaction between infected erythrocytes and the placental receptor is mediated by the parasite protein VAR2CSA. We aim to describe the genetic diversity of var2csa to help vaccine development.

Malaria infection during pregnancy, caused by the sequestering of *Plasmodium falciparum* parasites in the placenta, leads to high infant mortality and maternal morbidity. The parasite-placenta adherence mechanism is mediated by the VAR2CSA protein, a target for natural occurring immunity. Currently, vaccine development is based on its ID1-DBL2Xb domain however little is known about the global genetic diversity of the encoding *var2csa* gene, which could influence vaccine efficacy. In a comprehensive analysis of the *var2csa* gene in >2,000 P. falciparum field isolates across 23 countries, we found that var2csa is duplicated in high prevalence (>25%), African and Oceanian populations harbour a much higher diversity than other regions, and that insertions/deletions are abundant leading to an underestimation of the diversity of the locus. Further, ID1-DBL2Xb haplotypes associated with adverse birth outcomes are present globally, and African-specifnc haplotypes exist, which should be incorporated into vaccine design.



Population structure using the ID1-DBL2Xb protein sequences (A) Four distinct clades are identified, with some overlap to the clades found in18, where Clade 1 is 3D7-like and Clade 2 is FCR3-like. The PCA analysis (B and C) supports the separation of these clades and reveals the proximity of Clades 1 and 2. (D) The distribution of clades across the different regions and previous studies, with three of the clades present across all the populations (Clades 1, 2 and 4); 3D7-like clade associated with adverse outcome in pregnancy is in West Africa (41.2%), East Africa (27.5%), South East Asia (23.5%) and South America (20%); Clade 3 is present in African parasite populations and Clade 4 is predominantly in African populations.

A global analysis of copy number variation in *Plasmodium falciparum*

Location of study: LSHTM

LSHTM Investigators: **M**att Ravenhall, Ernest Diez Benavente, Colin Sutherland, Martin Hibberd, David Baker, Susana Campino, Taane G. Clark

External collaborators: Paola Florez de Sessions (Genome Institute Singapore)

Funding Body: BBSRC, MRC

Knowledge of copy number variants in the *P. falciparum* genome is incomplete, but they have been associated with antimalarial drug resistance. Using whole genome sequencing data for >3,000 isolates from 21 countries, we identified >70,000 specific deletions and >600 duplications, including in candidate drug resistance genes (e.g. *mdr1,* the *gch1* promoter region, and crt). Our work provides the largest catalogue of copy number variants in *P. falciparum* and will facilitate investigations into their functional roles.

Most studies of *Plasmodium falciparum* genetic diversity have focused on single-nucleotide polymorphisms (SNPs), enabling the identification of drug resistance-associated loci such as the chloroquine related *crt* and sulfadoxine-pyrimethamine related *dhfr.* Whilst larger structural variants are known to impact adaptation, for example, *mdr1* duplications with anti-malarial resistance, no large-scale, genome-wide study on clinical isolates has been undertaken using whole genome sequencing data. By applying a structural variant detection pipeline across whole genome sequence data from >3,000 clinical isolates in 21 malaria-endemic countries, we identified >70,000 specific deletions and >600 duplications. The majority of structural variants are rare, but a subset was present at high frequency in drug-resistance related genes including mdr1, the *gch1* promoter region, and a putative novel duplication of *crt.* Regional-specific variants were identified, and a companion visualisation tool has been developed to assist web-based investigation of these polymorphisms by the wider scientific community.

Novel genetic polymorphisms associated with severe malaria and under selective pressure in North-eastern Tanzania

Location of study: LSHTM

LSHTM Investigators: Matt Ravenhall, Susana Campino, Nuno Sepúlveda, Hugh Reyburn, Chris Drakeley, Eleanor M. Riley, Taane G. Clark

External collaborators: Alphaxard Manjurano, Behzad Nadjm, George Mtove, Hannah Wangai, Caroline Maxwell, Raimos Olomi (Joint Malaria Programme, Kilimanjaro Christian Medical College, Moshi, Tanzania), MalariaGEN (Sanger Institute, University of Oxford)

Funding Body: BBSRC, MRC

Some gene mutations in the human genome, including sickle cell trait, have been associated with reduced risk of developing severe malaria, and have increased in frequency through natural selection over generations. However, new genetic mutations remain to be discovered, and recent advances in human genome research technologies such as genome-wide association studies (GWAS) and fine-scale molecular genotyping tools, are facilitating their identification.

Here, we present findings of a GWAS of severe malaria performed in a well characterised Tanzanian population (n=914).

Significant selection pressure has been exerted on the genomes of human populations exposed to *Plasmodium falciparum* infection, resulting in the acquisition of mechanisms of resistance against severe malarial disease. Many host genetic factors, including sickle cell trait, have been associated with reduced risk of developing severe malaria, but do not account for all of the observed phenotypic variation. Identification of novel inherited risk factors relies upon high-resolution genome-wide association studies (GWAS). We present findings of a GWAS of severe malaria performed in a Tanzanian population (n=914, 15.2 million SNPs). Beyond the expected association with the sickle cell HbS variant, we identify protective associations within two interleukin receptors (IL-23R and IL-12RBR2) and the kelch-like protein KLHL3 (all P<10-6), as well as near significant effects for Major Histocompatibility Complex (MHC) haplotypes. Our approach demonstrates the potential of a joint genotyping-sequencing strategy to identify as-yet unknown susceptibility loci in an African population with well-characterised malaria phenotypes. The regions encompassing these loci are potential targets for the design of much needed interventions for preventing or treating malarial disease.

Inversions in the *Plasmodium falciparum* genome

Location of study: LSHTM

LSHTM Investigators: Matt Ravenhall, Ernest Diez Benavente, Martin Hibberd, David Baker, Susana Campino, Taane G. Clark

External collaborators: Paola Florez de Sessions (Genome Institute Singapore)

Funding Body: BBSRC, MRC

Knowledge of inversions in the *P. falciparum* genome is incomplete. Using whole genome sequencing data for 17 isolates from 14 countries, we identified 260 putative inversions, including in genes associated with anti-malarial resistance such as *gch1* and *pi4k*, and erythrocyte invasion such as *RH2b/RH2a*. Our work provides the first catalogue of polymorphic inversions in P. falciparum and will facilitate investigations into their functional roles.

Structural rearrangements, including deletions, duplications and inversions, in the *Plasmodium falciparum* malaria genome underpin a range of genetic variation associated with antimalarial resistance and host-pathogen interactions. In comparison, knowledge of inversions is incomplete, particularly as they are thought to exist within highly variable or repetitive regions, which are often excluded from genomic data analyses involving short read sequencing data. With the emergence of long read based technologies there is an opportunity to identify novel inversions genome-wide. We developed a pipeline for the robust detection of inversions and, using PacBio assemblies of 17 isolates from 14 countries, identified 260 putative inversions (median: 17; range: 7-20, per sample) in 117 specific forms. These inversions accounted for a median of 506 bp (range: 300-19,068 bp) per sample, compared to a median of 20,055 (range: 841-21,038) SNPs. Whilst 119 (45.8%) inversions were found in highly variable gene families (rifin: 75 (30 genes), stevor: 8 (5 genes), var: 36 (22 genes)), others involved significant rearrangement of genes associated with anti-malarial resistance such as gch1 and pi4k, and erythrocyte invasion such as *RH2b/RH2a*. Our work provides the first catalogue of polymorphic inversions in *P. falciparum* and will facilitate investigations into their functions.

Analysis of nuclear and organellar genomes of *Plasmodium knowlesi* in humans reveals ancient population structure and recent recombination among host-specific subpopulations

Location of study: LSHTM

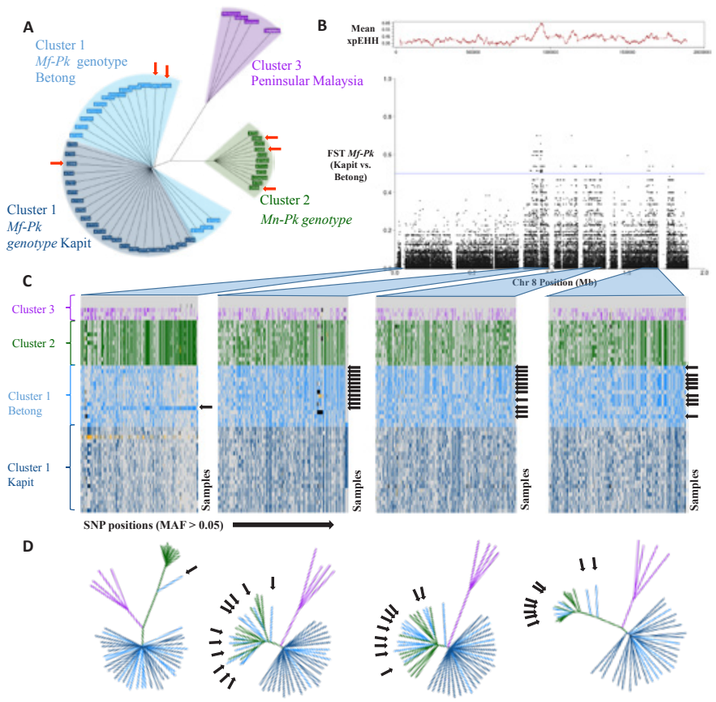
LSHTM Investigators: Ernest Diez Benavente, Rob Moon, Mike Blackman, Cally Roper, Chris Drakeley, Colin Sutherland, Martin Hibberd, Susana Campino, Taane G Clark

External collaborators: Ana Rita Gomes (Centre Hospitalier Universitaire de Montpellier, France), Jeremy Ryan De Silva and Lau Yee Ling (University of Malaya, Kuala Lumpur, Malaysia), Matthew Grigg, Sarah Auburn, and Nicholas Anstey (Menzies School of Health Research, Australia) , Paola Florez de Sessions (Genomics Institute, Singapore), Arnab Pain (KAUST, Kingdom of Saudi Arabia)

Funding Body: BBSRC, MRC

***Plasmodium knowlesi*, a common malaria parasite of long-tailed and pig-tailed macaques, is now recognized as a significant cause of human malaria, accounting for up to 70% of malaria cases in certain areas in Southeast Asia. Appropriate molecular tools are needed to assist surveillance by malaria control programmes, and to understand the genetics underpinning *Plasmodium knowlesi* transmission and switching of hosts from macaques to humans.**

The macaque parasite *Plasmodium knowlesi* is a significant concern in Malaysia where cases of human infection are increasing. Parasites infecting humans originate from genetically distinct subpopulations associated with the long-tailed (*Macaca fascicularis (Mf)*) or pig-tailed macaques (*Macaca nemestrina (Mn)*). We produced a high-quality reference genome generated using long-read technology and used this reference to re-evaluate previously described subpopulations among human and macaque isolates from Malaysian-Borneo and Peninsular-Malaysia. Nuclear genomes were dimorphic, as expected, but new evidence of chromosomal-segment exchanges between subpopulations was found. A large segment on chromosome 8 originating from the *Mn* subpopulation and containing genes encoding proteins expressed in mosquito-borne parasite stages, was found in *Mf* genotypes. By contrast, non-recombining organelle genomes partitioned into 3 deeply branched lineages, unlinked with nuclear genomic dimorphism. Subpopulations which diverged in isolation have re-connected, possibly due to deforestation and disruption of wild macaque habitats. The resulting genomic mosaics reveal traits selected by host-vector-parasite interactions in a setting of ecological transition. We are further exploring other genomic introgressions to understand their impact in parasite biology.



Whole genome population structure and evidence of genetic exchange in chromosome 8.
A) Neighbour joining tree constructed using 1,632,024 genome-wide SNPs across the 60 P. knowlesi (Pk) samples. The tree shows two levels of resolution involved in the clustering of genotypes. The first level differentiates Peninsular Malaysia samples (Cluster 3, purple) from the Malaysian-Borneo host-related Pk genotypes (Cluster 1, M. fascicularis macaques (Mf-Pk), blue; Cluster 2, M. nemestrinamacaques (Mn-Pk), green). The second level differentiates within Cluster 1, where Mf-Pk genotypes fall in subgroups from Betong (light blue) and from Kapit (dark blue). Samples from Sarikei have been highlighted using orange arrows. B) Allele frequency differences between Betong and Kapit regional subgroups of the Mf-Pk genotype in chromosome 8 SNPs using the population differentiation measure FST. There is high differentiation (FST > 0.4) in several regions across chromosome 8 (0.85-1Mb, 1.2Mb-1.35Mb, and 1.6–1.7Mb), and these signals overlap with strong evidence of recent positive selection, measured by the average XP-EHH calculated in 1kbp windows (red trace above). C) Haplotype plots for all samples (y-axis) at common SNP positions (MAF >5%, x-axis) highlighting the regions with abnormally high FST values (0.85-1Mb, 1.2Mb-1.35Mb, and 1.6–1.7Mb), as well as the low Fst region spanning from 0.1 to 0.2Mb for comparison. The black arrows indicate samples with the Mf-Pk genotype from Betong present with a Mn-Pk Cluster 2-like haplotype. These patterns are indicative of genetic exchange between the Mf-Pk and Mn-Pk genotype clusters, which is supported by the neighbour joining trees included in D). Missing calls are coloured in black and mixed calls are coloured in yellow. D) Neighbour joining trees constructed using SNPs in each of the regions in C). The trees show clear clustering of Mf-Pk Betong samples with the Mn-Pk genotype cluster in the genetic regions of abnormal FST (2nd, 3rd and 4th trees) compared to the 1st tree where only sample DIM2 presents introgression.

Selective whole genome amplification of *Plasmodium malariae* parasites

Location of study: LSHTM

LSHTM Investigators: Amy Ibrahim, Ernest Diez Benavente, Debbie Nolder, Julian Muwanguzi, Colin Sutherland, Jody Phelan, Paula Josefina Gomez Gonzalez, Daniel Ward, Matthew Higgins, Taane G. Clark, Susana Campino

External collaborators: Francois Nosten, Mahidol Oxford Tropical Medicine Research Unit, Thailand, Hans-Peter Fuerher, University of Veterinary Medicine Vienna, Austria

Funding Body: MRC UK

Despite *Plasmodium malariae* being one of the six parasite species that causes human malaria, it is greatly understudied. Whole genome sequencing (WGS) assists the investigation of the genomic diversity of *P. malariae,* as well as a comparative genome analysis with other Plasmodia, potentially unlocking the unique biology of parasite.

The genome of *Plasmodium malariae* is understudied, in part, due to difficulties in obtaining high quality DNA for WGS and downstream analysis. Selective whole genome amplification (SWGA) is a method that increases the relative levels of pathogen DNA in a clinical sample. Using bioinformatics software, we created a primer set ("pmset1"), which selectively amplifies the *P. malariae* genome from clinical samples. This primer set was validated using DNA extracted from clinical venous blood samples collected from 19 patients (in 8 countries) with a *P. malariae* infection. The 19 *P. malariae* DNA samples were amplified and sequenced. Two control unamplified samples were sequenced and directly compared to the SWGA-amplified version of the same sample.

The designed *P. malariae* primer set was extremely efficient, enabling the WGS of samples with low parasitaemias (~0.005%), with at least half of the genome covered to at least 5-fold, and a 14.5-fold increase in average genome coverage compared to unamplified samples. Across the 19 *P. malariae* we identified 225,340 high quality SNPs, and a population structure analysis revealed regional clustering, with African samples clustering independently to those from Thailand. Follow-up work is performing large-scale sequencing and genomic diversity analysis of *P. malariae*.

Identification and characterisation of *Plasmodium falciparum* merozoite associated Armadillo protein (PfMAAP), a novel armadillo repeat protein

Location of study: LSHTM, West African Centre for Cell Biology of Infectious Pathogens (WACCBIP) and the University of Ghana

LSHTM Investigators: David J. Conway and Kevin K. A. Tetteh

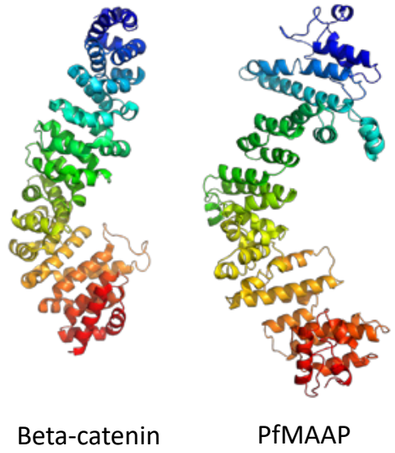
External collaborators: Yaw Aniweh[1], Prince B. Nyarko[1,2], Charles-Chess Essel[1,2], Felix Ansah[1,2], Evelyn Quansah[1,2] and Gordon A. Awandare[1,2]
[1]West African Centre for Cell Biology of Infectious Pathogens (WACCBIP),
[2]Department of Biochemistry, Cell and Molecular Biology, College of Basic and Applied Sciences, University of Ghana, Legon, Ghana

Despite recent successes in the reduction of the global burden, malaria remains a major threat to human health. Understanding the role of parasite proteins is crucial in the identification of putative drug targets or vaccines. As part of a joint venture between the LSHTM, the West African Centre for Cell Biology of Infectious Pathogens (WACCBIP) and the University of Ghana, we describe the identification of a novel parasite protein and attempt to dissect its role in parasite biology and host immunity.

It is important to understand the role played by proteins in the development of infectious organisms. Defining key characteristics such as the location, expression status and temporal association of the protein builds evidence to help define the role/function of the protein in parasite development, and potentially its importance as a putative therapeutic, diagnostic or vaccine target.

We recently identified the presence of an armadillo-repeat protein within *Plasmodium falciparum*. Members of the armadillo-repeat superfamily have been described in almost every organism, with a diverse range of fundamental functions ranging from cell signalling, T cell differentiation, cell invasion and organelle biogenesis, to name a few. To date, only a few armadillo-repeat containing proteins have been characterised in *Plasmodium*; PfARO (*P. falciparum* armadillo repeats only proteins; PF3D7_0414900), PfMOP (P. falciparum merozoite organizing protein; PF3D7_0917000) and Pf16 (role in *P. berghei* gamete flagella structure and function).

Here, we describe the characterization of another ARM repeat containing protein in *P. falciparum*. The protein shows a changing localization pattern during schizont maturation, from MSP-like surface expression, to a more apically located pattern. As a result, we propose the name *P. falciparum* Merozoite Associated Armadillo repeats protein (PfMAAP). Antibodies to three different domains of the protein demonstrated variable levels of merozoite invasion inhibition in in vitro assays. We have also shown that the protein domains elicit different levels of antibody recognition, with the N and C- termini associated with protection in malaria endemic settings.



Predicted structural model of the PfMAAP protein compared to the solved crystal structure for Beta-catenin, a multifunctional protein involved in cell-cell adhesion and cell signalling.

Serological profile of antibody responses to *P. falciparum* in Guapi, Columbia

Location of study: LSHTM, the Wellcome Trust Sanger Insitute (WTSI) and Universidad Nacional de Colombia in Bogotá (UNC)

LSHTM Investigators: Tate Oulton, Katherine Glass, Chris Drakeley and Kevin K. A. Tetteh

External collaborators: Angelica Knudson[1], Alena Pance[2], Julian Rayner[2], and Vladimir Corredor[1]
1. Universidad Nacional de Colombia in Bogotá (UNC)
2. Wellcome Trust Sanger Institute (WTSI)

The Guapi region, on the Pacific Coast of Columbia, is emerging from decades of armed conflict, forced displacement and a lack of basic healthcare infrastructure. Understanding the disease landscape after such a protracted period of conflict will be essential to addressing public health needs in the region.

A fifth of the malaria cases in South America occur in Columbia and of this, ~70% occur in the Pacific coast region. The ongoing peace process has allowed a window of opportunity to access and serologically profile malaria responses in the area. Guapi, a town and municipality in the Cauca department of Columbia, lies within this region. The population is of largely African decent, and therefore resistant to P.vivax infections. As such, malaria in this region is predominantly caused by Plasmodium falciparum.

Using a recently developed recombinant protein microarray, we have assayed serum samples from 1373 volunteers in Guapi, to obtain a serological profile of responses to a panel of >120 recombinant proteins, based on P. falciparum. The antigens were expressed in either E. coli or mammalian expression cells (HEK293E).

The most recognised antigens included GLURP R2, AMA1, a variant of MSP2 (15-38) and a conserved membrane antigen of unknown function (Pf3D7_1419400).

Work is ongoing to fully characterise antibody responses in the region, complimenting additional work focused on describing the factors unique to the locality that may act as barriers to malaria elimination.

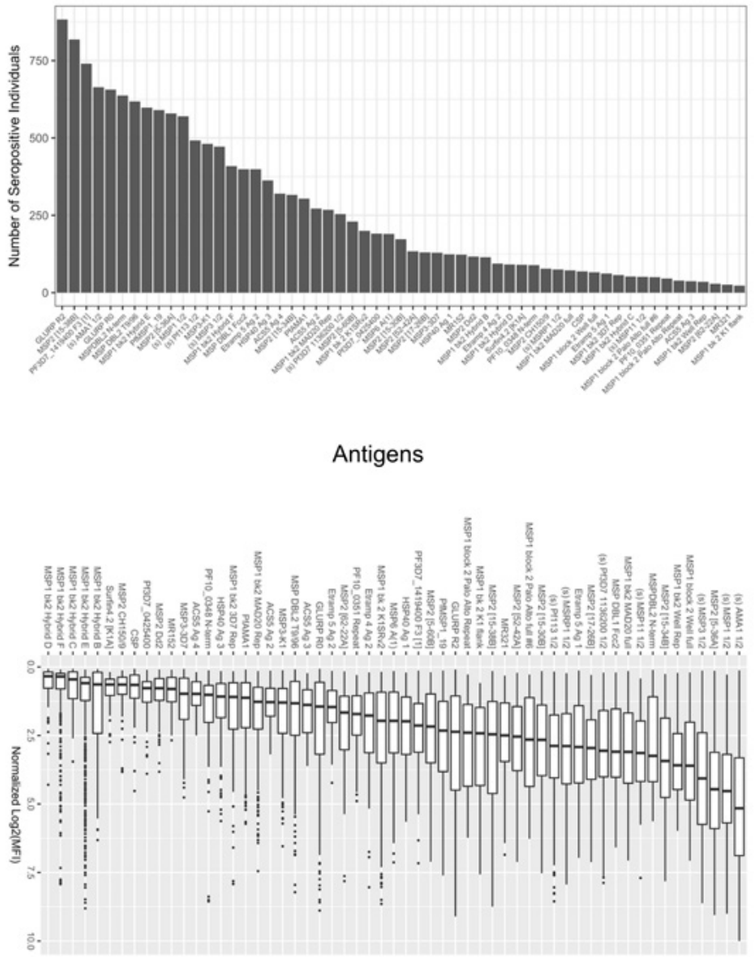


Fig 1A. Number of seropositive individuals responding to reactive malaria targets. 1B. Boxplots showing the normalised log2(MFI) responses, ranked in order of responsiveness.

Development of novel serological tools to measure exposure to the zoonotic parasite, *Plasmodium knowlesi*

Location of study: LSHTM, Malaysia, Australia

LSHTM Investigators: Lou S. Herman, Kim Fornace, Jody Phelan, Robert W. Moon, Mike J. Blackman[1], Chris J. Drakeley and Kevin K.A. Tetteh.
1. Malaria Biochemistry Laboratory, The Francis Crick Institute, London, United Kingdom.

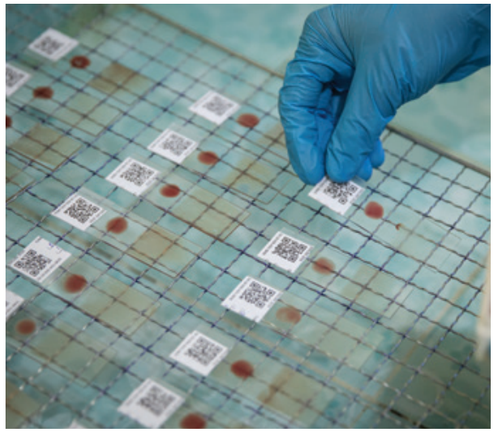
External collaborators: Grigg MJ[1,2], Anstey NM[1,2], William T[2,3,4],
1. Menzies School of Health Research and Charles Darwin University, Darwin, Northern Territory, Australia.
2. Infectious Diseases Society Sabah-Menzies School of Health Research Clinical Research Unit, Kota Kinabalu, Sabah, Malaysia.
3. Clinical Research Centre, Queen Elizabeth Hospital, Kota Kinabalu, Sabah, Malaysia.
4. Jesselton Medical Centre, Kota Kinabalu, Sabah, Malaysia.

Funding Body: Wellcome Trust and Medical Research Council

Plasmodium knowlesi is primarily an infection of primates found in Southeast Asia. However, infections of humans has become increasing common to the extent that malaria caused by _P. knowlesi_ is now the most common cause of malaria in Malaysian Borneo. This study focused on the identification of protein targets for use as serosurveillance tools to help measure exposure to this neglected disease.

A key requirement for serological studies of malaria is the identification and development of species-specific biomarkers. This is particularly important in regions where multispecies infections are prevalent. The use of such tools allows serological estimates of transmission to be made, and has been used to great effect with other malaria species to gather evidence on epidemiological patterns.

The recommendations of a WHO consultation panel, on the public health importance of *P. knowlesi*, recommended the urgent development of specific diagnostic tools. To date, few species-specific reagents with potential utility as serological tools exist for Pknowlesi; the few reagents that do exist are not suitable for measuring species-specific serological responses, due to the high degree of sequence homology (>85%) between *P. knowlesi* and *P. vivax* (e.g. *Pk*AMA1 and *Pk*SPATR). Here we describe the development of species-specific recombinant antigens based on an in silico analytical approach. Using publically available tools, we have developed a novel panel of reagents that will serve as potentially important serosurveillance biomarkers, to help monitor historical infections in endemic areas. It is intended that these tools will also help to define potential correlates of protection of immunity to *P. knowlesi.*



Blood samples collected in Malaysia. Photo credit: Joshua Paul for LSHTM

P. falciparum and *P. vivax* genetic barcodes for malaria surveillance.

Location of study: LSHTM

LSHTM Investigators: Ernest Diez Benavente, Jody Phelan, Debbie Nolder, Colin Sutherland, Martin Hibberd, Cally Roper, Susana Campino, Taane G. Clark

External collaborators: Paola Florez de Sessions (Genome Institute, Singapore), Claudio Marinho (University Sao Paolo, Brazil)

Funding Body: MRC UK

As more countries move towards reducing malaria cases and elimination, there is an increased need to understand the mobility of parasite populations, to avoid the introduction of malaria from high to lower burden and elimination areas.

Genetic monitoring can be used to identify and track parasite migration and source, for proactive and informed disease control.

The use of molecular epidemiology for tracking and studying parasite populations has been applied successfully to several malaria species. As the number of *P. vivax* and *P. falciparum* across endemic regions with whole genome sequences increase, improved molecular barcodes can be developed. By assembling large *P. vivax* and *P. falciparum* whole genome sequence datasets (n>4000), including genomic variants in nuclear, mitochondrial and apicoplast genomes, we have applied machine learning approaches to create SNP-based barcodes. These barcodes are highly predictive of parasite geographic origin, species and transmission intensity. When rolled-out in new and portable platforms, these SNP-based barcodes will be an invaluable tool to help malaria elimination efforts.

MRC Unit The Gambia, October 2019
Image Courtesy of: Louis Leeson for LSHTM

Human Pathology and Disease Response

The deployment of key interventions to prevent and treat malaria, such as long-lasting insecticide treated nets (LLINs) and artemisinin-combination therapies (ACTs), has led to a steady decrease in malaria cases reported worldwide over the past decade. Despite tremendous global efforts, this trend has now stalled. Novel approaches are therefore needed to continue curbing malaria transmission and mortality, including new vaccine strategies, parasite evolution surveillance, better tools to detect low parasitaemia and estimate malaria transmission levels, fast and reliable assessment of drug safety for patients, and effective adjunct therapies for severe cases. Researchers within the Malaria Centre are contributing to each of these fields, with promise for new intervention strategies.

While the safety and protective effect of the RTS,S vaccine shown in advanced clinical trials is currently being assessed by the WHO in real-life settings, its optimal efficacy requires 4 doses and has been reported to wane rapidly in high exposure areas. Several members of the Malaria Centre are evaluating the role of specific malaria parasite epitopes and host immune response pathways that lead to the building of an effective and long-lasting anti-malaria immunity, in order to optimise pre-erythrocytic or blood stage vaccine design. These could then be combined with an “altruistic” vaccine aimed at blocking the transmission of parasites from infected patients, another approach currently being evaluated at LSHTM.

Antigen-detecting rapid diagnostic tests (RDTs) play a key role in malaria control successes in many parts of the world, and most tests currently in use detect *Plasmodium falciparum* HRP2 and/or pLDH antigens. Researchers within the Malaria Centre are investigating the impact of recently reported *pfhrp2* gene deletions or mutations in African parasite populations on both false-negative results and clinical outcome.

The active detection of asymptomatic and sub-microscopic infections that can contribute to malaria transmission is a priority in current elimination programmes. However, these infections remain undetected by the standard diagnostic and surveillance tools. Members of the LSHTM staff are using antibodies as an indication of recent exposure to *Plasmodium* parasites, as well as current or past infections, through custom microarrays and bead-based malaria serology screening assays. These tools are also used by our researchers to estimate malaria transmission levels in specific settings, monitor the efficacy of treatment programs, and assess the impact of specific interventions such as targeted indoor residual spraying (IRS).

Once successfully detected, another challenge faced by clinicians is the effective treatment of asymptomatic and symptomatic infections by *P. vivax* and *P. falciparum*. Currently, the drug family that includes primaquine is only option available to clear individuals from *P. vivax* parasites and therefore reduce their transmission. Unfortunately, approximately 400 million people worldwide are affected by G6PD deficiency, a common enzyme defect leading to severe adverse reactions if exposed to these drugs at certain doses. Staff at LSHTM are investigating molecular mechanisms resulting in such reactions and evaluating reliable tools to detect this defect in patients before treatment. Similarly, a better understanding of the pathogenetic mechanisms leading to cerebral malaria, the most severe and lethal form of *P. falciparum* infections, is necessary to identify patients at risk and develop adjunct therapies to decrease mortality. Researchers from the Centre are using a combination of state-of-the-art neuroimaging techniques in patients with cerebral malaria, assessment of markers of severity, and *in vitro* modelling to this end.

Unravelling the immune signature of *Plasmodium falciparum* transmission reducing immunity

Location of study: UK, Netherlands, Burkina Faso, The Gambia, Cameroon

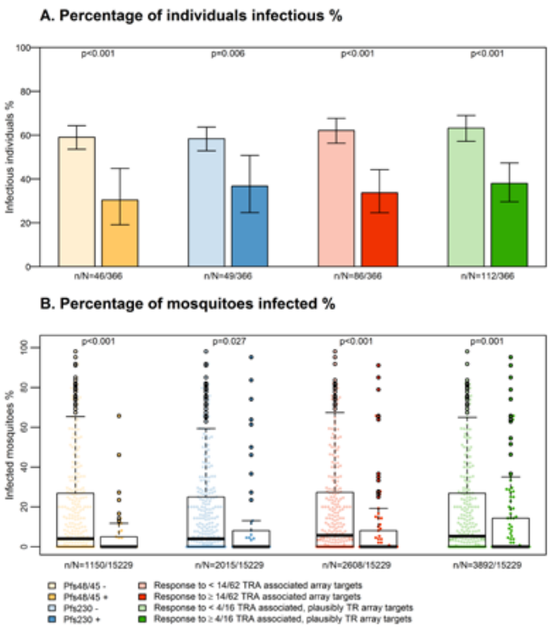
LSHTM Investigators: Will J. R. Stone, Colin J. Sutherland, Sophie Jones, John Bradley, Chris Drakeley

External collaborators: Joseph J. Campo, Adam D. Shandling, Jozelyn V. Pablo & Andy A. Teng, (Antigen Discovery Inc., US); André Lin Ouédraogo, (Institute for Disease Modeling, Washington, USA); Lisette Meerstein-Kessel, Robert Sauerwein & Teun Bousema, (Radboud Institute for Health Sciences, Nijmegen, The Netherlands); Isabelle Morlais & Anna Cohuet, (Institut de Recherche pour le Développement, Montpellier, France); Dari Da, (Institut de Recherche en Sciences de la Santé, Burkina Faso); Sandrine Nsango, (Organisation de Coordination pour la lutte contre les Endémies en Afrique Centrale, Yaoundé, Cameroon); Marga van de Vegte-Bolmer, Rianne Siebelink-Stoter, Geert-Jan van Gemert, Wouter Graumans, Kjerstin Lanke, Roos M. de Jong, Amanda Fabra-García, Will Roeffen & Matthijs Jore (Radboud Institute for Molecular Life Sciences, Nijmegen, The Netherlands); Edwin Lasonder, (Plymouth University, UK); Giuliana Gremo & Evelin Schwarzer, (University of Torino, Italy); Chris J. Janse, (Leiden University Medical Center (LUMC), The Netherlands); Susheel K Singh & Michael Theisen, (Statens Serum Institut, Copenhagen, Denmark); Phil Felgner, University of California, USA); Matthias Marti, (University of Glasgow, UK)

Funding Body: Marie Curie Career Integration Grant (SIGNAL, PCIG12-GA-2012-333936, award to Teun Bousema)

When people are infected with malaria parasites they develop antibodies. These help kill the parasites, and in rare cases also stop mosquitoes becoming infected when they take a blood-meal. We investigated this natural transmission blocking immunity, identifying antibody responses correlated with the phenomenon that could form the basis of a new transmission-blocking malaria vaccine.

Plasmodium infection can elicit antibodies that inhibit the parasites survival in the mosquito, when they are ingested in an infectious blood meal. We determined the transmission-reducing activity (TRA) of naturally acquired antibodies from more than 600 malaria infected individuals in lab based mosquito-feeding assays. Transmission inhibition was associated with antibody responses to the *Plasmodium* proteins Pfs48/45 and Pfs230, as well as 43 novel gametocyte proteins. In field-based transmission assays, mosquito infection was significantly lower when they fed on the blood of individuals with antibodies specific to Pfs48/45, Pfs230, or to combinations of the novel TRA associated proteins. We also showed that naturally acquired purified antibodies against Pfs48/45 and Pfs230 are mechanistically involved in TRA (i.e. they can block mosquito infection alone), while human sera depleted of these antibodies retained high-level, complement independent TRA. Together, our analysis indicates that humans naturally develop as yet uncharacterised transmission blocking antibodies in response to malaria infection, and that antibody responses to novel gametocyte proteins are associated with reduced malaria transmission efficiency from humans to mosquitoes. A number of these proteins are now being assessed as potential transmission-blocking vaccine candidates.



Seroprevalence to Pfs48/45, Pfs230, and novel transmission-reducing immunity (TRA) associated microarray proteins, and infectiousness in the direct membrane-feeding assay (DMFA) Individuals are categorised according to their possession of antibodies specific to: Pfs48/45 (positive [+]/negative [-]); Pfs230 (positive [+]/negative [-]); ≥14 of the 61 novel microarray proteins with TRA associated antibody responses (14 being the 75th percentile of the breadth of response to these microarray targets among the entire sample set); ≥4 of the 16 novel microarray proteins with TRA associated antibody responses that are also plausible targets of antibodies with TRA (4 being the 75th percentile of the breadth of response to these microarray targets among the entire sample set).

Antibody responses to antigenic targets of recent exposure are associated with low-density parasitemia in controlled human *Plasmodium falciparum* infections

Location of study: London, UK, Nijmegen, the Netherlands

LSHTM Investigators: Lotus L. van den Hoogen, Tate Oulton, Chris Drakeley & Kevin K.A. Tetteh

External collaborators: Jona Walk & Isaie J. Reuling, (Radboud University Medical Centre, The Netherlands); Linda Reiling, James G. Beeson & Ross L. Coppel (Burnet Institute, Australia); Susheel K. Singh, (Statens Serum Institut & University of Copenhagen, Denmark); Simon J. Draper, (University of Oxford, UK); Teun Bousema & Robert Sauerwein (Radboud University Medical Centre, The Netherlands)

Funding Body: The Global Good Fund I, LLC, Wellcome Trust, VIDI fellowship from The Netherlands Organization for Scientific Research and the National Health and Medical Research Council of Australia

The majority of malaria infections in low transmission settings are undetectable by conventional diagnostics (RDT and microscopy). They are thus a major challenge to malaria elimination.

We aimed to identify antibody responses that detect exposure to recent low-density infections which may lead to more sensitive methods of surveillance in these areas.

A powerful model to identify antibody responses that allow accurate detection of recent exposure to low-density infections is controlled human malaria infection (CHMI) studies in which healthy volunteers are infected with the *Plasmodium* parasite. We aimed to evaluate antibody responses in malaria-naïve volunteers exposed to a single CHMI using a custom-made protein microarray. All participants developed a blood-stage infection with peak parasite densities up to 100 parasites/µl in the majority of participants (50/54), the remaining participants had peak densities higher than 100 parasites/µl. There was a strong correlation between parasite density and antibody responses associated with the four most reactive blood-stage antigens one month after CHMI (Etramp 5, GLURP-R2, MSP4 and MSP1-19; Spearman's ρ=0.82, p<0.001). Most volunteers developed antibodies against a potential marker of recent exposure: Etramp 5 (37/45, 82%). Our findings justify validation in endemic populations to define a minimum set of antigens needed to detect exposure to natural low-density infections.

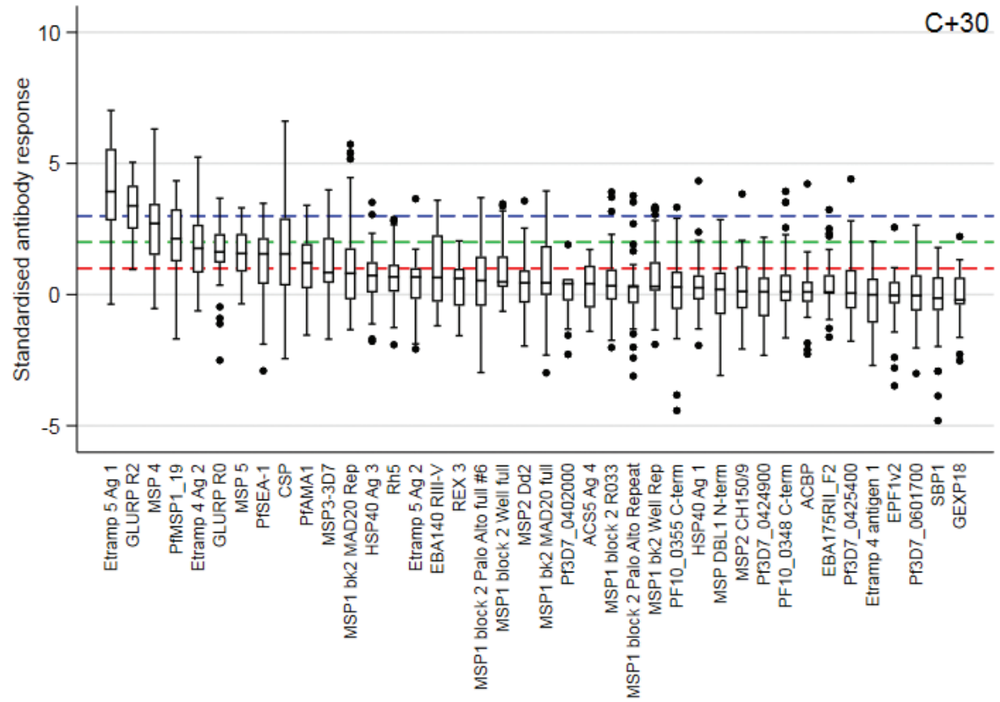


Figure 1: Standardised antibody responses 30 days post-challenge. Antibody responses were standardised by subtracting the mean of pre-challenge responses across 45 participants and dividing by its standard deviation (SD). Standardised antibody responses 30 days post-challenge are ordered by median reactivity on the x-axis. Dashed lines represent arbitrary thresholds at 1 SD (red – top 10 antigenic targets), 2 SD (green – top 4 antigenic targets) or 3 SD (blue – top 2 antigenic targets) greater than the mean of pre-challenge responses.

C+30: 30 days post-controlled human malaria infection.

India ICEMR – Pathogenesis of Cerebral Malaria in India

Location of study: Ispat General Hospital, Rourkela, India, LSHTM

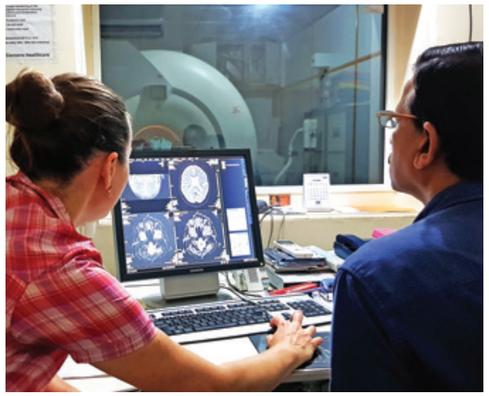
LSHTM Investigators: Sam Wassmer

External collaborators: Jane Carlton & Steven Sullivan (New York University, US); Sanjib Mohanty, (Centre for the Study of Complex Malaria, India); Rajyabardhan Pattnaik, Megharay Majhi & Rashmi Ranjan Mohanty (Ispat General Hospital, India); Praveen Sahu, (Centre for the Study of Complex Malaria, India); Akshaya Mohanty, (Institute of Life Sciences, India); Angelika Hoffmann, (Heidelberg University Hospital, Germany); Joe Smith, (Center for Infectious Disease Research, USA)

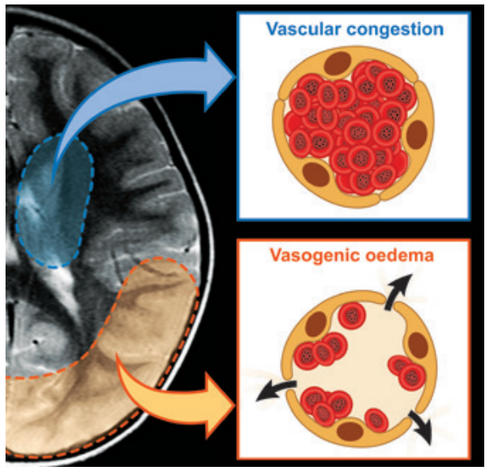
Funding Body: National Institutes of Health (NIH), USA

This project is part of an International Center of Excellence for Malaria Research (ICEMR) programme, which was created in July 2010 by the NIH. The aim of the programme is to establish a global network of independent research centres in malaria-endemic countries to provide knowledge, tools, and evidence-based strategies to support malaria researchers working in a variety of settings.

Plasmodium falciparum malaria is a major cause of mortality and morbidity in the developing world and cerebral malaria (CM), its most severe form, accounts for the majority of malaria-associated deaths.



Radiologists analysing the MRI scan of a patient with cerebral malaria at Ispat General Hospital, Rourkela, India



Visual summary of the two main mechanisms leading to brain swelling in our cohort of cerebral malaria patients

The pathophysiology and the molecular mechanisms underlying this complex neurologic syndrome are still poorly understood. Our team is using a unique combination of advanced neuroimaging techniques, post-mortem analyses, molecular biology approaches and machine-learning to explore the origin of brain swelling in CM patients from India. In a recent study carried out at Ispat General Hospital in Rourkela, we showed for the first time that increased brain volume during CM is predominantly due to posterior vasogenic oedema, indicating a local impairment of the blood-brain barrier integrity. In half of the scanned patients, we found that blood vessel congestion in the basal ganglia also contributed to the swelling, a phenomenon likely caused by a considerable accumulation of *P falciparum*-infected red blood cells. Our team is now looking into potential associations between specific PfEMP-1 variants in the blood of CM patients and the presence or absence of vasogenic oedema identified by MRI. We are also applying advanced machine-learning models of CM disease causation, informed by clinical and laboratory investigations. The primary outcome of this project will be a better understanding of the different pathogenetic processes involved in CM, which will guide the development of new adjunct therapies.

The India ICEMR team

Beta-catenin signalling in endothelial cells during cerebral malaria

Location of study: London School of Hygiene and Tropical Medicine, UK, New York University School of Medicine, USA, Ispat General Hospital, Rourkela, India

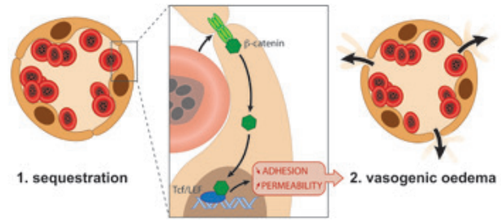
LSHTM Investigators: Sam Wassmer

External collaborators: Ana Rodriguez, (New York University School of Medicine, USA);
Ed Fisher, (New York University School of Medicine, USA); Julio Gallego-Delgado, (New York University School of Medicine, USA); Sanjib Mohanty, (Centre for the Study of Complex Malaria, India); Praveen Sahu, (Centre for the Study of Complex Malaria, India); Sanghamitra Satpathi, (Ispat General Hospital, India); Prativa Behera, (Ispat General Hospital, India); Sonia Joshi, (Ispat General Hospital, India); Krishna Pramanik, (National Institute of Technology, India); Sriti Kayal, (National Institute of Technology, India)

Funding Body: National Institutes of Health (NIH), USA

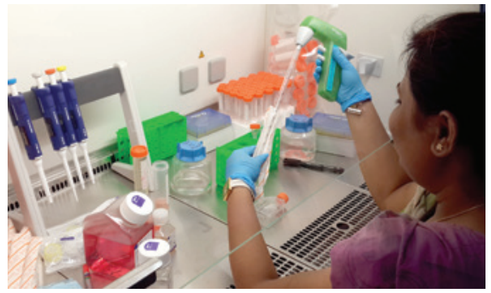
Cerebral malaria is the most severe complication of falciparum malaria. We discovered that strengthening the junctions between endothelial cells in the brain protects mice and human cells against damage induced by *Plasmodium falciparum*-infected red blood cells in vitro. The aim of this project is to investigate the molecular mechanisms responsible for this protective effect, with a view to identifying new treatments.

Cerebral malaria (CM) is caused by the interaction between *Plasmodium falciparum*-infected erythrocytes (PfIE) and host brain endothelial cells. Current management of CM only relies on anti-malarial drugs and while these are effective at clearing parasites from the blood, they do not protect again endothelial cell dysfunction. Using an in vitro model, we found that PfIE induce the activation of β-catenin in human brain microvascular endothelial cells, resulting in transcription of Tcf/LEF in their nucleus, a subsequent disruption of inter-endothelial cell junctions, and their detachment from the substrate. We showed that treatments that inhibit the activation of β-catenin result in protection both against this damage both in vitro, and from experimental CM in mice.



Beta-catenin signalling in endothelial cells during cerebral malaria

We discovered that the signalling induced by angiotensin II receptors (AT1 and AT2) modulates β-catenin in endothelial cells and, as a result, the integrity of the inter-endothelial junctions and blood-brain barrier integrity. Modulators of these receptors protect mice from experimental CM even when treatment is started after severe neurological symptoms develop. We are currently deciphering the signalling pathways triggered by PfIE in endothelial cells associated with the activation of β- catenin and leading to the disruption of inter-endothelial junctions, as well as its inhibition by the AT2 signalling cascade. Our aim is to evaluate potential targeted adjunct therapies in CM, which may also be relevant to the treatment of other brain haemorrhagic diseases.



Scientist isolating primary endothelial cells from the adipose tissue of a patient with cerebral malaria.

General view of Ispat General Hospital in Rourkela, Odisha, India, where part of the study is taking place.

Understanding the importance of the immunodominant CD8+ T cell epitope of *Plasmodium* circumsporozoite protein in parasite- and vaccine-induced protection

Location of study: LSHTM

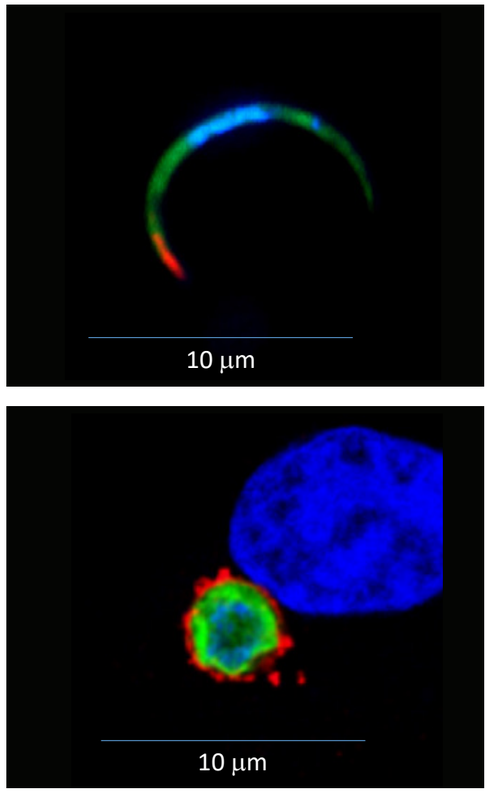
LSHTM Investigators: Matthew Gibbins, Maya Glover, Jasmine Liu Julius Clemence Hafalla

External collaborators: Karolis Bauza, Arturo Reyes-Sandoval, (The Jenner Institute, Nuffield Department of Medicine, University of Oxford, UK); Katja Müller, Kai Matuschewski, (Humboldt University, Berlin, Germany); Olivier Silvie, (Sorbonne Université, Paris, France)

Funding Body: NC3Rs and The Royal Society

The circumsporozoite protein (CSP), the surface coat of sporozoites, is at the forefront of malaria vaccine development for the last 30 years. CSP has been shown to elicit strong CD8+ T cell responses that eliminate the developing parasites in hepatocytes resulting in protection. In this study, we characterised the importance of SYIPSAEKI, the immunodominant CSP-derived epitope of *Plasmodium berghei* in both sporozoite- and vaccine-induced protection in murine infection models.

In BALB/c mice, where SYIPSAEKI is efficiently presented in the context of the major histocompatibility complex class I (MHC-I) molecule H-2-Kd, we show that epitope-specific CD8+ T cell responses contribute to parasite killing following sporozoite immunisation. Yet, sterile protection is achieved in the absence of this epitope reinforcing that other antigens are vital for parasite-induced protective immunity. Furthermore, we establish that SYIPSAEKI-specific CD8+ T cell responses induced by viral-vectored CSP-expressing vaccines efficiently target parasites in hepatocytes and the resulting sterile protection strictly relies on the expression of SYIPSAEKI. We further show that in C57BL/6 mice, which expresses an irrelevant MHC-I and therefore unable to express the immunodominant epitope, CSP-based vaccines are unable to confer protection. These results further demonstrate the importance of CSP in protection against malaria pre-erythrocytic stages and that a significant proportion of the protection against the parasite is mediated by CD8+ T cells that are specific for the immunodominant epitope of this sporozoite surface protein.



Plasmodium berghei sporozoite (A) and exo-erythrocytic form (EEF) (B). Green = GFP, Blue = Hoechst staining of the nucleus, Red = RON4 (for sporozoite) and UIS4 (for EEF)
(Olivier Silvie and Julius Hafalla)

Identification of novel CD8+ T cell epitopes from the pre-erythrocytic stages of malaria

Location of study: LSHTM

LSHTM Investigators: Matthew Gibbins, Julius Clemence Hafalla

External collaborators: Emilio Fenoy & Massimo Andreatta, (Instituto de Investigaciones Biotecnológicas, Universidad Nacional de San Martín, Buenos Aires, Argentina); Morten Nielsen (Instituto de Investigaciones Biotecnológicas, Universidad Nacional de San Martín & Technical University of Denmark, Denmark)

Funding Body: The Royal Society

CD8+ T cells mediate immunity against *Plasmodium* pre-erythrocytic stages. However, the paucity of parasite-specific epitopes of CD8+ T cells has limited our current understanding of the mechanisms influencing the generation, maintenance and efficiency of these responses

Using bioinformatics neural networks, which predict peptides that bind strongly to MHC class I molecules, we have identified and validated novel CD8+ T cell epitopes that induce strong IFN-gamma responses against proteins expressed in the sporozoite and pre-erythrocytic stages following sporozoite immunisation of mice under azithromycin prophylaxis. Feature analysis of these derivative proteins underscores a link between secreted or cell surface proteins and immunogenicity of antigens expressed in the pre-erythrocytic stages of malaria. A quarter of the induced CD8+ T cell response were found to express an antigen-experienced phenotype following immunisation. We propose that the majority of antigens that induce CD8+ T cell responses against the pre-erythrocytic stages of malaria still remain unknown.

Evidence of cross-stage CD8+ T cell epitopes in malaria pre-erythrocytic and blood stage infections

Location of study: LSHTM

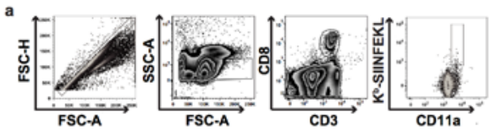
LSHTM Investigators: Matthew Gibbins, Julius Clemence Hafalla

External collaborators: Katja Müller, Kai Matuschewski (Department of Molecular Parasitology, Institute of Biology, Humboldt University, Berlin, Germany)

Funding Body: The Royal Society

Malaria parasites have a complex, multi-stage life cycle and there is a widely held view that each stage displays a distinct set of antigens presented to the immune system. Yet, molecular analysis of malaria parasites suggests that many putative antigenic targets are shared amongst the different stages. The specificities of these cross-stage antigens and the functions of the immune responses they elicit are poorly characterised.

It is well-known that CD8+ T cells play opposing immune functions following *Plasmodium berghei* (Pb) infection of C57BL/6 mice. Whilst these cells play a crucial role in protective immunity against pre-erythrocytic stages, they are implicated in the development of severe disease during blood stages.



Flow cytometry plots showing the gating strategy for identifying K^b-SIINFEKL+ CD11a+ CD8+ T cells. (Matt Gibbins and Julius Hafalla)

Recently, CD8+ T cell epitopes derived from proteins supposedly specific for either pre-erythrocytic or blood stages have been described. We have compiled and confirmed data that the majority of the mRNAs and/or proteins from which these epitopes are derived display expression across pre-erythrocytic and blood stages. Importantly, we provide evidence of cross-stage immune recognition of the majority of these CD8+ T cell epitopes. Hence, our findings provide a resource to further examine the relevance of antigen-specific cross-stage responses during malaria infections.

Investigating host-parasite interactions during symptomatic acute-febrile and asymptomatic malaria infections

Location of study: West African Center for Cell Biology of Infectious Pathogens (WACCBIP), University of Ghana - Legon

LSHTM Investigators: Matthew Gibbins, Julius Clemence Hafalla

External collaborators: Diana Ahu Prah & Gordon Awandare, (West African Center for Cell Biology of Infectious Pathogens, University of Ghana, Accra, Ghana); Linda Amoah, (Noguchi Memorial Institute for Medical Research, University of Ghana, Accra, Ghana); Aubrey Cunnington (Imperial College London, UK)

Funding Body: The Royal Society

Malaria is synonymous with its clinical symptoms – fevers, headache and chills. Individuals with "symptomatic" acute febrile infections present themselves to hospitals, where they are treated with anti-malarial drugs. In countries with high malaria transmission, most individuals with malaria parasites in their blood are "asymptomatic"; these individuals do not come to medical attention, do not receive treatment, and are a reservoir for ongoing spread of malaria by mosquitoes. Asymptomatic individuals have developed an imperfect immune response that is able to control but not completely eliminate the infection. Very little information is available on the immune response pathways that differentiate asymptomatic from symptomatic malaria infections.

The primary aim of this project is to investigate the interactions between the human host and the malaria parasite that may determine whether an infection is symptomatic or asymptomatic. We are identifying differences in the gene expression patterns of both the host and the parasite that distinguish symptomatic and asymptomatic infections. We are also comparing humoral and cellular immune responses to malaria in these individuals. In addressing these objectives, we are building research capacity for molecular biology, immunology and data analysis at our partner site in Ghana.

Regulating CD8+ T cell responses to malaria pre-erythrocytic stages

Location of study: LSHTM

LSHTM Investigators: Samuel Thorburn, Julius Clemence Hafalla

Funding Body: Medical Research Council, The Royal Society

CD8+ T cells have been implicated in protective immune responses in pre-erythrocytic malaria. Natural exposure to pre-erythrocytic malaria infection does not elicit sterile protection in humans, whereas multiple immunisations with large numbers of radiation attenuated sporozoites (RAS) can. We tested the hypothesis that the requirement for high doses of antigen at multiple intervals for sterilising protection is at least in part due to inhibition of CD8+ T cell activation via regulatory pathways that are stimulated during parasite exposure.

We ascertained the roles of the co-inhibitory molecules cytotoxic T lymphocyte antigen 4 (CTLA-4) and programmed death 1 (PD-1) in regulating CD8+ T cell responses and protection against malaria pre-erythrocytic stages. Antibody-blockade of CTLA-4, but not PD-1 ligand, concurrently with a normally non-protective *Plasmodium berghei* (single dose of RAS) immunisation led to a significant increase in antigen-specific interferon-gamma-producing CD8+ T cells upon restimulation. Additionally, this resulted in sterile protection following challenge with normal sprozoites. These experiments are a proof principle that certain regulatory pathways can significantly impact protective CD8+ T cell responses to sprozoite immunisation.

Optimisation and Standardisation of a Reverse Phase Protein array (RPPA) for Malarial Serological Screening

Location of study: LSHTM

LSHTM Investigators: Tate Oulton, Katherine Glass, Will Stone, Kevin Tetteh

Funding Body: Global Good Fund - Intellectual Ventures

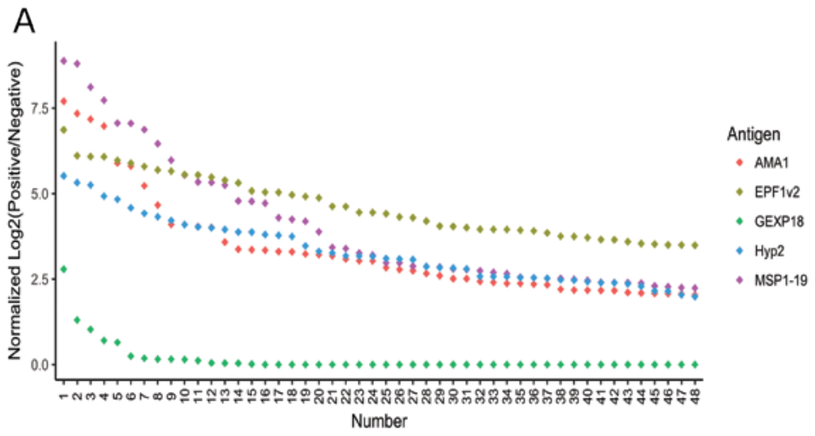
Serological screening for antibody responses in malaria is a proven approach for measuring exposure or investigating putative markers of protection, transmission and infectivity. The microarray platform is a high-throughput method for simultaneously detecting responses against hundreds to thousands of analytes, which we have rigorously optimised for our bespoke applications.

We have evaluated materials, reagents and approaches to the manufacture and screening of protein microarrays for human IgG reactivity, including slide substrates, printing buffers, blocking buffers and a range of protein concentrations.

A core panel of purified proteins, of varying immunogenicity in reference samples, were printed and screened under 480 unique conditions and the performance under each condition compared.

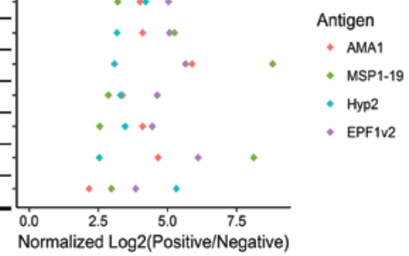
A ratio of reactivity (Normalised Log2(Positive/Negative)) for each condition was calculated based on signals detected in the positive control versus the corresponding negative control, with conditions then ranked for each antigen. The top 10% of ranked conditions were compared between antigens to determine any commonalities, resulting in a list of eight conditions that performed best across the board. Interestingly, no condition performed consistently well for all antigens, with considerable heterogeneity in the reactivity ratio seen between antigens, within each condition. These data highlight the diverse behaviour of proteins in the context of a microarray; a multitude of variables such as size, charge, conformation and epitope availability likely have a considerable impact on performance under different assay conditions. Despite this, we have demonstrated that we are able to tailor a protein microarray platform that performs effectively, and will allow a substantial increase in the throughput of the serological assessment of malarial antigens as biomarkers for potential utility in disease surveillance.

A



B

Slide Type	Blocking Buffer	Block Dilution	Print Buffer	Condition Number
SciChip_Ald	PA	1in2	Glycerol	318
AVID	AB	1in2	JBC	98
NOVA	GB	1in1	JBC	137
NOVA	PA	1in2	JBC	142
AVID	BB	1in2	JBC	102
AVID	PA	1in8	Nexterion_Spot	432
SciChip_Ald	PA	1in2	Nexterion_Spot	478
AVID	PA	1in8	JBC	112



A. The normalized log2(positive/negative) values ranked from highest to lowest for the top 10% (48) of conditions for each antigen printed at 100 µg/mL. Numbers 1-48 do not represent specific conditions, but rather a different set of the 48 conditions with the highest responses for each antigen. B. Normalized Log2(positive/negative) values for the 8 conditions which have a response in the top 10% for four antigens: AMA1, MSP1-19, Hyp2, and EPF1.

Using human sera to profile antibody isotype responses to a panel of *P. knowlesi*-specific recombinant antigens.

Location of study: Sabah, Malaysia

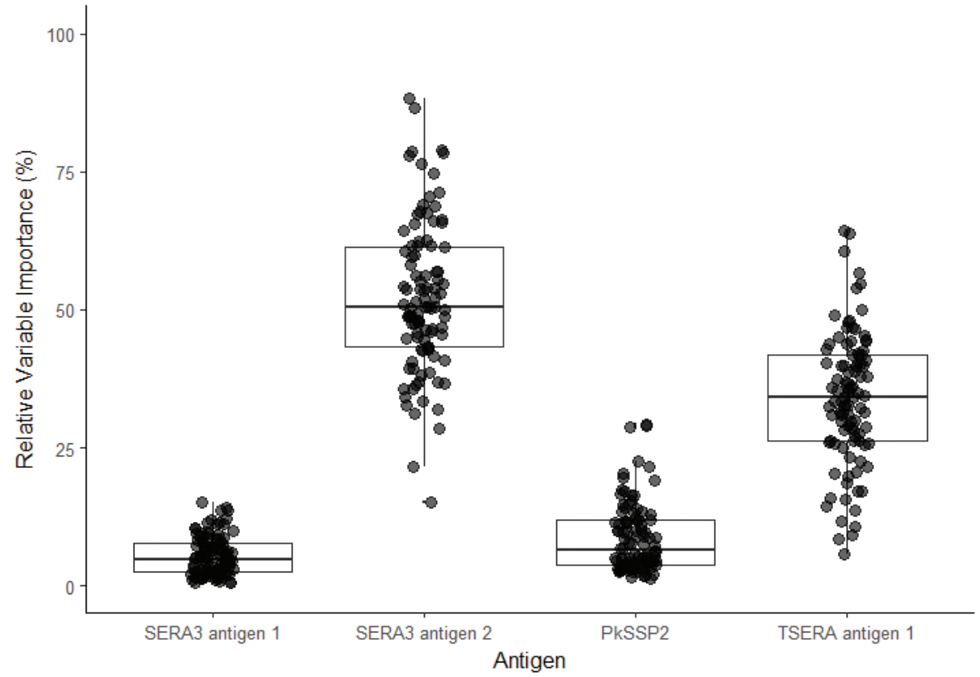
LSHTM Investigators: Lou Herman, Tate Oulton, Katherine Glass, Kimberly Fornace, Chris Drakeley, Kevin Tetteh

External collaborators: Matthew Grigg, Nicholas Anstey, Timothy William Michael Blackman

Malaria cause by *Plasmodium knowlesi* is the most common form of the disease in Malaysian Borneo. It is a zoonotic disease transmitted from macaques to humans through mosquito bite. The true extent of the geographical boundaries for *P. knowlesi* transmission is currently unknown. The ability to measure antibodies to infection is a powerful tool which would help address this problem. We have developed a panel of recombinant proteins to be used as serological tools and to determine the antibody profiles this pathogen elicits.

Antibody responses to *Plasmodium* spp. are known to play an important role in disease progression in infected hosts.

Much of the current data on antibody mediated protection and associated isotypes has come from human malaria but little is known about *Plasmodium knowlesi.* *P. knowlesi* is now the most common cause of malaria in Malaysian Borneo, with scarce data on the true extent of transmission and no data on the different reactivity profiles the disease elicits in endemic populations. A recently developed set of *P. knowlesi*-specific antigens (n=10) were included in a protein microarray panel to determine antibody isotype (IgM, IgG and IgA) reactivity patterns for three post-treatment time-points from a hospital clinical treatment trial in Sabah, Malaysia (n = 12 individuals; 36 total samples for all time points) using labelled Qdot fluorescent probes. Strong reactivity of all isotypes to *Pk*SERA3 antigen 2 was seen at all time points. IgG showed the strongest reactivity profile overall, followed by IgM which decreased over the course of follow up. IgA reactivity towards *Pk*MSP1 antigen 2 and *PK*H_031930 antigen 2 mirrored that of IgM, indicating possible class switching from IgM to IgA for some antigens. These reagents represent novel tools with which to measure seroincidence to *P. knowlesi.* The development of such tools would help to answer questions relating to population exposure and our understanding of the geographical boundaries of infection. The differing isotype profiles in infections may be related to disease progression and severity – further work is need in this area.



Plot showing the relative variable importance of responses to predicted P.knowlesi-specific antigens based on 100 boosted regression tree models predicting P. knowlesi seropositivity.
Median values for the relative variable importance and interquartile ranges are shown for all antigens tested: SERA3 ag 1 (4.8%; IQR 2.5–7.8%); SERA3 ag 2 (50.4%; IQR 43.3–61.4%); PkSSP2/TRAP (6.5%; IQR 3.7–11.8%) and TSERA ag 1 (34.2%; IQR 26.2–41.8%). (ref: Herman et al. PMID: 29902183)

Vector Biology

As *Plasmodium* parasites are transmitted by *Anopheles* mosquitoes, vector biology plays a major role in the battle against malaria, promoting a better understanding of the malaria life cycle, which in turn facilitates the discovery and use of more effectively targeted control strategies. LSHTM staff and their collaborators have been contributing to research on different aspects of vector biology and malaria transmission, as outlined in this section of the Report.

With a growing demand for rice in Africa and expansion of irrigated rice cultivation, there is a potential that vector densities will increase since since flooded rice fields are ideal breeding sites for the main malaria vectors. New research suggests that due to the universal access to insecticide treated nets the relationship between rice growing, vector abundance and malaria should be reassessed. Moreover, there is a need to use novel rice growing techniques that could potentially reduce vector densities. In order to be able to measure the effect, mosquito abundance should be a parameter included in rice research to estimate the adult vector productivity of rice fields.

Malaria transmission is also affected by the willingness of mosquitoes to feed on humans. Although mosquitoes are frequently described as “anthropophilic” or “zoophilic”, recent meta-analysis shows that human blood index is more associated with mosquitoes that were captured, rather than the species of mosquitoes. Furthermore, a recent study showed that attractiveness of humans to mosquitoes due to production of certain skin odour compounds is determined by human genes. Identifying genes linked to the production of these compounds could be used to develop novel malaria control methods. Another study showed that biting behaviour of mosquitoes, especially avoidance behaviour, together with insecticide resistance, can significantly impact malaria control efforts such as deployment and use of LLINs.

Different factors play a part in who or what the mosquitoes bite. Mosquito feeding choices partly depend on their dispersal but measuring mosquito dispersal in the field is extremely difficult. New molecular approach based on digestion of mosquito blood meals over time was developed by LSHTM researchers and can be used to estimate the dispersal rates of mosquitoes.

Presence of endosymbiotic bacteria such as *Wolbachia* can also affect transmission of vector-borne diseases. Until recently, *Wolbachia* was thought to be absent in *Anopheles* mosquitoes. A recent study described the presence of novel *Wolbachia* strains in five *Anopheles* species collected in different African countries. Further studies will be carried out to determine whether they inhibit *Plasmodium* transmission and could therefore be used for population replacement or suppression control strategies.

The ability to detect the presence of infectious mosquitoes is of vital importance for surveillance, control and elimination efforts. A study showed that sugar-feeding behaviour of mosquitoes can be exploited for surveillance purposes as mosquitoes expel sporozoites during sugar feeding. FTA cards can potentially be used as a simple and economical tool for collection of field samples for monitoring purposes.

Insecticide resistance remains a major threat to malaria control. A project in Uganda involves monitoring phenotypic and genotypic resistance across the country to determine the association between single nucleotide polymorphisms (SNPs) that confer resistance, mosquito survival post-insecticide-exposure, and sporozoite rates. Association between vector control interventions and the prevalence of SNPs that confer resistance is also being assessed.

Insectaries at MRC Unit The Gambia
Image Courtesy of: Louis Leeson for LSHTM

Novel *Wolbachia* strains in *Anopheles* malaria vectors from Sub-Saharan Africa and their role in malaria transmission.

Location of study: Guinea, Democratic Republic of the Congo (DRC), Ghana, Uganda , Madagascar

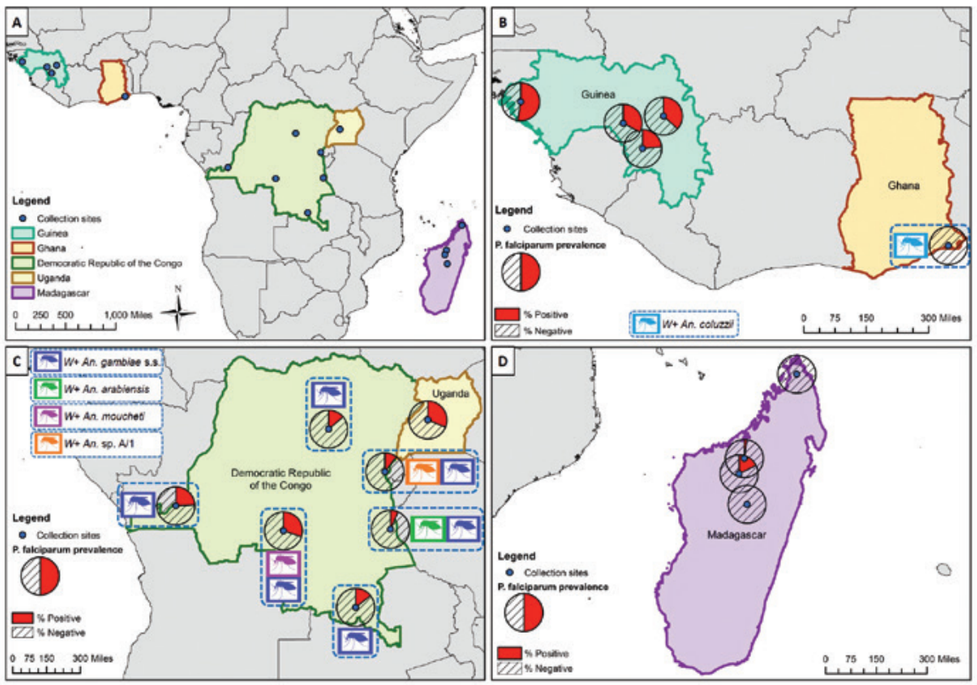
LSHTM Investigators: Thomas Walker, Claire Louise Jeffries, Mojca Kristan, James Orsborne, Kirstin Spence, Eliot Hurn

External collaborators: Gena G Lawrence and Seth R Irish, (Centers for Disease Control and Prevention, US); Janvier Bandibabone, (CRSN/LWIRO, DRC); Luciano M Tantely, Sébastien Boyer and Fara N Raharimalala, (Institut Pasteur de Madagascar, Madagascar); Kalil Keita, Denka Camara and Yaya Barry (Ministere de la Sante, Guinea); Francis Wat'senga and Emile Z Manzambi, (National Institute of Biomedical Research, DRC); Yaw A Afrane and Abdul R Mohammed, (University of Ghana, Ghana); Tarekegn A. Abeku, (Malaria Consortium, UK); Shivanand Hegde, Kamil Khanipov, Maria Pimenova and Yuriy Fofanov, George Golovko (University of Texas Medical Branch, US); Grant L Hughes (Liverpool School of Tropical Medicine, UK)

Funding Body: Wellcome Trust/Royal Society

***Wolbachia* are endosymbiotic bacteria found in ~40% of insects but historically thought to be absent in *Anopheles*. We discovered novel strains in species including *Anopheles moucheti* and we are currently investigating if high density strains can inhibit malaria transmission in *Anopheles* species and how they are maintained in mosquito populations.**

We have analysed a range of *Anopheles* species to determine *Wolbachia* prevalence rates, characterise novel *Wolbachia* strains and determine any correlation between the presence of *Plasmodium, Wolbachia*, and the competing endosymbiotic bacterium *Asaia*. *Anopheles* adult mosquitoes were collected from five malaria endemic countries: Guinea, DRC, Ghana, Uganda and Madagascar, between 2013 and 2017. Molecular analysis has revealed a novel *Wolbachia* strains in species including *An. coluzzii*, *An. gambiae* s.s., *An. arabiensis*, *An. melas, An. moucheti*, *An.* species 'A' (A potentially new distinct species, as yet unnamed). Variable prevalence rates in different locations have been shown and novel strains are phylogenetically diverse. *Wolbachia* is the dominant member of the microbiome in *An. moucheti* and *An.* species 'A', but present at lower densities in *An. coluzzii*. The important discovery of novel *Wolbachia* strains in *Anopheles* provides greater insight into the prevalence of resident *Wolbachia* strains in diverse malaria vectors. Further experiments are planned to determine if high density *Wolbachia* strains inhibit malaria transmission and we will determine how these strains are being maintained in wild mosquito populations. Novel *Wolbachia* strains (particularly high density strains) are ideal candidate strains for transinfection to create stable infections in other *Anopheles* mosquito species, which could be used for population replacement or suppression control strategies.



A) Map showing the five malaria-endemic countries where mosquito collections were undertaken. B) High malaria prevalence rates in Guinea, and Wolbachia-infected An. coluzzii from Ghana. C) Wolbachia strains in An. gambiae s.s., An. arabiensis, An. species A and An. moucheti from DRC and variable P. falciparum prevalence rate in DRC and Uganda. D) Low P. falciparum infection rates in Madagascar and no evidence of resident Wolbachia strains. (W+; Wolbachia detected in this species).

Malaria vector biting behaviour

Location of study: Ghana and Guinea

LSHTM Investigators: James Orsborne, Thomas Walker, Laith Yakob

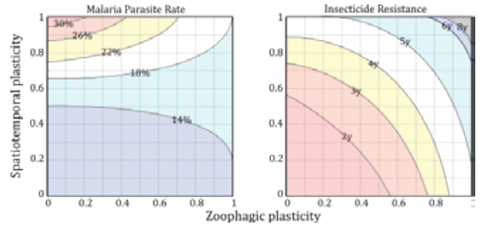
External collaborators: Yaw Afrane, (University of Accra, Ghana), Kalil Keita, (National Malaria Control Program, Guinea); Seth Irish, (CDC, USA)

Funding Body: MRC and Royal Society

This research looks into how the mosquito vectors of malaria choose which host species to bite. Understanding how the environment can alter biting behaviour can inform how an environment could be altered to deflect bites away from people. It can also be used to inform targeting of different control tools.

A systematic review and meta-analysis of the human blood index of the major African malaria vectors revealed that the host choice was more associated with location of mosquito captures (indoors versus outdoors) than with mosquito (sibling) species, calling into question the dogma of 'anthropophilic' versus 'zoophilic' vector species.

Using data-driven mathematical models, it was shown how understanding this biting behaviour is central to being able to project insecticide resistance frequency. It also underlies malaria control efficacy of bednets both in the short- and long-term.



Impact of spatiotemporal and zoophagic plasticity on malaria infection prevalence (averaged over the fourth bednet distribution cycle; left) and on the number of years (y) following bednet distribution before insecticide resistance reaches 50% frequency in the local vector population (right).

A novel method for assessing vector dispersal

Location of study: Ghana, Burkina Faso

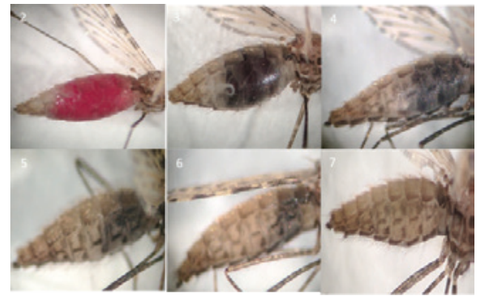
LSHTM Investigators: James Orsborne, Thomas Walker, Laith Yakob

External collaborators: Yaw Afrane, (University of Accra, Ghana), Abdoulaye Diabate, (Institut de Recherche en Science de la Santé/Centre Muraz, Burkina Faso)

Funding Body: MRC

Measuring vector dispersal in the field is extremely difficult. Within his PhD, James Orsborne (supervised by Laith Yakob and Thomas Walker) is developing a completely new method for measuring dispersal. This new method could be broadly applicable to other blood-feeding disease vectors.

Using quantitative PCR, the digestion of mosquito blood-meals was quantified for field-caught mosquitoes and calibrated according to timed blood digestion in colony mosquitoes. It was demonstrated how this new 'molecular Sella score' approach can be used to estimate the dispersal rate of blood-feeding vectors caught in the field.



Images of Anopheles gambiae mosquitoes fed on human blood periodically removed to produce a Sella score panel on which PCR assay sensitivity could be tested. Image credit: J Orsborne

Science Officer Pa Modou Gaye collects male mosquitoes in a test tube, Wali Kunda, The Gambia - October 2019

Malaria sporozoite detection during mosquito sugar feeding

Location of study: LSHTM and Guinea

LSHTM Investigators: Thomas Walker, Mojca Kristan, Victor Brugman

Funding Body: Royal Society

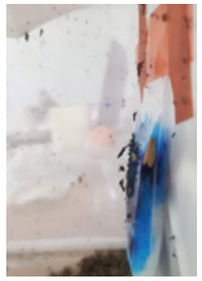
Rapid detection of infectious mosquitoes able to transmit malaria is of vital importance. Our aim was to determine if *Plasmodium*-infected *Anopheles* mosquitoes expel sporozoites during sugar feeding and if these sporozoites can be detected with sugar-coated FTA cards, providing a simple tool for sentinel site surveillance in malaria endemic settings.

Modified CDC light trap with a sugar-and-dye-soaked FTA card inside a mosquito cage was used in Guinea.

The ability to detect the presence of infectious mosquitoes is of vital importance for surveillance, control and elimination efforts, but can be labour-intensive and has to be carried out by trained personnel.

In laboratory experiments we demonstrated that *Anopheles* mosquitoes infected with *Plasmodium berghei* and *P. falciparum* expel sporozoites during sugar feeding.

Sporozoite DNA was detected on sugar-soaked cotton wool and FTA cards using real-time PCR even when low numbers of sporozoites were ejected. These results demonstrate an effective and rapid methodology for detecting the presence of infectious mosquitoes with sporozoites and highlight potential laboratory applications for investigating mosquito-malaria interactions.



Mosquitoes caught with a modified CDC light trap at night feeding on a sugar-and-dye-soaked FTA card.

Sugar feeding behaviour of adult female mosquitoes could also be exploited to detect malaria sporozoites in the wild, with FTA cards as a simple and economical tool used to enhance field surveillance activities for malaria. They preserve RNA and DNA without the need for a cold storage chain which is often problematic in tropical field settings. They were tested in a small field trial in Guinea, using modified CDC light traps but this was not effective due to low sporozoite rates. However, FTA cards have a potential to be used if further modified, allowing contact with larger numbers of infected mosquitoes over a longer period, not just a single night.

An Anopheles mosquito female which fed on a sugar-and-dye-soaked FTA card.

Expression of Insecticide Resistance in Indoor and Outdoor-resting *Anopheles gambiae* sl in Northern Ghana

Location of study: Ghana, The Gambia

LSHTM Investigators: Majidah Hamid-Adiamoh (MRC Gambia, LSHTM)

External collaborators: Alfred Amambua-Ngwa & Davis Nwakanma, (Medical Research Council Unit, The Gambia at LSHTM); Yaw Afrane, (West African Centre for Cell Biology of Infectious Pathogens (WACCBIP), University of Ghana, Legon, Ghana)

Funding Body: West African Centre for Cell Biology of Infectious Pathogens (WACCBIP)-Wellcome Trust-DELTAS

Selection pressure from continued exposure to insecticides seems to be driving development of resistance and changes in resting behaviour of malaria vectors, which could contribute significantly to residual malaria transmission. The differences in the expression of insecticide resistance within *Anopheles gambiae sl* resting indoors and outdoors in Northern Ghana was examined to understand their contribution to 'residual malaria transmission.

Live adult mosquitoes were collected indoors and outdoors and progenies generated were assessed for phenotypic and genotypic resistance to DDT, deltamethrin, Malathion and bendiocarb using WHO insecticide susceptibility tests and molecular assays.

A significant difference in phenotypic resistance to DDT and deltametrin, correlating with differential expression of *Vgsc-1014F* and *GSTe2-114T* was observed between the indoor and outdoor mosquito populations. Carriage of *Vgsc-1014F* mutation was strongly associated with resistance to deltamethrin (OR =5.46, p= 0.001) but not to DDT (OR =0.69, p= 0.75). Expression of *Vgsc-1575Y* was also significantly higher in the deltamethrin-resistant outdoor mosquitoes than the indoor mosquitoes (X2 = 7.09, p =0.008).

Both mosquito populations were fully susceptible to malathion with indoor and outdoor mortality of 100% and 98% respectively. A suspected resistance was found to bendiocarb with no significant difference in mortality between the indoor (90%) and outdoor (95%) mosquito populations (X^2 = 1.07, p =0.30). However, a positive association was observed in the indoor bendiocarb-resistant mosquitoes and carriage of *Ace1-119S* mutation, but this was not significant (OR =2.22, p= 0.29).

Program for Resistance, Immunology, Surveillance, and Modelling of malaria (PRISM2): Insecticide Resistance Project

Location of study: Uganda

LSHTM Investigators: Henry Ddumba Mawejje, Sarah Staedke, Jo Lines

External collaborators: Martin Donnelly, (Liverpool School of Tropical Medicine, UK); Sam Nsobya and Moses Kamya, (Makerere University/Infectious Diseases Research Collaboration Uganda); Grant Dorsey, (University of California, San Francisco, US)

Funding Body: National Institutes of Health (NIH) - National Institute of Allergy and Infectious Diseases (NIAID)

Insecticide resistance remains a major threat to malaria control. This project will assess the status and intensity of insecticide resistance, evaluate its interaction with vector control and malaria transmission, and identify novel resistance-associated genes in local malaria vector populations.

This study will answer fundamental questions about insecticide resistance, mosquito survival and sporozoite infection using single nucleotide polymorphism (SNP) associations. The study will involve repeated cross-sectional monitoring of measures of phenotypic and genotypic insecticide resistance in different mosquito populations throughout Uganda. Mosquitoes will be collected from the catchment area of the Uganda Malaria Surveillance Project (UMSP) Malaria Reference Centers (MRCs), and other sites of national interest. The specific objectives of the project include (1) to evaluate the association between SNPs that confer insecticide resistance (*Cyp4j5, Coeae1d, kdr* L1014S, *kdr* L1014F, & Ace - 1 119S) and mosquito survival in insecticide exposed *An. gambiae s.l.* adults; (2) to evaluate the association between resistance SNPs and sporozoite infection in wild caught *An. gambiae s.s.*; and (3) to evaluate the association between vector control interventions and the prevalence of SNPs that confer insecticide resistance in *An. gambiae s.l.* mosquitoes across time and space. Initially, we will screen for resistance-associated polymorphisms in *An. gambiae* and *An. arabiensis,* as these are likely the primary vectors in most study locations, but this may change over time. The results of this project will provide an improved understanding of the scope and intensity of insecticide resistance in Uganda.

The mechanisms underlying the production of natural mosquito repellents by human beings

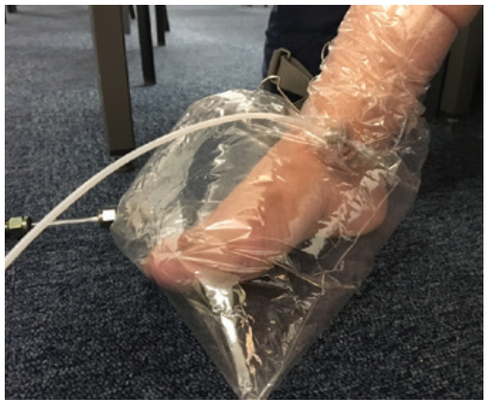
Location of study: LSHTM, UK; MRC, The Gambia

LSHTM Investigators: James Logan, Umberto D'Alessandro, Julien Martinez, Catherine Oke, Thomas Ant, Elizabeth Pretorius

External collaborators: John Armour, (University of Nottingham, UK); Steven Lindsay, (Durham University, UK); John Pickett, (Rothamsted Research, UK)

Funding Body: MRC

A recent study demonstrated that human genetics plays an important role in determining our attractiveness to mosquitoes. We are measuring the attractiveness of identical and non-identical twins to malaria-transmitting mosquitoes, and using genome sequence data to identify the genes involved. If the genes can be identified, we can use this information to develop novel methods to protect against malaria.



Air entrainment captures the volatile odour compounds released from the skin

Wind tunnels can be used to quantify mosquito attractiveness to different hosts

We have collected odour compounds from the skin of 90 pairs of identical and non-identical twins using two methods: 1) asking the twins to wear a nylon sock for 24 hours, and 2) using a technique called air entrainment by which skin volatiles are captured and dissolved in a solvent.

Levels of attractiveness of the different socks are quantified in wind tunnel behavioural experiments, in which female mosquitoes are presented with the choice of flying towards a sock worn by a participant or an unworn sock. Gas-chromatography analysis is being used in parallel to identify and quantify individual volatile compounds from the solvent solutions, to determine which compounds are associated with attractiveness.

In collaboration with Nottingham University, we plan to use existing genomic data from the twins to perform an association analysis and identify genes linked to the production of volatile compounds and attractiveness to mosquitoes.

With the MRC Unit The Gambia we are conducting a similar study throughout 2019, measuring attractiveness in a human population naturally exposed to malaria. The aim here is to investigate whether genes play a role in the modulation of attractiveness, caused by infection.

Kota Marudu MONKEYBAR Project
Image Courtesy of: Joshua Paul for LSHTM

Epidemiology and Transmission

With malaria, even more than with other infectious diseases, patterns of transmission are extraordinarily variable from place to place. For example, in Africa, malaria is transmitted in both savannah and forest areas, whereas in Southeast Asia, malaria exposure is closely associated with forest, and is now more or less confined to the provinces where there are still areas of dense forest. Moreover, transmission can be observed as the prevalence or incidence of human infection, or as the rate at which people are bitten by infectious mosquitoes. We need techniques to measure these processes, and an understanding of how they interact, in order to identify risk factors, to measure the effectiveness of interventions, and to track the progress of malaria control.

LSHTM Malaria Centre members are engaged in a wide range of studies on the epidemiology and transmission of malaria, across different continents and in varying malaria transmission settings. Many projects have taken into account of the increased intervention coverage and the subsequent declining transmission of malaria worldwide, thus focusing on sources of remaining transmission. At the Gambian MRC Unit The Gambia, Malaria Centre membersare trying to determine the significance of asymptomatic malaria as a source of infections. On the other side of the continent, the Ugandan PRISM2 project is also trying to characterise the 'human reservoir of infection', by examining the dynamics of gametocyte production and human infectivity to mosquito vectors. Simultaneously, data from these two countries are being used to evaluate the accuracy of a set of novel *Plasmodium falciparum* antigens as measures of sero-incidence. In Northern Sumatra, where humans are exposed to several *Plasmodium* species, active and passive methods of care detection have been combined to better understand the mix of infections present in humans.

The effect of environmental factors such as land-use change on malaria has also been investigated. In Africa, rice fields provide ideal breeding conditions for malaria vectors.

A CGIAR Agriculture for Nutrition and Health (A4NH)-funded project in Cote d'Ivoire is investigating how certain rice-growing techniques can increase or reduce vector abundance, with the hope that a suitable combination of conventional techniques could give effective vector control. In Borneo, zoonotic malaria *P. knowlesi* is also associated with environmental change. The MONKEYBAR project showed that dependent on the spatial scale, forest use and house construction and agricultural and forest cover were associated with increased risks of exposure. The significance of spatial scale was illustrated in another Malaysian study: spatial scale determined which landscape factors (proportion of cleared land around a household, aspect, slope and canopy regrowth and fragmentation of deforested areas) could best predict the incidence of zoonotic malaria.

In the future, as malaria density decreases worldwide, studies performed in low transmission settings will become increasingly useful. Novel approaches in risk mapping and serological surveys can help identify remaining areas of high malaria burden and allow for more impactful control and elimination programme planning.

Validating serological markers of recent malaria exposure as measures of sero-incidence

Location of study: The Gambia, Uganda

LSHTM Investigators: Lindsey Wu, Chris Drakeley, and Kevin K.A. Tetteh

External collaborators: Isabel Rodriquez, Bryan Greenhouse, Inna Gerlovina (UC San Francisco, USA)
Isaac Ssewanyana (Infectious Disease Research Collaboration, Uganda)

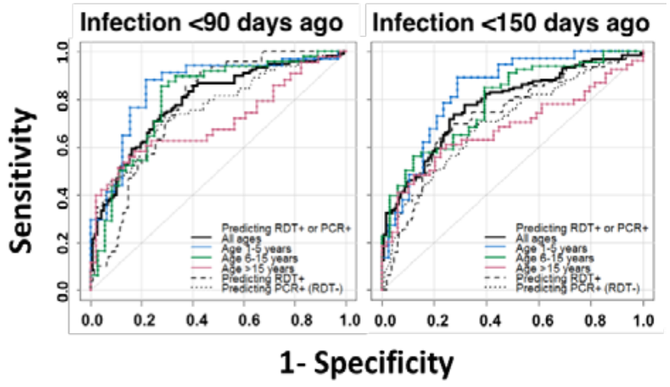
Funding Body: UK Medical Research Council, US National Institutes of Health, Intellectual Ventures

Serology has been widely used to measure historical malaria exposure based on antibody responses to *Plasmodium falciparum* parasite proteins. Recent studies have also identified markers correlated with recent malaria infection via protein microarray. Using the Luminex quantitative suspension array technology (qSAT) and individual-level longitudinal data from The Gambia and Uganda, this project aims to evaluate a panel of novel *Pf* antigens and their accuracy as measures of sero-incidence.

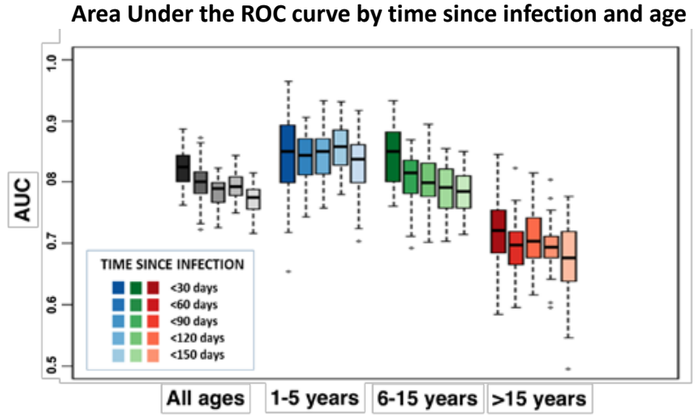
With the Luminex multiplex immunoassay (MAGPIX) and a panel of over 20 *Pf* antigens, we quantified the temporal dynamics of antibody responses in an all-age longitudinal cohort of 2,688 individuals in rural Gambia. We estimated their sensitivity/specificity for identifying rapid diagnostic test (RDT)- positive or polymerase chain reaction (PCR)-positive infections in the last 150 days. Combined antibody responses to several antigens showed high sensitivity/specificity as markers of *Pf* infection in the previous six months. Area under the curve (AUC) values ranged from 0.85 (95%CI 0.72-0.91) and 0.85 (0.77-0.92) to 0.77 (0.62-0.82) in ages 1-5 years, 6-15 years, and >15 years respectively.

Research is currently ongoing to compare these outcomes with longitudinal data in Uganda amongst individuals with confirmed malaria infection in the previous year. The predictive power of these serological markers is being assessed with respect to epidemiological covariates such as age, time since last infection, geographical region, parasite density, and repeat exposure.

Receiver Operating Characteristics by time since infection and age



Area Under the ROC curve by time since infection and age



Receiver Operating Characteristic (ROC) curves for a combined panel of serological markers estimating time since last infection by age category and RDT- or PCR-positive infection (top) and Area under the ROC curve (AUC) for a combined panel of serological markers to estimate time since last infection by age category, based on cross-validated ROC analysis (bottom).

Rice and Malaria Vectors in West Africa

Location of study: Cote d'Ivoire

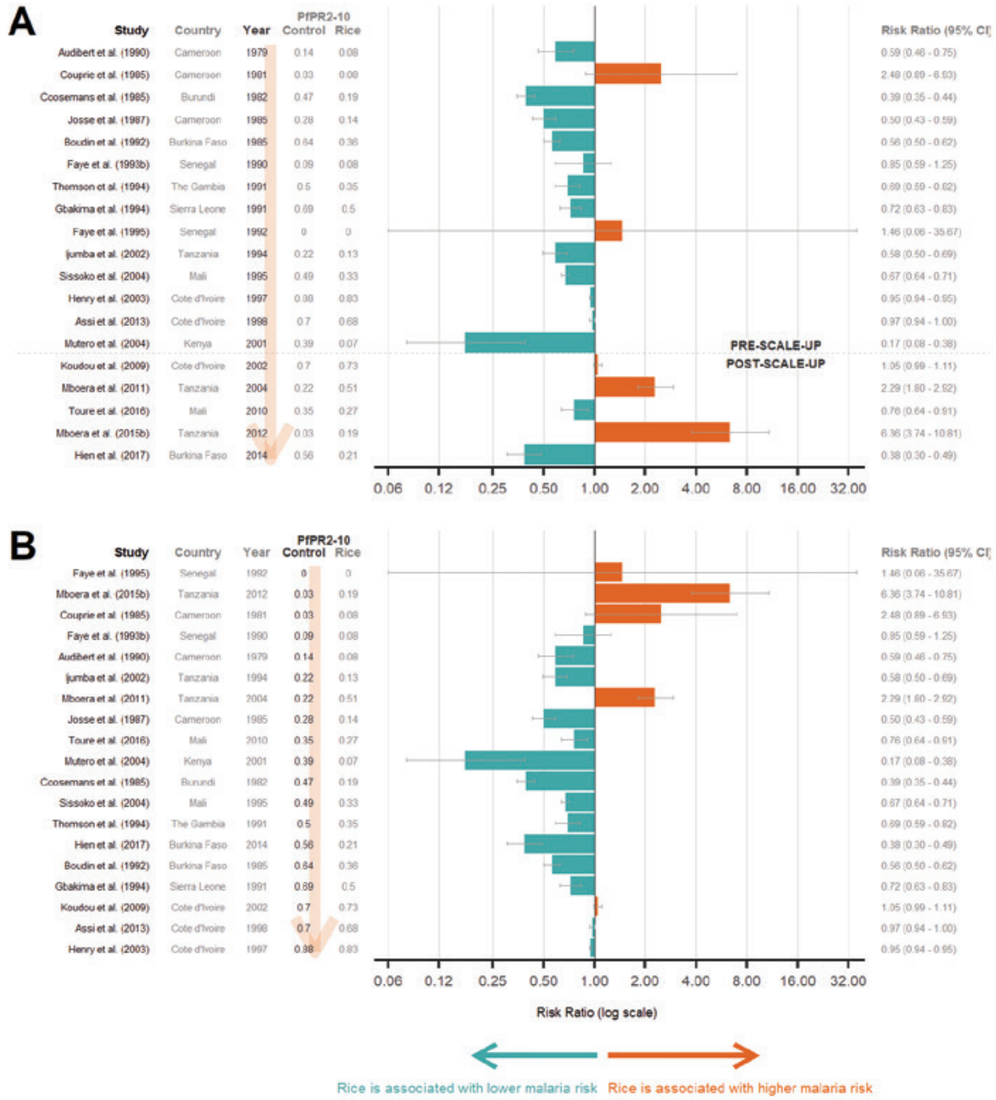
LSHTM Investigators: Jo Lines, Jeff Waage, Kallista Chan, Raphael N'Guessan

External collaborators: Kazuki Saito (AfricaRice, Cote d'Ivoire), Rousseau Djouaka (International Institute of Tropical Agriculture)

Funding Body: CGIAR Agriculture for Nutrition and Health Research Programme

Rice fields are ideal breeding sites for African malaria vectors and can bring increased malaria risk amongst rice-growing communities. However, for a growing African population, rice cultivation is a necessity and cannot be stopped. Thus, to minimise malaria risk, we are searching for rice-growing techniques that can significantly minimise vector densities.

Rice can be grown in vast and variable methods and the techniques that could potentially affect vector densities are endless. Some of these techniques include water management (alternate wet-dry irrigation vs. continuous flooding), crop establishment, weeding, hoeing and pesticide and herbicide use. At present, the main outcomes of rice research include yield, water use and weed production. However, we want to find out how current and prospective rice-growing methods could affect (increase or decrease) malaria vector densities. Thus, we collaborate with AfricaRice on their on-farm trials, and include mosquito abundance as an additional parameter to record in their rice research. This will involve developing an efficient and representative method of sampling larvae in rice fields, and using that to estimate the adult vector productivity of rice fields. We will also explore rice farmers' views and perspectives on rice cultivation and its effect on mosquitoes and health.



Predictive analysis across spatial scales links zoonotic malaria to deforestation

Location of study: Sabah, Malaysia

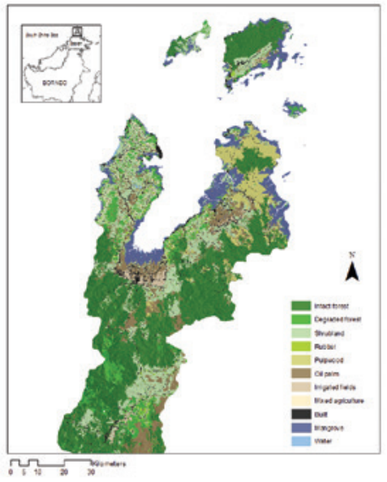
LSHTM Investigators: Kimberly Fornace, Jon Cox, Chris Drakeley

External collaborators: Paddy Brock, Heather Ferguson, Rowland Kao (University of Glasgow, UK); Timothy William (Infectious Diseases Society Kota Kinabalu, Malaysia); Matthew Grigg, Nicholas Anstey (Menzies School of Health Research, Australia)

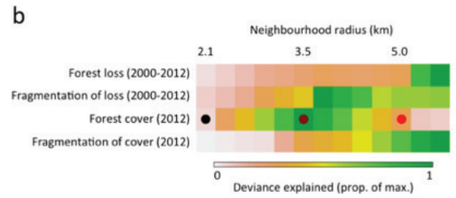
Funding Body: UK Research Councils

Incidence of the zoonotic malaria *Plasmodium knowlesi* has been linked to deforestation. As most people are infected outside of the house, household risk may be determined by environmental factors at different spatial scales due to the movements and distribution of people, mosquito vectors and wildlife hosts.

We utilise a machine learning pathway to identify landscape factors defining the relationship between zoonotic malaria and environmental change. Using data from satellite imagery, GPS locations of *P. knowles* cases and a cross-sectional survey, we fit models of household occurrence of *P. knowlesi* at 11 spatial scales, from 100m to 5km around the house. The proportion of cleared land around the household was most predictive at 1km while aspect, slope and canopy regrowth were all important at small spatial scales. In contrast, fragmentation of deforested areas influenced *P. knowlesi* at larger scales (4 and 5km). Models were most predictive at multiple spatial scales, illustrative of the complex nature of transmission of this disease and relationships with environmental change.



Three example neighbourhood sizes around a case household showing % of forest cover



Deviance explained by forest variables at different distances

Preparatory study for an assessment of the relative contribution of symptomatic malaria cases and asymptomatic infections to onward malaria transmission in The Gambia

Location of study: The Gambia

LSHTM Investigators: Marta Moreno, Chris Drakeley, Umberto D'Alessandro

External collaborators: Teun Bousema (Radboud University Nijmegen Medical Centre, The Netherlands)

Funding Body: The Bill and Melinda Gates Foundation

There is increasing awareness that a large fraction of malaria-infected individuals do not experience symptoms that elicit treatment-seeking and, thus, these individuals may constitute a major source of new infections. Understanding the significance of asymptomatic malaria is particularly relevant for low-transmission and elimination settings and for improving malaria transmission-reduction interventions.

An observational study across different malaria transmission intensities will be performed and we will examine the relative contribution of symptomatic and asymptomatic malaria infections in the Upper River Region in The Gambia.

First, we have to ensure that the system is sufficiently sensitive for meaningful assessments of the human infectious reservoir for malaria. For this, a preparatory study that involves the validation of xenodiagnostic surveys is currently underway at the MRC Unit The Gambia.

We recruited participants (>2 years old) with patent *P. falciparum* gametocytemia (≥1 gametocyte/µl) from September 2018 to January 2019. Then, we conducted direct membrane feeding assays (DMFAs) to assess their infectiousness to *Anopheles coluzzii* lab-reared mosquitoes.

We will examine the association between parasite and gametocyte density and infectiousness to mosquitoes. Furthermore, we will integrate infectivity data from feeding assays, parasite quantification (confirmation by qPCR), gametocyte sex ratio and maturity to assess the likelihood to infect local malaria vectors.

The team of the insectary in Basse Station (The Gambia) where the malaria vector Anopheles coluzzi is reared to conduct the feeding experiments.

MONKEYBAR: Understanding environmental risk factors for the zoonotic malaria *Plasmodium knowlesi:* a cross-sectional survey

Location of study: Sabah, Malaysia

LSHTM Investigators: Chris Drakeley, Kimberly Fornace, Jon Cox, Kevin Tetteh, Lynn Grignard, Lou Herman, Katie Patterson, Tom Hall

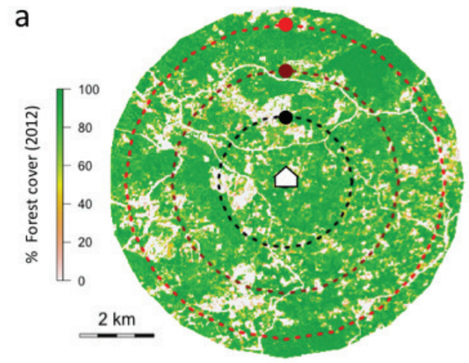
External collaborators: Paddy Brock (University of Glasgow, UK); Tommy Abidin, Tock Hing Chua, Sylvia Daim (Universiti Sabah Malaysia, Malaysia); Timothy William (Infectious Diseases Society Kota Kinabalu, Malaysia); Matthew Grigg, Nicholas Anstey (Menzies School of Health Research, Australia)

Funding Body: UK Research Councils

Increases in the numbers of human infections with the zoonotic malaria *Plasmodium knowlesi* are believed to be driven by environmental change, increasing the proximity between people, macaques and mosquitoes. We conducted a cross-sectional survey to identify environmental risk factors for *P. knowlesi* exposure in Malaysian Borneo.

Monkeybar fieldworker Dellroy Donny flies drone for land use mapping in Sabah, Malaysia

We sampled over 10,000 people in 180 villages across Northern Sabah Malaysia and tested for infection and serological exposure to *P. knowlesi* and other malaria species. To identify how the landscape affects risk, we used a drone to map land cover in selected areas and derived detailed land cover maps for Northern Sabah around the sampled houses. Although few infections were detected, approximately 5% of the population had been exposed to *P. knowlesi* in the past year. Higher ages, male gender, forest use and house construction were all associated with increased risks of exposure while elevation and insecticide use were protective. As well, agricultural and forest cover at different spatial scales from sampled households were associated with exposure. Results suggest agricultural expansion and forest fragmentation impact on *P. knowlesi* exposure, supporting linkages between land use change and knowlesi transmission.



Final land cover map derived from satellite and drone imagery

Program for Resistance, Immunology, Surveillance, and Modelling of malaria (PRISM2): Transmission Project

Location of study: Tororo district, Uganda

LSHTM Investigators: Chris Drakeley, Sarah Staedke

External collaborators: Teun Bousema and Chiara Andolina, (Radboud Institute for Health Sciences, Netherlands); Grant Dorsey, (University of California, San Francisco, USA); Moses Kamya, (Makerere University / Infectious Diseases Research Collaboration, Uganda)

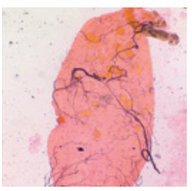
Funding Body: UK Research Councils

A better understanding of how malaria is transmitted from human hosts to mosquito vectors is needed. We aim to characterize factors associated with gametocyte production, evaluate the impact of human and parasite factors on parasite infectivity to mosquito vectors, and characterize the human infectious reservoir for malaria in Tororo, Uganda.

Between October 2017 and October 2018, we followed 492 participants from 80 randomly selected households in Tororo, Uganda. Participants are seen monthly to estimate the incidence of malaria and assess parasite densities in relation to characteristics of the human host and the infection. Blood samples from individuals who were qPCR positive for *P. falciparum* at the prior monthly visit are fed to female

PRISM2 Transmission team

An. gambiae mosquitoes, which are dissected 9-10 days later to examine for oocysts. Of 200 membrane feeding experiments, 26 (13.0%) resulted in mosquito infections. Stratified by clinical presentation, 13 of the 47 feeds (27.7%) done on asymptomatic participants with microscopic parasitaemia were infectious, compared to 11 of the 126 feeds (8.7%) done in individuals with sub-microscopic parasitaemia and 1 of 8 (12.5%) participants with clinical malaria. The quantification of male and female gametocytes and genetic characterization of gametocyte populations are ongoing. This unique study design, with intensive monitoring of natural malaria infections in an all-age cohort, will help us to understand the dynamics of gametocyte production and infectivity over time.



Oocysts in an infected mosquito

Contribution of *Plasmodium knowlesi* to multi-species human malaria infections in North Sumatera, Indonesia

Location of study: North Sumatera Province, Indonesia

LSHTM Investigators: Inke Lubis, Paul Divis, Khalid Beshir, Colin Sutherland

External collaborators: Hendri Wijaya, Munar Lubis, Chairuddin P. Lubis (Department of Paediatrics, Faculty of Medicine, University of Sumatera Utara, Medan, Indonesia)

Funding Body: Directorate General of Higher Education, Indonesia

Advances in DNA-based detection of malaria parasites has enabled better understanding of the infections present in human communities, important information to assist malaria elimination. We tested volunteers in 3 Regencies in Sumatera and found a complex mix of four malaria species contributing to the burden of infection. These included the monkey parasite *Plasmodium knowlesi.*

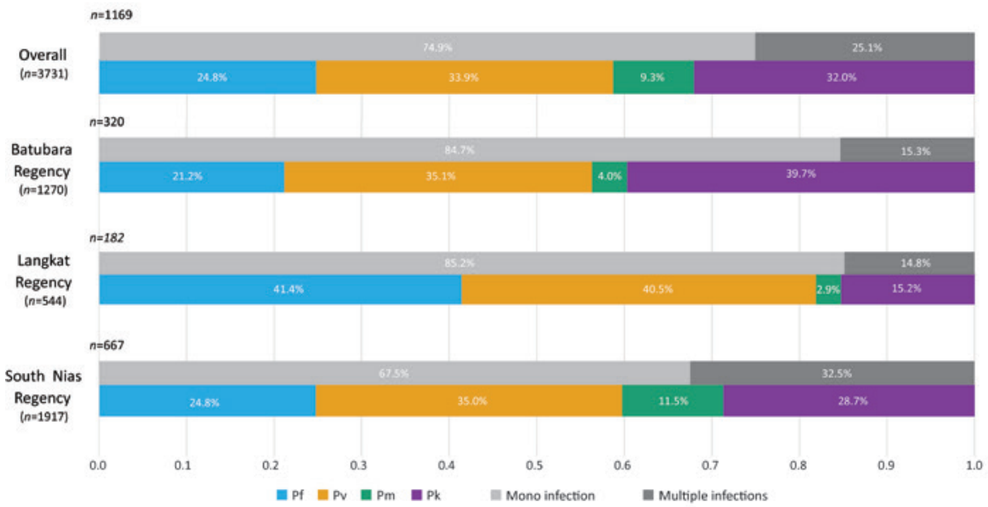
As Indonesia works towards the goal of malaria elimination, information is lacking on malaria epidemiology from some western provinces.

As a baseline for studies of antimalarial efficacy, we surveyed parasite carriage in three communities in North Sumatera Province.

A combination of active and passive detection of infection was carried out among communities in Batubara, Langkat and South Nias regencies. Finger-prick blood samples from consenting individuals of all ages provided blood films for microscopic examination and blood spots on filter paper. Plasmodium species were identified by nested polymerase chain reaction (PCR) of rRNA genes, and a novel assay which amplifies a conserved sequence specific for the sicavar gene family of *P. knowlesi*.

614 of 3,731 participants (16.5%) were positive for malaria parasites by microscopy. PCR detected parasite DNA in samples from 1,169 individuals (31.3%). In total, 377 participants (11.8%) harboured *P. knowlesi*. Also present were *P. vivax* (14.3%), *P. falciparum* (10.5%) and *P. malariae* (3.4%).

Amplification of sicavar is a specific and sensitive test for the presence of *P. knowlesi* DNA in humans. Subpatent and asymptomatic multi-species parasitaemia is relatively common in North Sumatera, and so PCR-based surveillance (PCR) -based surveillance is required to support control and elimination activities.



Prevalence of Four Plasmodium species in 3 Regencies of North Sumatera Province, Indonesia.
Denominators for each site (total number of individuals tested) are given under each regency name. The number of parasite-positive individuals is shown at top-left of each bar graph. Coloured bars denote species, grey bars denote proportion of mixed-species infections identified in each site.
Pf = P. falciparum, Pv = P. vivax, Pm = P. malariae, Pk = P. knowlesi.

Kota Marudu MONKEYBAR Project
Image Courtesy of: Joshua Paul for LSHTM

Vector Control

Long term research by LSHTM on long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) of houses have contributed substantially to the reductions in malaria burden attributable to vector control over the last 20 years. However increasing insecticide resistance threatens progress, and LSHTM researchers are actively engaged in research to counter this threat - conducting trials to evaluate new resistance-breaking products and exploring alternative methods of vector control.

Recognising the threat that resistance presented to the future of pyrethroid LLIN and the long lead time required to develop safe and effective alternatives, Malaria Centre researchers with in strategic partnership with WHO and pesticide manufacturers to identify and test new active ingredients for nets and IRS. As early as 2008 a new type of LLIN, the PBO-pyrethroid LLIN, which contained a chemical synergist to neutralise metabolic resistance, had demonstrated the entomological potential of PBO synergist LLIN over pyrethroid-only LLIN, in experimental hut studies. Uptake of PBO-LLIN by malaria control agencies remained slow due to the extra cost entailed and the absence of definitive evidence that pyrethroid resistance was an obstacle to control.

In some countries standard LLIN have remained partially protective, as demonstrated in a multi-country study which investigated whether insecticide resistance was associated with loss of effectiveness of LLIN and increased malaria burden. No evidence was found for an association between insecticide resistance and infection prevalence. Child users of nets, although better protected than non-users, were nevertheless subject to high malaria infection risk especially in Africa.

In order to demonstrate the added benefit and protection of PBO-pyrethroid LLIN, more rigorous evidence was sought. The cluster randomised trial (CRT) conducted in NW Tanzania demonstrated that PBO - LLIN would reduce malaria infection prevalence by 44% compared to standard pyrethroid LLIN for over two years. This coincided with the new WHO policy requiring RCT evidence for improved malaria control impact before recommending any new class of vector control product. Armed with the new evidence, WHO in 2017 recommended PBO-pyrethroid LLIN as a new product class to control malaria transmitted by pyrethroid resistant vectors. The Global Fund and President's Malaria Initiative responded by placing significant country orders for PBO LLIN in 2018. A second CRT of two types of PBO LLIN and two standard LLIN was initiated in Uganda on a larger scale in over a hundred sub-districts. It is due to report at the end of 2019.

Dual AI LLIN combining mixtures of insecticide with a differing modes of action developed through the LSHTM/PAMVERC/IVCC alliance have completed Phase 1 laboratory and 2 experimental hut trials and are now ready for community-scale CRT evaluation.

The trial in Tanzania is comparing the LLIN that combines pyrethroid and chlorfenapyr with a LLIN that combines pyrethroid and pyriproxifen and a PBO-LLIN. A parallel CRT has started in Benin. Two CRTs are required to generate the evidence for the full WHO recommendation. These Dual AI LLIN will form the next generation of LLIN to continue malaria control progress.

House improvements for malaria control has been another major movement. Eave tubes are a novel vector control tool which aims to prevent malaria by bringing insecticide resistant mosquitoes into contact with a high dose of insecticide as they try to enter though screened ports fixed in the house walls. A CRT is currently taking place in central Cote d'Ivoire. Other studies have investigated the use of insecticide treated wall linings, an alternative to IRS, designed to make interiors more aesthetic while providing a long-lasting substrate for insecticide.

Improved housing should improve malaria prevention. A study which analysed national survey data from 21 countries found that modern housing, built with metal or tiled roofs and concrete or brick walls, was associated with up to 14% reduction in the odds of malaria infection in children compared to more traditional housing. The effect of ITNs was additive; among the same children, the odds of malaria infection were 15% lower among ITN users than non-users.

The Building out Vector-Borne Diseases (BOVA) network is focused on control of malaria and other vector-borne diseases by improving the built environment in sub-Saharan Africa. It is providing small-scale funding for relevant research among African scientists.

A cohort study in rural Uganda, in relation to a mass scale-up of control efforts, found a rapid increase in the prevalence of modern housing between 2011-17. Implementation of IRS led to declines in malaria transmission, and housing changes were associated with additional reductions in mosquito density and parasite prevalence.

A CRT in neighbouring Tanzania also found that a single application of IRS using long-lasting Actellic CS (pirimiphos methyl) would reduce the prevalence of malaria for over one year. LSHTM has generated a wealth of entomological information on IRS and is working with modellers from Imperial College to examine whether this will judge IRS effectiveness in countries other than the location of the CRTs that generated the initial evidence.

A modelling approach is also being taken to examine the conditions whereby the application of systemic insecticides to domestic cattle will control malaria transmitted by partially zoophilic vectors. Interactions between mosquito biting behaviours and relative availabilities of alternative blood-host species are important to success. The outcomes indicate this systemic treatments will complement distributions of LLINs.

In order to achieve malaria elimination, it will be necessary to compliment indoor interventions with others such as larviciding. Larviciding can be difficult to implement, in part because water bodies are often numerous and can be challenging to locate. A trial is comparing spatial intelligence system comprising satellite data and drones, combined with spatial modelling, to improve the detection of breeding sites.

Rapid improvements to rural Ugandan housing and their association with malaria from intense to reduced transmission: a cohort study

Location of study: Uganda

LSHTM Investigators: Lucy Tusting, Sarah Staedke, Emmanuel Arinaitwe, Jim Todd

External collaborators: Grant Dorsey (University of California San Francisco, USA), Moses Kamya (Makerere University, Uganda), Agaba Katureebe, Maxwell Kilama (Infectious Disease Research Collaboration, Uganda),, Steve Lindsay (Durham University, UK), Victor Alegana (University of Southampton, UK).

Funding Body: U.S. National Institutes of Health, Bill & Melinda Gates Foundation, Medical Research Council UK

Rapid population growth in Africa requires an urgent expansion and improvement of housing options. Improving housing presents a promising opportunity for malaria control, by reducing indoor exposure to mosquitoes. This study measured recent changes in house design in rural Uganda and evaluated their association with malaria in relation to a mass scale-up of control efforts.

The study was nested within the Program for Resistance, Immunology, Surveillance and Modelling of malaria (PRISM) cohort study in Tororo, Uganda.

Children aged six months to ten years (n=384) living in 107 households were given long-lasting insecticide treated nets and followed between August 2011 and June 2017. Repeat rounds of indoor residual spraying (IRS) were initiated in December 2014. Socioeconomic data were collected in 2013 and 2016.

The study, published in The Lancet Global Health (2018), found a rapid increase in the prevalence of modern houses (with a cement, wood or metal wall, tiled or metal roof and closed eaves) from 23% in 2013 to 45% in 2016, possibly linked to economic development in Uganda. The implementation of IRS was associated with significant declines in malaria transmission. Following IRS, housing changes were associated with additional reductions in mosquito density (73% reduction) and parasite prevalence (57% reduction), but not malaria incidence.

The findings suggest that rapid changes in house design are occurring in parts of rural Africa and that these changes may further benefit existing malaria control interventions. As endemic Africa continues to undergo unprecedented population growth, economic change and urbanisation, there is a need to establish how efforts to meet increased housing demand may be leveraged to support malaria control and elimination.

Evaluation of a novel long lasting insecticidal net (treated with the synergist PBO) and a indoor residual spray product, separately and together, against malaria transmitted by pyrethroid resistant mosquitoes: a cluster randomised controlled trial

Location of study: Muleba district, Kagera Region, Tanzania

LSHTM Investigators: Natacha Protopopoff, Mark Rowland, Jacques Derek Charlwood, Alexandra Wright, Catherine Pitt, David Bath, Immo Kleinschmidt

External collaborators: Franklin W Mosha, Alphaxard Manjurano & Jacklin Mosha, (Kilimanjaro Christian Medical University College, Tanzania)

Funding Body: Global Health trial DFID/MRC/NIHR/ Wellcome Trust

This cluster randomised controlled trial aimed to evaluate the efficacy of four vector control interventions for reducing malaria transmission and controlling malaria vectors resistant to pyrethroid insecticide.

We allocated 48 clusters to four intervention arms using a factorial design: 1/ a standard long lasting insecticidal net (Olyset Net), 2/ a LLIN incorporating the synergist PBO (Olyset Plus), 3/ a standard LLIN and long lasting indoor residual spray formulation (Actellic 300CS), 4/ a PBO LLIN and Long-lasting IRS.

In the PBO LLIN arm the prevalence of malaria was reduced by 44% and 33% in the first and second year respectively compared to the standard LLIN arm treated with pyrethroid only. The addition of IRS to the standard LLINs provided a 44% additional protection against malaria compared to the standard LLIN alone, whilst the addition of IRS to PBO LLIN did not significantly improve protection over PBO LLIN alone.

This was the first clear evidence that PBO LLINs are more effective than standard LLINs for malaria prevention in areas of high pyrethroid resistance and justifies their increased deployment and use. As a direct consequence of the trial, WHO gave interim endorsement to PBO LLIN in September 2017 as new WHO class of vector control product, and recommended that PBO LLINs are deployed for malaria prevention in areas where vectors are resistant to pyrethroids.

Analysis of the third year trial data and laboratory testing of the residual bio-efficacy of the LLINs are ongoing.

Evaluating the impact of eave tubes plus house screening on malaria transmission

Location of study: Bouake, Cote d'Ivoire

LSHTM Investigators: Jackie Cook, Raphael N'Guessan, Immo Kleinschmidt, Welbeck Achille Oumbouke

External collaborators: Matt Thomas (Penn State, US), Eleanore Sternberg (Penn State, US), Serge Assi (Institute Pierre Richet, Cote d'Ivoire), Alphonsine Koffi (Institute Pierre Richet, Cote d'Ivoire), Marit Farenhorst (In2Care, The Netherlands), Anne Osinga (In2Care, The Netherlands), Malal Diop (In2Care, The Netherlands)

Funding Body: Bill and Melinda Gates Foundation

Vector control has resulted in large reductions in malaria transmission across sub-Saharan Africa; however, in some countries transmission remains high and insecticide resistance threatens current progress. Eave tubes are a novel vector control tool which aims to overcome resistance through bringing mosquitoes into contact with a high dose of insecticide as they try to enter a household. This project aims to evaluate the impact of eave tubes on epidemiological and entomological outcomes.

Eave tubes being installed to a house in Bouake, Cote d'Ivoire

A large cluster randomised control is currently taking place in Bouake, central Cote d'Ivoire, an area with high levels of insecticide resistance.

Example of a house with eave tubes and screening installed, Bouake, Cote d'Ivoire

The trial incorporates 40 villages (20 per arm), with households in the intervention arm receiving screening and eave tubes, with an insert with a high dose of pyrethroid inserted, as well as receiving new long-lasting insecticide treated bed nets (LLIN). The control arm households have received LLIN. The trial involves an active case cohort in children aged 6 months to 10 years who are visited every two weeks during the transmission season, and every month during the dry season. During the visit, an RDT is taken if the child is febrile and a blood spot sample is taken for future analysis. The trial will run for two years. Secondary outcomes include malaria infection prevalence, measured in a cross-sectional survey 18 months post-installation of eave tubes; mean mosquito density and entomological inoculation rate (EIR). There are also important anthropological and economical considerations in this new type of vector control which need to be explored.

Spatial intelligence system for precision larvicing in Zanzibar

Location of study: Zanzibar, Tanzania

LSHTM Investigators: Jackie Cook

External collaborators: Andy Hardy, Chris Thomas (Aberystwyth University, UK), Khamis Haji (Zanzibar Malaria Elimination Programme), Arnon Yafin (Sight DX, Israel), Eve Worrall (Liverpool School of Tropical Medicine, UK), Silas Majambere (Mosquito Consulting, Norway)

Funding Body: IVCC

Indoor interventions, such as nets and indoor residual spraying, have limited effect for species that show a tendency for feeding and resting outdoors. In order to achieve malaria elimination, it will be necessary to compliment indoor interventions with others which target residual transmission, such as larviciding. Larviciding can be difficult to implement, in part because water bodies are often numerous and can be challenging to locate. This project aims to use a combination of a state-of-the-art remote sensing system (satellite and drone-based), coupled with an innovative use of smartphone technology, to offer a more efficient and cost-effective approach to larval source management compared to conventional larviciding.

Our Spatial intelligence system (SIS) comprises of satellite data and drones, combined with spatial modelling, to determine where productive larval sites are located on Unguja island, Zanzibar.

This data will be collated and downloaded onto an easy-to-use app for field staff to locate breeding sites. We will conduct a 3 arm cluster randomised study comparing SIS larviciding and standard larviciding (using currently used methods for locating water bodies) to no larviciding. Our cluster will be a 'hotspot' community (determined through previous malaria incidence). We aim to determine whether a) larviciding has an impact on epidemiological (passive case detection) and entomological (vector density) outcomes and b) if SIS larviciding is more cost-effective than standard larviciding.

A Zanzibar Malaria Elimination Programme staff member learning how to use a drone

Malaria vector control using systemic insecticides

Location of study: Papua New Guinea

LSHTM Investigators: Hannah Meredith, Laith Yakob

External collaborators: Luis Furuya-Kanamori, (Melbourne University, Australia), Greg Devine, (Queensland Institute of Medical Research, Australia)

Funding Body: Whitaker International

Systemic insecticides are insecticides that are applied directly to hosts so that mosquitoes are exposed to them when they take a bite. Optimising their implementation requires extensive knowledge of local mosquito biting behaviour. Models help inform their strategic combination with other, standard control tools such as bednets.

Interactions between mosquito biting behaviours and relative availabilities of alternative blood-host species have largely been neglected in malaria programmatic strategy but will increasingly underlie sustaining the successes of vector control initiatives.

Models demonstrate that targeting blood-feeding mosquitoes by treating livestock with endectocides offers a potentially useful complement to existing malaria control programmes centred on LLIN distribution.

Cattle used for testing systemic insecticides. Image credit J. Orsborne

Implications of insecticide resistance for malaria vector control with long-lasting insecticidal nets

Location of study: Benin, Cameroon, India, Kenya, Sudan

LSHTM Investigators: Immo Kleinschmidt, John Bradley, Jackie Cook, Jo Lines, Pippa West

External collaborators: Tessa Knox, (WHO Global Malaria Programme); Martin Donnelly, (Liverpool School of Tropical Medicine); Hmooda Toto Kafy, (Federal Ministry of Health, Khartoum, Sudan); Charles Mbogo, KEMRI, Nairobi, Kenya); Martin Akogbeto, (Centre de Recherche Entomologique de Cotonou, Cotonou, Benin); J D Bigoga,(Biotechnology Centre, University of Yaoundé, Yaoundé, Cameroon); K Raghavendra, (Indian Council of Medical Research, Department of Health Research, New Delhi, India)

Funding Body: Bill & Melinda Gates Foundation

Scale-up of insecticide-based interventions has averted more than 500 million malaria cases since 2000. Increasing insecticide resistance could herald a rebound in disease and mortality. The study investigated whether insecticide resistance was associated with loss of effectiveness of long-lasting insecticidal nets and increased malaria disease burden.

This WHO-coordinated, prospective, observational cohort study was carried out in 279 clusters (villages or groups of villages in which phenotypic resistance was measurable) in Benin, Cameroon, India, Kenya, and Sudan. Pyrethroid long-lasting insecticidal nets were the principal form of malaria vector control in all study areas.

Cohorts of children from randomly selected households in each cluster were recruited and followed up by community health workers to measure incidence of clinical malaria and prevalence of infection. Mosquitoes were assessed for susceptibility to pyrethroids using the standard WHO bioassay test. Country-specific results were combined using meta-analysis.

Between June 2, 2012, and Nov 4, 2016, 40 000 children were enrolled and assessed for clinical incidence during 1·4 million follow-up visits and 80 000 mosquitoes were assessed for insecticide resistance.

Long-lasting insecticidal net users had lower infection prevalence (adjusted odds ratio [OR] 0·63, 95% CI 0·51–0·78) and disease incidence (adjusted rate ratio [RR] 0·62, 0·41–0·94) than did non-users across a range of resistance levels.

We found no evidence of an association between insecticide resistance and infection prevalence (adjusted OR 0·86, 0·70–1·06) or incidence (adjusted RR 0·89, 0·72–1·10). Users of nets, although significantly better protected than non-users, were nevertheless subject to high malaria infection risk (ranging from an average incidence in net users of 0·023, [95% CI 0·016–0·033] per person-year in India, to 0·80 [0·65–0·97] per person year in Kenya.

Irrespective of resistance, populations in malaria endemic areas should continue to use long-lasting insecticidal nets to reduce their risk of infection. As nets provide only partial protection, the development of additional vector control tools should be prioritised to reduce the unacceptably high malaria burden.

Efficacy of novel bi-treated long lasting insecticidal nets for control of malaria transmitted by pyrethroid resistant vectors in Tanzania: A cluster randomised controlled trial

Location of study: Misungwi district, Mwanza Region, Tanzania

LSHTM Investigators: Natacha Protopopoff, Mark Rowland, Immo Kleinschmidt

External collaborators: Franklin W Mosha, Alphaxard Manjurano & Jacklin Mosha (Kilimanjaro Christian Medical University College, Tanzania); Manisha Kulkarni, (University of Ottawa, Canada)

Funding Body: Global Health trial DFID/MRC/NIHR/ Wellcome Trust

Malaria control progress is being undermined by the selection of insecticide resistance in the malaria vectors around the Great Lakes region of East Africa. Long Lasting Insecticidal Nets (LLIN) which combine with pyrethroid either the synergist PBO or the insecticides chlorfenapyr and pyriproxifen have the potential to control malaria transmitted in this region. A cluster randomised trial is comparing 3 bi-treated LLIN - Olyset Plus, Interceptor G2, Royal Guard – to a standard pyrethroid LLIN Interceptor in an area of northwest Tanzania where the vectors are resistant to pyrethoids. Findings will be used by WHO to develop new public health policy on the use of these new classes of LLIN.

The project has several objectives. The cluster randomised trial compares the efficacy of the 3 bi-treated LLINs to standard LLIN across the three-year lifespan of the nets with respect to: 1. Malaria infection prevalence in children under 15 years, 2. Prevalence of anaemia in children under 5 years, 3. Incidence of malaria cases in children under 10 years, 4. Entomological inoculation rate (EIR) as proxy for malaria transmission. Other objectives are:

1. LLIN durability (net survivorship, fabric integrity, duration of partner insecticidal activity).

2. Incremental cost-effectiveness, usage, adverse effects, and equity implications of each net type. An additional objective will examine whether experimental hut trial outcomes can serve as a surrogate for epidemiological and transmission outcomes of CRT through transmission modelling (Imperial College). The study is conducted in the district of Misungwi where the main malaria vectors, *Anopheles gambiae s.s.* and *A. funestus* are resistant to pyrethroids. Baseline data collection was completed in 2018 and LLIN were distributed in January 2019.

Demonstration of the mosquito trap (CDC light trap) by PAMVERC field assistant during consent procedure, Misungwi, Tanzania

Efficacy of two dual active ingredient long lasting insecticidal nets for control of malaria transmitted by pyrethroid resistant vectors in Benin: A cluster randomised controlled trial

Location of study: Zagnanado & Ouinhi, Zou, Benin

LSHTM Investigators: Jackie Cook, Immo Kleinschmidt, Corine Ngufor, Natacha Protopopoff, Mark Rowland

External collaborators: Martin Akogbeto, (Centre de Recherche Entomologique de Cotonou), Aurore Hounto, (Programme National de Lutte contre le Paludisme de la République du Bénin, Tom Churcher, Imperial College)

Funding Body: UNITAID through IVCC

While the scale-up of LLIN has led to major reduction in malaria burden in many African countries, progress is threatened by the selection of insecticide resistant malaria vectors. LLIN with new active ingredients (AI) chlorfenapyr and pyriproxifen which kill or sterilize resistant vectors require evidence of malaria control before they can be recommended by the World Health Organisation. A cluster-randomised controlled trial and concurrent experimental hut trials compares two dual AI LLIN Interceptor G2 (BASF) and Royal Guard (Disease Control technology) to a standard LLIN (only pyrethroid) to prevent malaria in Benin.

This project has two aims. The first is a three-arm superiority, cluster-randomized trial (CRT) to be conducted in highly resistant area of West Africa where *Anopheles coluzzii* is the predominant vector. The second is to establish whether entomological outcomes generated by experimental hut trials can predict accurately the outcomes of CRT and ultimately replace the need for CRT of future dual AI LLIN. The CRT will be conducted in the Zou department in Benin. The following outcome will be followed incidence of malaria cases in children aged 6 months to 10 years followed for 24 months. Secondary outcomes are cross-sectional community prevalence of malaria infection in the study population at 6- and 18-months post-intervention, prevalence of anaemia in children under 5, entomological inoculation rates (EIR), vector density and insecticide resistance intensity. Insecticide durability and chemical content In addition, LLIN survivorship, physical durability (hole index), shall be measured at intervals. Data from concurrent experimental hut trials shall be incorporated into malaria transmission models to determine whether these could be used as a proxy for epidemiological trial evidence. A meeting in Benin was held in October 2018 to finalise the protocol and baseline data collection began in 2019.

LLIN Evaluation in Uganda Project (LLINEUP)

PBO Net Study – Impact of long-lasting insecticide treated bednets with and without piperonyl butoxide (PBO) on malaria indicators in Uganda: a cluster-randomised trial

Location of study: Uganda (48 districts)

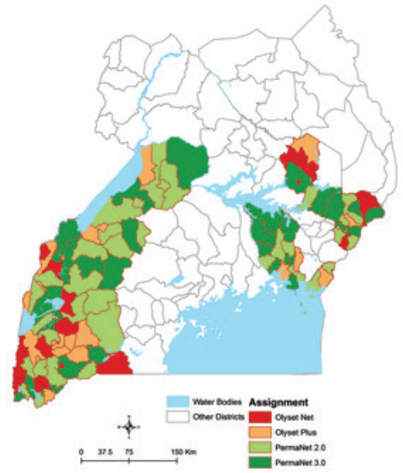
LSHTM Investigators: Sarah Staedke

External collaborators: Janet Hemingway, (Liverpool School of Tropical Medicine, UK); Moses Kamya, (Makerere University / Infectious Diseases Research Collaboration, Uganda); Grant Dorsey, (University of California San Francisco, USA); Martin Donnelly, (Liverpool School of Tropical Medicine, UK); Yeka Adoke, (Infectious Diseases Research Collaboration, Uganda); Jimmy Opigo, (National Malaria Control Division, Ministry of Health, Uganda); Catherine Maiteki-Sebuguzi, (National Malaria Control Division, Ministry of Health, Uganda)

Funding Body: The Against Malaria Foundation; Department for International Development (DfID) via the Innovative Vector Control Consortium

Long-lasting insecticidal nets (LLINs) are a key malaria control intervention, but their effectiveness is threatened by resistance to pyrethroid insecticides. Some new LLINs combine pyrethroids with piperonyl butoxide (PBO), a synergist that can overcome resistance. In 2017-18, the Ugandan Ministry of Health distributed LLINs with, and without, PBO through a national mass-distribution campaign, providing a unique opportunity to rigorously evaluate PBO LLINs across different epidemiological settings.

Together with the Ministry of Health, we embedded a cluster-randomised trial to evaluate the impact of LLINs delivered in the 2017-18 national campaign. A total of 104 clusters (health sub-districts) in Eastern and Western Uganda were included, covering 48 of 121 (40%) districts. Using adaptive randomisation driven by the number of LLINs available, clusters were assigned to receive one of 4 types of LLINs, including 2 brands with PBO: (1) PermaNet 3.0 [n=32] and (2) Olyset Plus [n=20]; and 2 without PBO: (3) PermaNet 2.0 [n=37] and (4) Olyset Net [n=15]. We are conducting cross-sectional community surveys in 50 randomly selected households per cluster (5200 households per survey) and entomological surveillance for insecticide resistance in up to 10 randomly selected households enrolled in the community surveys per cluster (1040 households per survey), at baseline, and 6, 12, and 18 months after LLIN distribution. Net durability and bio-efficacy will be assessed in 400 nets withdrawn from households with replacement at 12 months. The primary trial outcome is parasite prevalence as measured by microscopy in children aged 2-10 years in the follow-up surveys. PBO LLINs are a promising new tool to reduce the impact of pyrethroid resistance on malaria control. The results of this innovative, large-scale trial will make an important contribution to malaria control policy in Uganda, and throughout Africa. This model of evaluation could be a paradigm for future assessment of malaria control interventions.



Map of the study area, showing allocation of nets by cluster (health sub-district)

Insecticide-treated durable wall lining for control of malaria: cluster randomized trial

Location of study: Muheza, Tanzania

LSHTM Investigators: Mark Rowland, Louisa Messenger, Sophie Weston, Immo Kleinschmidt, Jackie Cook

External collaborators: William Kisinza, George Mtove, Dr. Joseph Mugasa and Robert Malima (National Institute for Medical Research, Amani Research Centre, Muheza, Tanzania), Frank Mosha, Robert Kaaya (Kilimanjaro Christian Medical College, Moshi, Tanzania)

Funding Body: TUS Agency for International Development

Malaria prevention through vector control is threatened by the development of pyrethroid resistance insecticide. Insecticide treated wall lining (ITWL) represents a potentially more sustainable control method. A non-pyrethroid ITWL containing abamectin and fenpyroximate (PermaNet® lining, Vestergaard-Fransen) was developed in rapid time when it was realised that pyrethroid resistance was becoming prevalent in Eastern Africa. 3-6 months after installation it was evident the ITWL was no longer effective, the project was stopped and the ITWL removed.

On realisation that pyrethroid resistance was prevalent in *Anopheles gambiae* and *funestus* in Tanzania, the planned community randomised trial of pyrethroid insecticide-treated wall lining (ITWL) was postponed until a new ITWL was prepared using non-pyrethroid active ingredients. A short 6-week experimental hut study with the new ITWL yielded quite promising efficacy data. A two-armed cluster randomized controlled trial in 44 village clusters (22 ITWL+LLINs and 22 LLINs only) was initiated concurrently. Study children, aged 0.5-14 years, were followed monthly to estimate cumulative incidence of malaria parasitaemia. Insecticide durability was monitored in situ using cone bioassay. The durability monitoring indicated low insecticidal activity within 3-6 months of installation. The incidence of malaria infection was 2.8 episodes per person-year in the intervention and 1.7 in the control arm. After adjusting for ITWL coverage and socio-economic status, the random effect model revealed no significant difference in rate of infection between the two arms (adjusted hazard ratio = 1.1, 95% CI 0.9-1.4, p=0.5). The overall effectiveness of ITWL was not modified by reported net use which was consistently high in the cohorts throughout the 8 month follow-up period. The trial was stopped by the independent data monitoring committee and the ITWL removed. The project highlights the importance of taking a phased, evidence-based approach to vector control product development and to not ruin a promising technique, perhaps irrevocably, before the prototype had demonstrated its true potential.

Indoor residual spraying against *falciparum* malaria in Africa: a systematic review of efficacy and effectiveness

Location of study: Tanzania, Benin, Ivory Coast

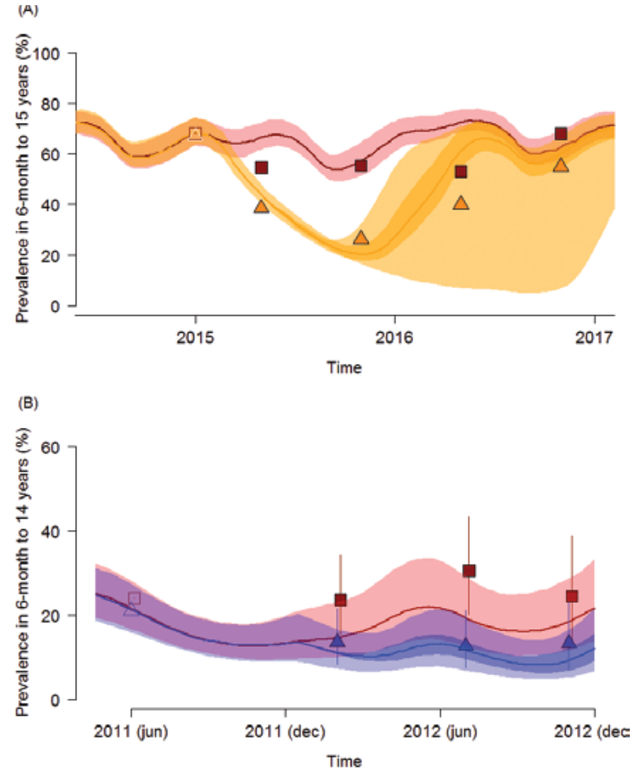
LSHTM Investigators: Mark Rowland, Natacha Protopopoff, Raphael N'Guessan, Corine Ngufor, Richard Oxborough

External collaborators: Tom Churcher, Ellie Sherrard-Smith, (Imperial College London)

Funding Body: MRC, DFID, Wellcome Trust, IVCC, UNITAID

Indoor residual spraying (IRS) constitutes one of two main tools for malaria vector control. LSHTM has helped to identify and develop several new IRS insecticide formulations in partnership with pesticide industry and has been evaluating these at phase 2 experimental-hut and phase 3 community randomised trial levels. Transmission modelling of hut-trial entomological outcomes may prove useful to predict malaria control impact epidemiological elsewhere.

Indoor residual spraying is an important method of malaria control. Several classes of insecticide are in use by national control programmes in Africa. Pyrethroid use is now restricted to LLINs. There are a growing number of insecticide classes used for IRS. Many of these have been developed by pesticide industry in collaboration with LSHTM and evaluated in Phase 2 experimental hut trials at our sites in Tanzania, Benin and Ivory Coast. These generate entomological outcomes typical of those occurring at household level. Subsequent to this we have carried out more definitive cluster randomised trials in Tanzania to control malaria using these insecticides. We are collaborating with the malaria vector control modelling group of Imperial College to use our experimental hut trial data to characterise the entomological efficacy of the IRS insecticides and predict their impact on malaria control transmitted by pyrethroid-resistant mosquitoes. This should provide a framework to help decision makers judge IRS product effectiveness in countries other than Tanzania.



Comparison of the predicted impact of IRS on malaria prevalence compared to that measured in randomised control trials. A: Comparison of best fit standard LLINs (solid red line) vs standard LLINs + a single round of Actellic® 300CS (solid orange line). B. Comparison of standard LLINs (solid red line) vs standard LLINs+ two rounds of bendiocarb (solid blue line). The observed estimates for prevalence obtained during cross-sectional surveys of each RCT are plotted as closed symbols. Shaded area indicates uncertainty in model predictions. The paler shaded area around the IRS lines shows additional uncertainty driven by variability in IRS efficacy from experimental hut trials.

Image Courtesy of: Save the Children for LSHTM

Chemoprevention

First used in pregnant women, intermittent preventive treatment (IPT) with antimalarial drugs is now recommended by WHO as an effective means to reduce the incidence of malaria in infants and young children under 5 years. Research by the LSHTM Malaria Centre, in partnership with our collaborators in malaria-endemic countries, has been pivotal in providing the vital scientific evidence to underpin these new policy recommendations. The approach, collectively termed chemoprevention, now represents the third mainstay of malaria control, alongside strategies to ensure timely access to effective treatment and use of insecticide treated nets (ITNs) to protect against mosquito bites.

Since 2012, seasonal malaria chemoprevention (SMC) in young children has been rolled out across 12 countries in West Africa, protecting an estimated 15.7 million children in 2017. Malaria Centre researchers have been involved in monitoring the consequences of scaling up SMC, measuring the frequency of malaria cases, adverse drug reactions, and drug resistant alleles over time. Researchers at LSHTM also continue to investigate new ways to improve preventive chemotherapy – exploring the effectiveness and safety of alternative drugs for IPT in pregnancy, and whether IPT could also benefit other population groups. Trials led by LSHTM were the first to show the promise of IPT in reducing asymptomatic malaria infection and anaemia in older school-aged children; a finding recently confirmed by a meta-analysis which combines data from 11 trials across Africa.

Efforts to combine the preventive use of antimalarial drugs with other interventions are also being evaluated. Studies in pregnant women in Zambia have shown that IPTp can have benefits beyond malaria, also reducing the incidence of sexually-transmitted and reproductive tract infections. A trial is now underway to investigate whether adding the antibiotic/antiprotozoal drug metronidazole to preventative antimalarial treatment has further benefits.

In Mali and Burkina Faso, we are working with our local collaborators to evaluate the utility of combining seasonal malaria chemoprevention with monthly azithromycin (a broad spectrum antibiotic) in preventing childhood mortality. Whereas this provided some benefit in lowering the incidence of non-malaria fevers, there was no measurable benefit in reducing overall mortality in the target group. Studies are also underway to investigate whether seasonal boosters of the RTS,S vaccine, recently licensed for use in Africa, could be used as an alternative to SMC; or whether the combination of the two interventions could provide added benefit.

Finally, the Malaria Centre is involved in projects which examine the use of antimalarial drugs to treat entire populations with the aim of reducing the infectious reservoir of asymptomatic *Plasmodium* infections that maintain transmission. This approach, known as mass drug administration (MDA), is currently being explored as a key component within malaria elimination strategies in many countries around the world. Projects which include MDA are summarised in the malaria elimination section.

"ASPIRE Trial" – Aiming for Safe Pregnancies by Reducing Malaria and Infections of the Reproductive Tract

Location of study: Nchelenge District, Zambia

LSHTM Investigators: Matthew Chico, Daniel Chandramohan, Elizabeth Allen, Joanna Sturgess, Philippe, Mayaud, Suzanna Francis

External collaborators: Nigel Klein, (University College London, UK), David MacIntyre, (Imperial College London, UK), Antonieta Medina-Lara, (Exeter Medical School, UK), Mike Chaponda, (Tropical Disease Research Centre, Zambia), Modest Mulenga, (Tropical Disease Research Centre, Zambia), Enesia Chaponda, (University of Zambia)

Funding Body: Medical Research Council

LSHTM carried out an observational study study in Nchelenge, Zambia between 2013-14 and found that 58% of pregnant women had a malaria infection, 48% were diagnosed with bacterial vaginosis (BV), and 26% had *Trichomonas vaginalis* (TV); 29% had malaria and BV co-infection, and 15% were diagnosed with malaria and TV. In response to this LSHTM is leading a three arm clinical trial designed to reduce the burden of malaria, BV, and TV.

The trial is a partially placebo-controlled individually randomised, superiority trial comparing three intermittent chemoprevention therapies among pregnant women: (1) intermittent preventive treatment (IPTp) using sulphadoxine-pyrimethamine (SP) plus metronidazole (MTZ) versus (2) IPTp with dihydroartemisinin-piperaquine (DP) plus MTZ versus (3) IPTp-SP plus placebo MTZ .

HIV-negative pregnant women between 16-28 gestational weeks will receive one of these three treatments while attending antenatal care at three health facilities in Nchelenge, Zambia. Therapy will be administered during visit 1 (gestational week 16-19) and, again, during visit 4 prior to delivery (week 30-34). SP or DP will be administered without MTZ on visit 2 (week 20-24) and visit 3 (week 25- 29). The primary outcome is any adverse pregnancy outcome defined as the composite of foetal loss (spontaneous abortion or stillbirth; WHO ICD-11 definitions), or singleton live births born small for gestational age (SGA), or low birth weight (LBW; <2.5kg), or preterm delivery (<37 weeks), or neonatal death (day 28 post-partum). Secondary outcomes include: single outcomes combined in the composite pregnancy outcome; incidence of clinical malaria; and incidence of curable STIs/RTIs.

We will also conduct cost-effectiveness analyses and discrete choice experiments, as well as two sub-studies that are key to interpreting results from the main trial and understanding microbiological mechanics of chemoprevention against adverse birth outcomes and antimicrobial resistance.

Achieving Catalytic Expansion of Seasonal Malaria Chemoprevention in the Sahel (ACCESS-SMC)

Location of study: Burkina Faso, Guinea, Chad, Mali, Niger, Nigeria, The Gambia

LSHTM Investigators: Paul Milligan , Susana Scott, Matt Cairns, Colin Sutherland, Khalid Beshir, Julian Muwanguzi, Sham Lal, Paul Snell, Raoul Mansukhani, Yolanda Fernandez, Rhosyn Tuta

External collaborators: Malaria Consortium; Catholic Relief Services; Management Sciences for Health (MSH); Medicines for Malaria Venture (MMV); Institut de Recherche en Sciences de la Sante (IRSS), Burkina Faso; Centre de Support en Sante Internationale (CSSI), Chad; The University Gamal Abdel Nasser, Conakry (GANU), Guinea; Malaria Research Training Centre (MRTC), Mali; Epicentre, Niger; Le Centre de Recherche Médicale et Sanitaire (CERMES), Niger; Epidemiological Resources and Investigation Consultancy (ERIC), Nigeria; Jedima International Health Consult Ltd. (JIHCL), Nigeria; Medical Research Council-The Gambia (MRC), The Gambia; Universite Cheikh Anta Diop, Senegal; TDR, Switzerland; WHO, Switzerland

Funding Body: UNITAID through Malaria Consortium and Catholic Relief Services

Seasonal Malaria Chemoprevention (SMC) is the administration of effective antimalarial drugs once a month to children under 5 years of age, during the rainy season, in order to prevent malaria. The ACCESS-SMC project was undertaken to improve availability of SMC drugs, to expand access to SMC, and evaluate the effectiveness of the strategy at scale, in seven high-burden countries: Burkina Faso, Chad, The Gambia, Guinea, Mali, Niger and Nigeria.

SMC was implemented in a phased manner in 2015 and 2016 to a total of about 7million children. Children were surveyed each year to verify uptake, national pharmacovigilance systems in each country were strengthened to monitor adverse drug reactions, case control studies were used to estimate the protective efficacy of monthly treatments, the frequencies of molecular markers of drug resistance were measured in large-scale surveys, and the impact in terms of the reduction in the number outpatient cases of malaria and the number of children dying from malaria in hospital, was assessed using cases reported in national health management information systems and by collecting individual patient data before and after SMC introduction from health facilities. Costs of SMC were estimated in each country and cost effectiveness and net cost savings were estimated.

The study showed that high, equitable coverage was achieved overall, in 2016 90% of eligible children received at least one SMC treatment and 54% received all four treatments, but coverage varied with some countries performing better than others. Each monthly treatment provided a high degree of protection for 4 weeks. Molecular monitoring showed that drug resistant infections are very uncommon, but the situation may change and needs careful monitoring. Monitoring of safety was strengthened during the project but these efforts need to be sustained. Serious side effects were rare. SMC cost $3.8 per child per year, and was highly cost-effective. There was a substantial reduction in the number of malaria cases, and the number of deaths from malaria in hospital, when SMC was introduced. In two countries with a DHIS2 (District Health Information System 2) databases established prior to SMC scale-up, Burkina Faso and The Gambia, introduction of SMC was associated with a reduction in the number of malaria deaths in hospital during the high transmission period, of 44% (Burkina Faso) and 57% (The Gambia). Despite the complexity of delivery, SMC has proved highly effective.

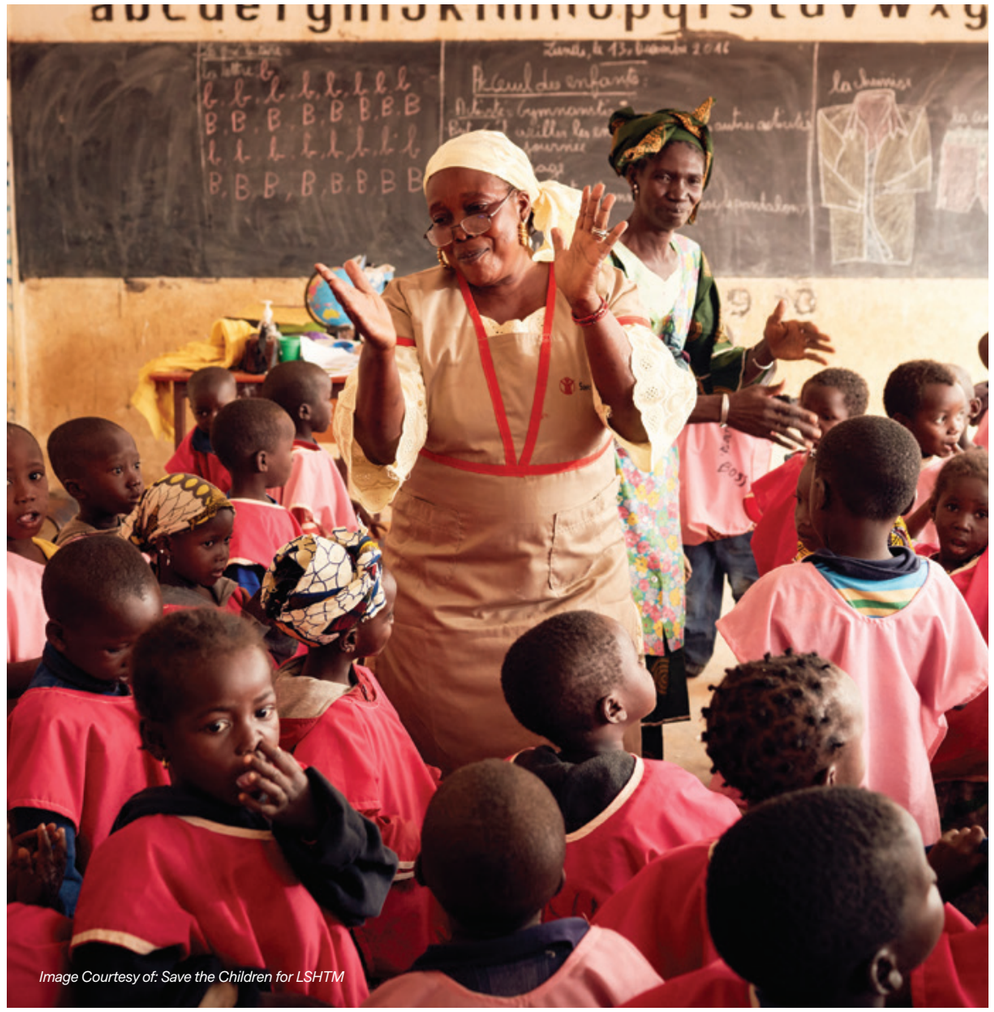


Image Courtesy of: Save the Children for LSHTM

A trial of seasonal malaria chemoprevention plus azithromycin in African children

Location of study: Bougouni district, Mali, and Hounde district, Burkina Faso

LSHTM Investigators: Brian Greenwood, Daniel Chandramohan, Paul Milligan, Matthew Cairns, Irene Kuepfer

External collaborators: Ogobara Dumbo, Alassane Dicko & Issaka Sagara (MRTC, Bamako, Mali); Jean-Bosco Ouedraogo, Issaka Zongo & Halidou Tinto (IRSS, Bobo-Dioulasso, Burkina Faso)

Funding Body: MRC/DFID/NIHR/Wellcome Trust Joint Global Health Trials scheme

Recent studies have shown that mortality is reduced among African children who received azithromycin during mass treatment programs for the control of trachoma. We investigated whether the addition of azithromycin to monthly sulphadoxine-pyrimethamine plus amodiaquine, used for seasonal malaria chemoprevention, reduces mortality and morbidity in young African children.

We randomised nearly 20,000 children aged 3-59 months by household to receive either a 3-day azithromycin or placebo course four times each year, alongside monthly seasonal malaria chemoprevention. Mortality and morbidity were recorded through active and passive surveillance. During the malaria transmission seasons from 2014-2016 the incidence of deaths or hospital admissions (not due to trauma or elective surgery) was similar in the two study groups. The incidence of febrile illnesses not due to malaria, gastrointestinal and respiratory infection was lower among children who received azithromycin. In conclusion, the findings of this study do not support the addition of azithromycin to the antimalarials used for malaria chemo prevention to reduce child mortality and severe morbidity in the areas of sub-Saharan Africa where seasonal malaria chemoprevention is implemented.

A Phase IIIB comparative trial of seasonal vaccination with the malaria vaccine RTS,S/AS01, seasonal malaria chemoprevention and of the two interventions combined

Location of study: Bougouni district, Mali, and Hounde district, Burkina Faso

LSHTM Investigators: Brian Greenwood, Daniel Chandramohan, Paul Millligan, Matthew Cairns, Irene Kuepfer

External collaborators: Ogobara Dumbo, Alassane Dicko & Issaka Sagara (MRTC, Bamako, Mali); Jean Bosco Ouedraogo, Issaka Zongo & Halidou Tinto (IRSS, Bobo-Dioulasso, Burkina Faso)

Funding Body: MRC/DFID/Wellcome Trust Global Health Trials program

Seasonal Malaria Chemoprevention (SMC) reduces malaria morbidity and mortality in children below 5 years of age. However, SMC administration is challenging and does not provide full protection. We are assessing whether seasonal vaccination with RTS,S/AS01 could be used as an alternative to SMC and whether the combination of the two interventions would provide added benefit.

Seasonal Malaria Chemoprevention (SMC), which involves monthly administration of sulphadoxine-pyrimethamine plus amodiaquine (SP+AQ) given at monthly intervals during the high malaria transmission season, is currently being implemented in 12 countries in the Sahelian and sub-Sahelian regions of Africa. SMC is having a major impact on malaria in the populations where it is being given, but it does not provide complete protection and there are concerns over the possible emergence of resistance to the drugs currently being used for SMC. At the moment, there are no other long-acting anti-malarial combinations available to replace them. The malaria vaccine RTS,S/ASO1 has shown promise, but it provides only a modest level of protection during a three or four year period of follow-up. However, efficacy during the first few months after administration of a three dose course is high, and waning protection can be partially restored by a booster dose. Thus, a potential way of using this vaccine in areas of seasonal malaria transmission is to vaccinate children who have been primed during infancy at the beginning of each malaria transmission season.

A child receiving Seasonal Malaria Chemoprevention (SMC) treatment

Impact of malaria in pregnancy and intermittent preventive treatment of malaria in pregnancy on the risk of malaria in infancy

Location of study: Busia district, Eastern Uganda

LSHTM Investigators: Abel Kakuru, Sarah Staedke & Daniel Chandramohan

External collaborators: Grant Dorsey, (University of California San Francisco, USA), Moses R. Kamya, (Makerere University School of Medicine, Uganda), Prassana Jagannathan, (Stanford University, USA)

Funding Body: NICHD, Bill and Melinda Gates Foundation

Infants born to mothers with placental malaria may be at increased risk of malaria. However, evidence is limited. We aim to evaluate the effect of intermittent preventive treatment of malaria in pregnant women (IPTp) and malaria in pregnancy (MiP) on the incidence of malaria during infancy.

Between September 2016 and May 2017, 782 HIV-uninfected pregnant women were enrolled at 12-20 weeks of gestation and randomised in a ratio of 1:1 to IPTp with monthly sulfadoxine-pyrimethamine (SP) or monthly dihydroartemisinin-piperquine (DP); women were followed up monthly. At delivery, placental malaria (PM) status was determined from placental tissue or placental blood. Infants born are followed up to 12 months of age. Overall, 678 infants were born during the study. More infants were born to mothers with PM in the IPTp-SP group (60.9%) than in the IPTp-DP group (28.3%, p<0.001). Preliminary results show a non-significant difference in the incidence of malaria among infants born to mothers in the two groups (IPTp-SP: 1.96 episodes per person year [PPY] vs IPTp-DP: 1.72 episodes PPY, p=0.13). However, on stratifying for infant age and sex, IPTp-DP is associated with lower malaria incidence compared to IPTp-SP among infants aged 3-9 months (IRR 0.79, 95% CI 0.64-0.98, p=0.03), and among male infants (IRR 0.76, 95% CI 0.58-0.99, p=0.04). Monthly IPTp-DP may be protective against malaria in male infants and those 3-9 months of age.

Sulfadoxine-pyrimethamine exhibits dose-response protection against adverse birth outcomes related to malaria and sexually transmitted and reproductive tract infections

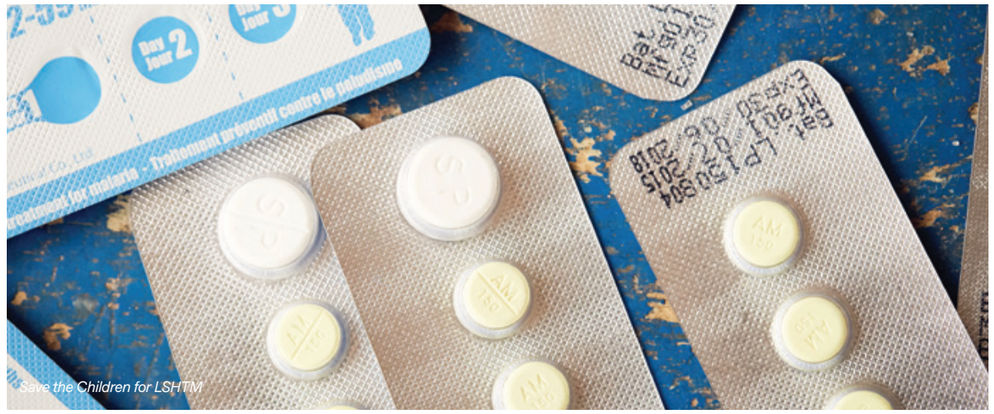
Location of study: Nchelenge District, Zambia

LSHTM Investigators: Matthew Chico, Cono Ariti, Enesia Chaponda, Daniel Chandramohan

LSHTM carried out a secondary analysis of data from an observational study Nchelenge, Zambia. Women received standard antenatal care and provided samples for retrospective diagnosis of malaria and curable sexually transmitted and reproductive tract infections (STIs/RTIs). We then analysed the relationship between maternal infection, doses of intermittent preventive treatment using sulfadoxine-pyrimethamine (IPTp-SP) and adverse birth outcomes. The results provide the first evidence to our knowledge that IPTp-SP is protective against curable STIs/RTIs as well as malaria

We calculated the odds ratios (ORs) of adverse birth outcomes by IPTp-SP exposure, 0–1 dose (n = 126) vs ≥2 doses (n = 590) and ≥2 doses (n = 310) vs ≥3 doses (n = 280) in 7 categories of malaria infection and sexually transmitted and reproductive tract infections (STIs/RTIs).

We found no significant differences in baseline prevalence of infection across IPTp-SP exposure groups. However, among women given 2 doses compared to 0–1 dose, the odds of any adverse birth outcome were reduced 45% (OR, 0.55; 95% confidence interval [CI], 0.36, 0.86) and 13% further with ≥3 doses (OR, 0.43; 95% CI, 0.27, 0.68). Two or more doses compared to 0–1 dose reduced preterm delivery by 58% (OR, 0.42; 95% CI, 0.27, 0.67) and 21% further with ≥3 doses (OR, 0.21; 95% CI, 0.13, 0.35). Women with malaria at enrolment who received ≥2 doses vs 0-1 had 76% lower odds of any adverse birth outcome (OR, 0.24; 95% 0.09, 0.66), and *Neisseria gonorrhoeae* and/or *Chlamydia trachomatis* had 92% lower odds of any adverse birth outcome (OR, 0.08; 95% CI, 0.01, 0.64). Women with neither a malaria infection nor STIs/RTIs who received ≥2 doses had 73% fewer adverse birth outcomes (OR, 0.27; 95% CI, 0.11, 0.68).



Save the Children for LSHTM

Antimalarial treatment of asymptomatic school-age children to decrease *Plasmodium falciparum* infection and anaemia: A systematic review and meta-analysis

Location of study: Multiple countries in Africa

LSHTM Investigators: Matthew Chico, Charles Opondo, Katherine Halliday, Jorge Cano, Elizabeth Allen, Sian Clarke

External collaborators: Lauren Cohee, Andrea Shipper, Miriam Laufer (University of Maryland, USA)

We have conducted the first meta-analysis to our knowledge of published and unpublished data from school-based malaria intervention trials. We present study-level results and an individual participant meta-analysis with effects stratified by treatment type, follow-up period, and intervention strategy to draw important conclusions about the utility of school-based interventions on malaria infection and anaemia. We also examine the effect of malaria transmission intensity on the treatment outcomes.

Preliminary results based on data from 16,551 children and 11 studies show that the risk of malaria infection is significantly lower in intervention arms compared to control arms. There was a small but statistically significant reduction in risk of anaemia. Despite historic levels of funding over the past dozen years and progress towards malaria elimination, these gains are in jeopardy. New interventions are urgently needed that target, in particular, populations that are responsible for human to mosquito transmission. Overall, this analysis supports consideration of policy on and programmatic implementation of antimalarial treatment of asymptomatic school-age children as an intervention to decrease the burden of malaria in this critical age group.

Malaria prevention in schoolchildren: a cluster-randomised trial in schools in Mali

Location of study: Sikasso district, Mali

LSHTM Investigators: Sian Clarke, Saba Rouhani, Rebecca Jones, Simon Brooker

External collaborators: Natalie Roschnik, Seybou Diarra, Bamadio Modibo (Save the Children, Mali); Moussa Sacko, Renion Saye (National Institute for Public Health Research, Ministry of Health, Mali); Diahara Traore, Klenon Traore (National Malaria Control Programme, Ministry of Health, Mali); Matthew Jukes (RTI International, USA); Josselin Thuilliez (Centre d'economie de la Sorbonne, France)

Funding Body: The trial was funded by Save the Children. Sian Clarke and Simon Brooker were supported by the Wellcome Trust.

School-aged children are rarely targeted by malaria control, yet the prevalence of malaria infection in this age group often exceeds that seen in younger children and could affect haemoglobin concentration and school performance.

This cluster-randomised trial in 80 schools in Mali examined the impact of a teacher-led malaria control programme which combined participatory education activities to promote the use of insecticide treated nets, with intermittent parasite clearance in schools (IPCs). In this setting, where malaria is highly seasonal, a single annual antimalarial treatment was given at the end of the transmission season.

Teachers administering an annual treatment to clear malaria infections in schoolchildren in Mali

Delivery of a single round of IPCs was associated with striking reductions in malaria parasitaemia and gametocyte carriage in intervention compared to control schools; OR=0.005 (95% CI 0.002-0.011), p<0.001 and OR=0.02 (0.00-0.17), p<0.001, respectively. This effect was sustained for six months until the beginning of the next transmission season. The prevalence of anaemia also decreased; OR=0.56 (0.40-0.78), p=0.001. The intervention program was also found to result in significant improvements in tests of sustained attention; difference +0.23 (0.10-0.36), p<0.001, confirming findings from an earlier trial of IPCs in western Kenya. These findings highlight the impact of asymptomatic malaria infection on cognitive performance in schoolchildren, and the benefit of intermittent parasite clearance in reducing this burden. Additionally, malaria control in schools can help diminish the infectious reservoir that sustains *Plasmodium* transmission.

Seasonal malaria chemoprevention combined with micronutrient supplementation delivered through community pre-schools in Mali

Location of study: Sikasso district, Mali

LSHTM Investigators: Sian Clarke, Karla Smuts, Isobel Stanley, Hans Verhoef

External collaborators: Natalie Roschnik, Hawa Diarra, Yahia Dicko (Save the Children, Mali); Moussa Sacko, Renion Saye (National Institute for Public Health Research, Ministry of Health, Mali); Rebecca Jones (University College London, UK); Yvonne Griffiths (University of Leeds, UK); Michael Boivin (Michigan State University, USA)

Funding Body: World Bank Strategic Impact Evaluation Fund, UBS Optimus Foundation and Save the Children

Anaemia is common in preschool African children, and is mostly due to iron deficiency and *Plasmodium* infection. Studies of seasonal malaria chemoprevention (SMC) have previously reported reductions in anaemia. This study was undertaken to test whether combining preventive interventions against malaria with micronutrient supplementation might be more efficacious than either intervention alone.

A cluster-randomised trial in 60 rural communities in Mali examined the impact of home fortification with micronutrient powders (MNP), in combination with SMC, and parenting education through community-based preschools, on malaria, anaemia and cognitive development in early childhood. Intervention communities received SMC from August-November, followed by daily home fortification with micronutrient powders for four months January-April each year (SMC+MNP). In the second year, SMC was scaled up to also include the 30 control communities.

The combined impact of the interventions was examined in two age groups of children (aged 3 and 5 years). A significant reduction in malaria infection was recorded in intervention compared to control villages after the first year of implementation; 21% vs 45% in children aged 3 years, and 32% vs 55% in children aged 5 years (both p<0.001). However, this did not translate into any evident benefit in terms of anaemia or nutritional outcomes. Cross-sectional surveys after three successive years of the intervention, again found no discernible difference in anaemia between children receiving SMC and SMC+MNP respectively; and negligible effects for stunting. In conclusion, this study found that the combination of SMC and micronutrient supplementation had limited impact on childhood anaemia in an area of high prevalence, despite good uptake of both interventions.

Community members administering seasonal malaria chemoprevention to children in Mali

Image Courtesy of: Anne Koerber for LSHTM

Clinical Research, Drug Efficacy & Resistance

Malaria Centre researchers are making a concerted contribution to improving two key aspects of malaria case management – diagnosis and chemotherapy. Detection of parasites remains the essential step in confirming a patient has malaria but this can be particularly challenging in low transmission and near-elimination areas where the majority of fever presentations are not caused by malaria. Once identified, effective treatment requires that malaria drugs are of good quality, and our work has pioneered methods for ensuring that substandard, degraded or falsified malaria medicines are identified and tracked. Finally, treatment will not be fully effective if the patient harbours resistant malaria parasites, and we are recognised as world leaders in identifying antimalarial drug resistance in the lab, and monitoring treatment failure in the field.

The appropriate treatment of malaria in endemic countries can be hindered by several factors. Malaria patients with G6PD deficiency are unable to tolerate drugs of the 8-aminoquinoline class, which includes primaquine and tafenoquine, particularly important for preventing *Plasmodium vivax* relapses. Malaria Centre members are leading the development and implementation of a high-throughput standardisable platform for identifying patients with G6PD deficiency, who are at much greater risk of post-treatment haemolytic anaemia when 8-aminoquinolines are used, so that alternative regimens can be deployed. The platform also enables identification of other haemoglobinopathies such as HbC and HbS. One drug now being more heavily used across the malaria endemic world is piperaquine, which is associated with a level of cardiac toxicity. Piperaquine is now being considered (in a combination) for prophylactic treatment to protect pregnant women from *P. falciparum*, and the Malaria Centre is leading a study in northern Tanzania, where other options are precluded by the presence of resistant parasites, to evaluate the safety of regular piperaquine use in this important at-risk group.

Drug resistance emerging in SE Asia, associated with mutations in the gene for the K13 propeller protein that enable a proportion of ring stage parasites to survive artemisinin, is of concern, but the few documented treatment failures in African parasites do not bear this genetic signature. In Indonesian studies of ACT efficacy, we also find no evidence of artemisinin resistance, although the complexity of multi-species infections makes efficacy studies more difficult to carry out. New work in the Malaria Centre has been investigating two candidate genes, *pfubp1* and *pfap2mu,* originally identified in artemisinin-resistant rodent malaria parasites, and demonstrated that mutations introduced by CRISPR do indeed generate a ring-stage resistance phenotype *in vitro*. Further, and exploration of the cellular functions of the AP2-μ protein in *P. falciparum* has provided clear evidence that its role in the parasite is distinct from its normal endocytic trafficking role in other eukaryotic cells.

Drug quality is also key to successful management of malaria, and the Malaria Centre team have been conducting surveillance in Equatorial Guinea to monitor this potential problem. 91% of artemisinin-based therapies were found to be of acceptable quality, but the majority of the remainder were falsified. These data are vital for international efforts to curtail the criminal activity leading to sale of these products in malaria endemic areas.

Finally, the Malaria Centre is also involved in projects combining antimalarial therapy with other interventions. In Mali and Burkina Faso we are working with our local collaborators to investigate the impact of combining seasonal malaria chemoprevention in children under 5 with vaccination, using the RTS,S vaccine recently licensed for use in Africa. We have also been evaluating the utility of combining seasonal malaria chemoprevention with monthly azithromycin (a broad spectrum antibiotic) in preventing childhood mortality. Whereas this provided some benefit in lowering the incidence of non-malaria fevers, there was no measurable benefit in reducing mortality in the target group.

The role of G6PD polymorphisms in haemolysis after treatment with primaquine: an analysis of six studies in Africa

Location of study: Burkina Faso, The Gambia, Uganda, Kenya and Mali

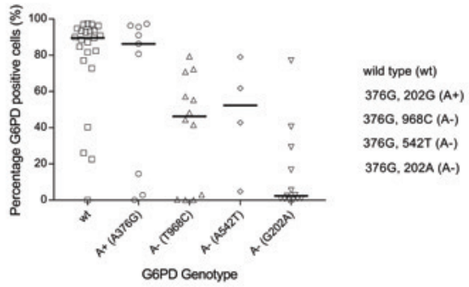
LSHTM Investigators: Nuno Sepúlveda, Lynn Grignard, Bronner Gonçalves, Alice Eziefula, Taane Clark, Susana Campino and Chris Drakeley

External collaborators: Jonathan Curry and Laleta Mahey, (LGC genomics, UK); Guide Bastiaens, Kjerstin Lanke and Teun Bousema, (Radboud University, The Netherlands); Alfred Tiono, Sam Coulibaly, Alphonse Ouédraogo, Edith Bougouma, Guillaume Sanou, Issa Nébié and Sodiomon Sirima, (CNRFP, Burkina Faso); Joseph Okebe, Muna Affara and Umberto d'Alessandro, (MRC, The Gambia); Alassane Dicko, (University of Science, Techniques and Technologies of Bamako, Bamako, Mali); Ingrid Chen and Roly Gosling, (UCSF, Global Health Group, US)

Funding Body: The Gates Foundation

The study aimed to identify human genetic changes associated with the rupture of red blood cells in the presence of an anti-malarial drug called primaquine (PQ).

Primaquine (PQ), an 8 – aminoquinoline, is being considered for wide scale use as it is the only active agent against the sexual stages of *P. falciparum,* gametocytes. However, this drug induces haemolysis during treatment, which might pose a potential risk to individuals with Glucose-6-phospate dehydrogenase deficiency (G6PDd), a common haemoglobinopathy in African populations.



Genetic association between different G6PD SNPs and haemolyis at day 7 after adjusting for gender, age, parasitaemia and baseline Hb where SNPs with log10(p-values) above the dashed line (false discovery rate) are statistically associated with the variation in haemolysis at day 7.

The investigation of genetic associations with haemolysis after treatment will then enable control programmes to better assess putative risks of using the drug. We retrospectively tested the association between haemolysis after PQ treatment and 20 SNPs in the *G6PD* gene in 957 individuals from 6 clinical trials on the safety and efficacy of this drug in 5 African countries. When adjusted for age, sex and baseline Hb, the known rs1050829 SNP (A376G) as well as 2 lesser described SNPs (rs28470352 and rs2230037) were moderately associated with drops in haemoglobin (Hb). Data suggests that haemolysis is transient. Nevertheless, control programmes using PQ might consider including genetic screening for these three SNPs for any associated pharmacovigilance.

Bead based assays to simultaneously detect multiple human inherited blood disorders associated with malaria

Location of study: Burkina Faso and The Gambia

LSHTM Investigators: Lynn Grignard, Catherine Mair, Bronner P. Gonçalves, Taane G. Clark, Susana Campino, Chris Drakeley

External collaborators: Jonathan Curry and Laleta Mahey, (LGC genomics, UK); Kjerstin H.W. Lanke, Guido J.H. Bastiaens and Teun Bousema, (Radboud University, The Netherlands); Alfred B. Tiono, Sam A. Coulibaly, Alphonse Ouédraogo, Edith C. Bougouma, Guillaume Sanou, Issa Nébié and Sodiomon B. Sirima, (CNRFP, Burkina Faso); Joseph Okebe, Muna Affara and Umberto d'Alessandro, (MRC The Gambia)

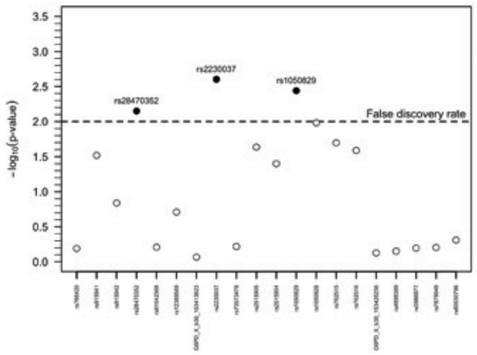
Funding Body: The Bill and Melinda Gates Foundation

We aimed to develop a new, cheap and quick method to simultaneously detect human genetic changes in a single reaction. The genetic changes of interest are associated with the severity of malaria or the treatment of the disease.

Glucose-6-phosphate dehydrogenase deficiency (G6PDd), haemoglobin C (HbC) and S (HbS) are inherited blood disorders (IBD) common in populations in malaria endemic areas. All are associated to some degree with protection against clinical malaria whilst additionally G6PDd is associated with haemolysis following treatment with 8-aminoquinolines. Current methods in epidemiological studies are time consuming and relatively low throughput.

Our new assay detects G6PD SNPs and common haemoglobin mutations (HbS and HbC). In a pilot study, seventy-five samples from Burkina Faso (n=75/78, 96.2%) and fifty-eight samples from The Gambia (n=58/61, 95.1%) had a G6PD and a HBB genotype successfully assigned by the assay. Flow cytometry data further supported the concordance between G6PD enzyme activity and genotype.

The bead based assay compares well to alternative measures of genotyping and phenotyping for G6PD. The screening is high throughput, and easily standardised to include more targets.



G6PD enzyme activity by flow cytometry and G6PD genotype.

Quality of Artemisinin-Based Combination Antimalarial Medicines in Sub-Saharan Africa and Cambodia

Location of study: Tanzania, Ghana, Nigeria, Rwanda, Equatorial Guinea and Cambodia

LSHTM Investigators: Harparkash Kaur

External collaborators: Facundo M Fernandez, (Georgia Tech, School of Chemistry and Biochemistry, Atlanta, US); Michael D. Green, (US Centers for Disease Control and Prevention, Atlanta, US)

Funding Body: ACT Consortium, funded through a grant from the Bill & Melinda Gates Foundation to the London School of Hygiene & Tropical Medicine

There is increasing international awareness of the threat posed by falsified and substandard antimalarials in malaria endemic countries. Nonetheless, evidence is largely based on studies of small sample numbers collected using the convenience sampling approach, which does not generate reliable or replicable estimates of medicine quality for regulators to take action.

Malaria is a leading cause of morbidity and mortality in tropical countries. artemisinin combination therapy (ACTs) are the first line medicines endorsed by the World Health Organization for the treatment of malaria, and have been adopted in endemic countries.

Woman obtains medicines in Ghana

Both patients and health professionals assume that their medicines are of good quality, but reports have drawn attention to the presence of up to 35% poor quality antimalarial medicines from 21 sub-Saharan African countries that had failed content analysis.

The ACT Consortium drug quality programme purchased over 10,000 artemisinin containing medicines, using representative sampling approaches (convenience survey using mystery clients, randomised survey using mystery clients and overt sampling), from six malaria endemic countries and assessed their quality in three independent laboratories. Falsified ACTs were found in just two of the countries, whilst substandard ACTs were present in all six countries, and, artemisinin-based monotherapy tablets were widely available.

Overall, our Drug Quality programme has provided reassuring results, but there is no room for complacency.

- One falsified antimalarial drug is one too many.
- Substandard medicines are present in every country that we studied.
- Monotherapy tablets of artesunate and dihydroartemisinin are still available.

Our research showed how representative methods to sample medicines are important for generating reliable estimates of the prevalence of poor quality drugs in a given country. However, this type of study is cost intensive, both for the purchase and analysis of drugs.

It is important to work with affordable but robust, representative sampling methods as well as laboratory techniques to assess the quality of medicines on a regular basis.

Quality of antimalarial medicines sold in Bioko Island, Equatorial Guinea

Location of study: Bioko Island, Equatorial Guinea

LSHTM Investigators: Harparkash Kaur, Elizabeth Louise Allan, Ibrahim Mamadu, Zoe Hall

External collaborators: Michael D Green and Isabel Swamidoss, (Division of Parasitic Diseases and Malaria, US Centers for Disease Control and Prevention, Atlanta, US); Facundo M Fernández, Prabha Dwivedi and Maria Julia Culzoni, (Georgia Institute of Technology, School of Chemistry and Biochemistry, Atlanta, US); Feliciano Monti, Dianna Hergott and Guillermo Garcia, (Bioko Island Malaria Control Project, Equatorial Guinea)

Funding Body: Ministry of Health and Social Welfare Malabo, Equatorial Guinea (through Medical Care Development International Bioko Island Malaria Control Project)

Access to good quality, efficacious medicines is essential to treat malaria. Poor-quality medicines, including falsified, substandard, and degraded medicines, pose serious health concerns in malaria endemic countries, with potential lead to drug resistance and kill patients. Systematic assessment of drug quality will provide evidence for Ministries of Health to take action.

The first line medicines to treat malaria, namely the artemisinin-containing antimalarials (ACAs) were purchased using three sampling approaches on Bioko Island, Equatorial Guinea. Samples (677 of from 278 outlets) were purchased as follows: convenience survey using mystery client (n=16 outlets, 31 samples), full island-wide survey using mystery client (n=174 outlets, 368 samples) and randomised survey using an overt sampling approach (n=88 outlets, 278 samples).

The stated active pharmaceutical ingredients (SAPIs) were assessed using high-performance liquid chromatography and confirmed by mass spectrometry at three independent laboratories.

Content analysis showed 91.0% of ACAs were of acceptable quality, 1.6% were substandard and 7.4% falsified. No degraded medicines were detected. The prevalence of medicines without the SAPIs was higher for ACAs purchased in the convenience survey compared to the estimates obtained using the full island-wide survey-mystery client and randomised overt sampling approaches. Comparable results were obtained for full-island survey mystery client and randomised overt.

Falsified ACAs were found on Bioko Island, with the prevalence ranging between 6.1-16.1%, depending on the sampling method used. These findings underscore the vital need for national authorities to track the scale of ineffective medicines that jeopardise treatment of life-threatening diseases, and value of a representative sampling approach to obtain/measure the true prevalence of poor quality medicines in a given geographical region.

Examples of falsified artemisinin containing antimalarials from Bioko

Identification of *Plasmodium falciparum* with histidine-rich protein (*pfhrp2* and *pfhrp3*) deletions in east Africa: Unravelling the genomic Profile and impact on parasite fitness and clinical outcome

Location of study: Eritrea, Ethiopia, Tanzania, Sudan

LSHTM Investigators: Khalid B Beshir, Nuno Sepulveda, Susana Campino, Taane Clark, Colin Sutherland

External collaborators: Robert Kayaa (Tanzania); Sindew Mekesha (Ethiopia); Araia Berhane (Eritrea)

Funding Body: ACT Consortium; Infection and Immunology Department

***Plasmodium falciparum* parasites lacking histidine-rich protein 2 (HRP2) have recently emerged in South America, Southeast Asia and east Africa. HRP2 is species-specific target antigen in rapid diagnostic test (RDTs) for diagnosis of P. falciparum parasites as well as predictor of disease outcome.**

The distribution of such deletions in east Africa and the impact on RDT performance as well as on malaria control and elimination programs has not yet been fully elucidated. Our study in western Kenya has revealed that the presence of *P. falciparum* parasites with *hrp2* deletion. We have also published a global analysis of *pfhrp2/3* deletions using publicly available genomic data and we found that *hrp2* and *hrp3* deletions have been well represented in genomic datasets from in south east Asia and south America collected over the last decade. Using publicly available genomic data generated from genetic crosses, we have recently reported the absence of fitness cost for *hrp2*-deleted parasites. The consequence of HRP2 deletion for parasite fitness and virulence, as well as the impact on biomarker and clinical outcome, require urgent attention. We seek to measure the contribution of HRP2 deletions to severe malaria. In addition, integration of HRP2 status with patients' clinical data permits us to investigate the threat posed to HRP2 plasma level as a useful prognostic biomarker in severe malaria. Using whole genome sequencing, we examine the consequence of *pfhrp2* deletion to the overall parasite genome, particularly to the regions reported to have associations with severe malaria.

K13-independent mechanisms of reduced artemisinin susceptibility in *Plasmodium falciparum*

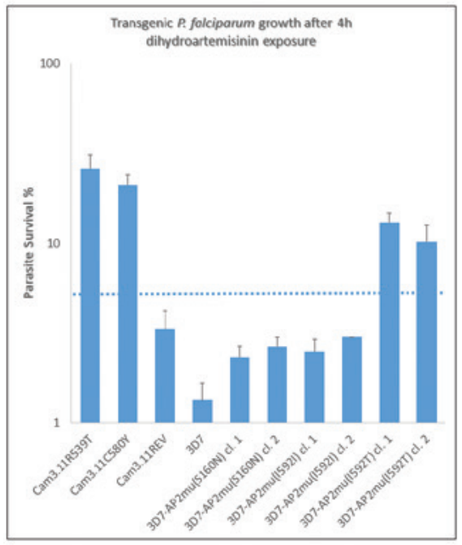
Location of study: LSHTM

LSHTM Investigators: Ryan Henrici, Donelly van Schwalkwyk, Colin Sutherland

Funding Body: UK Foreign and Commonwealth Office through the Marshall Scholarship Programme

The effectiveness of artemisinin, a current malaria drug, has been falling in SE Asia over the last decade. This is linked to mutations in the gene encoding the parasite protein K13. We have been examining other ways that parasites can become less susceptible to artemisinin, as these may be relevant in Africa.

We have been examining the effects of mutations in two *P. falciparum* genes, *pfap2mu* (Figure) and *pfubp1*, on artemisinin susceptibility *in vitro*. We have used CRISPR-Cas9 gene editing to introduce mutations of interest, and have succeeded in generating parasite lines with a significantly enhanced ability to survive a 4h pulse of 700 nM dihydroartemisinin. We have also shown that transient exposure to low termperature, and the use of agents that inhibit intracellular trafficking produce a similar affect. In each case, the parasites did not carry mutations in the gene encoding the K13 protein. Interestingly, none of these genes or other factors, including K13, can generate artemisinin resistance beyond the ring-stage of parasite development; all parasite stages are fully susceptible from 8-10h post-invasion onwards. Reduced parasite susceptibility is thus a partial adaptation to the brevity of artemisinin exposure *in vivo*, giving a proportion of early parasites an improved chance of surviving in the treated host. Work continues to unravel the biological basis of this interesting phenomenon.



Transgenic P. falciparum 3D7 harbouring the I592T variant of the pfap2mu locus demonstrate significantly enhanced survival of a 4h 700 nM pulse of DHA in vitro. This level of survival is compared to that of other transgenic parasites, parental 3D7 and Cambodian isolates carrying pfk13 variants.

ACT treatment of uncomplicated *Plasmodium falciparum* infections, and co-infecting *P. knowlesi, P. malariae* and *P. vivax,* in North Sumatera

Location of study: North Sumatera, Indonesia

LSHTM Investigators: Inke Lubis, Sarah Staedke, Colin Sutherland

External collaborators: Hendri Wijaya, Munar Lubis, Chairuddin Lubis (Department of Paediatrics, University of Sumatera Utara, Indonesia)

Funding Body: Directorate General of Higher Education, Indonesia

Growing evidence of drug resistant malaria in the Greater Mekong Region has led to concerns that this problem may spread to neighbouring countries of SE Asia. We set out to test the effectiveness of current malaria drugs in Nth Sumatera Province, Indonesia, as data from this region has been unavailable.

Plasmodium falciparum with reduced susceptibility to the artemisinin family of drugs, and harbouring variant alleles of *pfk13,* are present in the SE Asian region. We conducted an open-label, randomised comparison of dihydroartemisinin-piperaquine and artemether-lumefantrine for the treatment of uncomplicated *falciparum* malaria, with or without other co-infecting *Plasmodium*, in North Sumatera province, Indonesia. 153 and 149 individuals with microscopy-confirmed *P.falciparum* malaria were randomised into the two treatment arms, respectively. In the evaluable intention to treat population (n=144, 146 respectively) drug efficacy at day 42 was 84.0% for dihydroartemisinin-piperaquine and 90.4% for artemether-lumefantrine (P=0.09). The PCR-corrected efficacy at day 42 was 99.3% and 100% (P=0.31), respectively. *Post hoc* species-specific PCR analysis at all time-points identified a significant proportion of parasite-negative individuals among the randomised population, indicating poor specificity of microscopic diagnosis at enrolment. Nine cases of microscopically-detected parasitaemia in follow-up were confirmed by PCR as *P. malariae, P. knowlesi, P. vivax* or a mixture of species. qPCR estimates of parasite-clearance up to 72h post-treatment indicated that, in our study area, *P. falciparum* is susceptible to artemisinin. However, *P. falciparum* DNA was detected at day 28 or day 42 in 35.1% of evaluable individuals, suggesting a failure of prophylactic protection from lumefantrine and piperaquine. Pre-treatment analysis found the SVMNT *pfcrt* haplotype at codons 72-76 dominant in these parasite populations; the *pfk13* propeller domain variant T474A, of unknown phenotypic significance, was identified in 3 individuals. We found strong evidence of post-treatment selection for the *Pfmdr1* NF haplotype at codons 86 and 184 in both drug groups.

Map of North Sumatera province, Indonesia.
The three studied regencies (Batubara regency, Langkat regency, South Nias regency) are indicated.

Efficacy of DHP and AL against P. falciparum and P. malariae over 42 days of follow-up

Use of malaria rapid diagnostic tests by community health workers in Afghanistan: cluster randomised trial of effective diagnosis and treatment

Location of study: Eastern Afghanistan

LSHTM Investigators: Toby Lelie, Mark Rowland, Chris Whitty, Amy Mikhail, Bonnie Cundill, Barbara Willey, Baptiste Laurent

External collaborators: Ismail Mayan, Nader Mohammed, Asif Alokozai (Health Protection and Research Organisation, Kabul, Afghanistan)

Funding Body: UK Foreign and Commonwealth Office through the Marshall Scholarship Programme

Use of malaria rapid diagnostic tests (mRDT) by community health workers (CHW) has not been tested within health services in Afghanistan and south Asia. RDTs could improve the diagnosis of falciparum and vivax malaria more accurate and improve treatment of these co-endemic diseases.

We have been examining the effects of mutations in two P. CHWs are often the first point of contact for those with fever. CHWs in Afghanistan and much of south Asia have limited access to laboratory testing and diagnose using clinical signs and symptoms alone.

CHWs from 22 clusters received standard training on clinical diagnosis and treatment of malaria; 11 clusters randomised to the intervention arm were provided with RDTs. Cases of suspected malaria were enrolled by CHWs and the diagnoses and treatments were compared to PCR diagnosis. In the intervention mRDT arm, 75% (828/1099) were treated appropriately vs. 17% (185/1055) in the control arm ($p < 0.001$). In the control arm, 86% (164/191) with confirmed Plasmodium vivax received chloroquine compared to 45% (70/155) in the intervention arm ($p < 0.001$). There was high concordance between the RDT result and CHW prescription decisions. Overuse of chloroquine in the control arm resulted in 88% of PCR negative patients being treated vs. 10% in the intervention arm. In the intervention arm, 71% (30/42) of patients with P. falciparum did not receive ACT partly because of low RDT sensitivity. Antibiotics were prescribed to 63% of malaria negative cases in the intervention and to 15% in the control arm. Introducing RDT to CHWs reduced overuse of antimalarials but many malaria cases were missed using current RDTs. Overtreatment with antimalarial drugs in the control arm was replaced with probable overuse of antibiotic prescription to malaria negative patients in the RDT arm.

Cardiac safety of dihydroartemisinin-piperaquine amongst pregnant women in Tanzania

Location of study: Handeni, Tanzania

LSHTM Investigators: Matthew Chico, Daniel Chandramohan, Brian Greenwood

External collaborators: Frank Mosha, Reginald Kavishe (Kilimanjaro Christian Medical Centre, Tanzania); Jacklin Mosha, Alphaxard Manjurano (National Institute of Medical Research, Tanzania)

Funding Body: Medicines for Malaria Venture

Dihydroartemisinin-piperaquine (DP) is a potential chemoprophylaxis against adverse pregnancy outcomes attributable to malaria infection. The purpose of this trial is to confirm that DP does not adversely affect cardiac function of pregnant women, with and without asymptomatic parasitaemia, compared to pregnant women who receive sulphadoxine-pyrimethamine, with and without asymptomatic parasitaemia.

The WHO currently recommends the provision of intermittent preventive treatment in pregnancy (IPTp) using sulphadoxine-pyrimethamine (SP) during scheduled antenatal care visits from the second trimester to delivery in moderate to high transmission areas.

However, SP is compromised as antimalarial treatment and dihydroartemisinin-piperaquine (DP) is a possible replacement. Piperaquine, however, is known to alter cardiac function, although dosing levels recommended by the WHO do not pose any clinical consequences to non-pregnant individuals. Malaria infection can independently alter cardiac function. Thus, the purpose of this trial is to confirm that DP does not adversely alter cardiac function of pregnant women, with and without asymptomatic parasitaemia, compared to pregnant women who receive SP, with and without asymptomatic parasitaemia,

The trial is being conducted in the Tanga Region of Tanzania where malaria parasites have a high prevalence of the 581G mutation which greatly compromises the efficacy of SP. The trial is being conducted in collaboration with Kilimanjaro Christian Medical Centre and the National Institution of Medical Research in Tanzania. In total, 200 pregnant women will participate in the trial. Recruitment is ongoing. More details can be found in the trial registry and results database maintained by the U.S. National Institutes of Health at ClinicalTrials.gov using the Identifier: NCT02909712

Fever case management in urban slums in Uganda

Location of study: Kampala, Uganda

LSHTM Investigators: Sian Clarke , Eleanor Hutchinson, Daniel Chandramohan

External collaborators: Anthony Mbonye (Ministry of Health, Uganda); Phyllis Awor, Miriam Kayendeke, Elizeus Rutebemberwa & Esther Buregyeya (Makerere University School of Public Health, Uganda); Pascal Magnussen & Kristian Hansen (University of Copenhagen, Denmark)

Funding Body: MRC/ESRC/DfID/Wellcome Trust: (Development Grant, Health Systems Research)

Malaria, pneumonia and diarrhoea are major causes of death in African children, yet if diagnosis and treatment are available most of these deaths can be prevented. Integrated community case management (iCCM) by community health workers (CHWs) can improve access to treatment in rural areas. However use of CHWs in urban settings is low, and other ways to provide health care for the urban poor are needed. Growing cities are characterised by a vibrant private sector of formal and informal providers, who potentially could be trained to deliver iCCM.

Snowball sampling was used to identify all treatment providers in two high-density urban slums in Kampala, Uganda. Data on treatment practices and quality of care were collected through interviews with private sector providers, audit of drug stocks, and observations of consultations for childhood fever. We also conducted focus group discussions with health providers and community members.

Our findings revealed the vast number and diversity of private providers that operate in urban settings, even in low-income areas – with 72 private clinics, drug shops, and pharmacies identified in the two sites.

Drug shops are an important source of malaria treatment in Uganda

Almost all were located within 30 minutes' walk of a public health centre. Over 70% of premises were licensed and staffed by a qualified health worker (nurse or medical doctor). All reported selling antimalarials, and over 70% reported selling amoxicillin and other antibiotics. Treatment for malaria and diarrhoea were consistent with national guidelines, but the diagnosis and treatment of pneumonia was poor, across all types of providers. In brief, though premises were usually operated by a qualified health worker, there remains ample scope for treatment services to be improved.

Image Courtesy of: Anne Koerber for LSHTM

Image Courtesy of: Joshua Paul for LSHTM

Malaria Elimination

Malaria elimination is an explicit goal for a significant number of malaria-endemic countries, including some where nation-wide elimination may seem unrealistic but sub-national elimination is possible due to the natural heterogeneity of malaria transmission.

The Malaria Centre works in several African countries where transmission is uniformly low (The Gambia, Namibia, South Africa), a number of south east Asian countries coping with varied parasite species, human movement and drug resistance (Cambodia, Malaysia, Philippines, Indonesia) and the only country in the Caribbean with indigenous transmission (Haiti).

These different study sites have in common a need to identify the driving forces behind persisting transmission, so that spatial and/or demographic foci (hot spots and hot pops) can be better understood and targeted. Research in Cambodia highlights the multifaceted nature of work required to understand ongoing transmission. Building on strong links with the national control programme, Malaria Centre researchers have investigated different approaches to identify and target infected individuals in the face of the threat of drug resistance. This involves proactive detection of infections, including among high-risk forest-goers, and attempting to increase efficacy by involving community members. These efforts are coupled with the evaluation of new rapid diagnostic tests with reportedly higher sensitivity in detecting individuals with low-density parasite infections, which have been implicated in the maintenance of transmission.

A key component for any intervention is the cost that would ultimately have to be borne by control programmes. Both the proactive infection detection in Cambodia and reactive household detection in The Gambia have core economic components that will help guide intervention choice. Similarly, rationalising choice and use of existing interventions is equally important. In South Africa, a study comparing targeted versus blanket IRS showed no difference in efficacy but a significant reduction in cost with targeted interventions.

The Malaria Centre at LSHTM has been at the forefront of investigating the use of specific antimalarial antibody responses as markers of exposure and transmission. Antibodies are longer-lived than infections in either mosquitoes or humans and therefore have unique potential as a marker of previous infection, particularly at low transmission. Malaria Centre projects located in Indonesia, South Africa and Haiti are examining the utility of serological markers of infection to describe hotspots of transmission, and to stratify areas for interventions. Studies in Namibia and The Gambia have extended the use of these markers to post-intervention evaluation, and in particular to include antigens that are associated with recent infections. This offers an alternative approach to measure incidence which has considerable potential for the future.

Non-inferiority of targeted reactive IRS compared with generalised routine IRS and determination of transmission hotspots

Location of study: South Africa

LSHTM Investigators: Immo Kleinschmidt, Jackie Cook, David Bath, Catherine Pitt, Joseph Biggs

External collaborators: Maureen Coetzee (Wits Research Institute for Malaria, University of the Witwatersrand, South Africa); Natashia Morris, Rajendra Maharaj (South African Medical Research Council, South Africa); Aaron Mabuza (Mpumalanga Provincial Malaria Control Programme, South Africa)

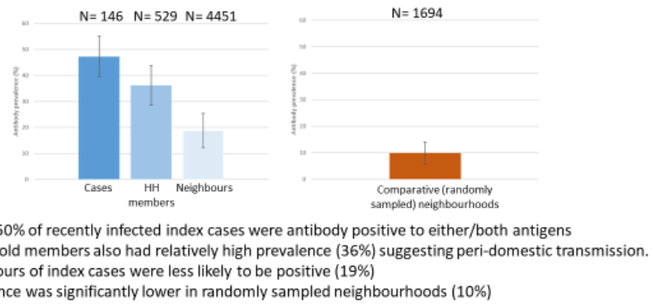
Funding Body: MRC/DFID/Wellcome Trust Joint Global Health Trials

In areas of very low, pre-elimination malaria transmission the low number of incident cases may call into question the resources needed for spraying all houses annually with insecticide (generalised indoor residual spraying (GIRS)). An alternative that may be more cost-effective is to target IRS, reactively spraying only houses in the immediate neighbourhood of incident cases as they arise.

62 clusters consisting of settlements of approximately 5000 persons each in the provinces of Mpumalanga and Limpopo in South Africa, were randomly allocated to receive the currently practised GIRS whilst the other half received reactive targeted IRS (TIRS) in the immediate neighbourhood (about 8 houses) of every passively reported local case. Non-inferiority of TIRS was assessed as passive incidence of no more than 1 case/1000 per year above that of the GIRS arm. Dried blood spots were collected from neighbourhoods of index cases, and in randomly selected neighbourhoods where there were no cases occurred, to determine, using serology, whether new cases arise predominantly in areas of previous exposure to parasites.

Incidence in the targeted IRS was higher than in the generalised IRS arm by 0.29 cases per 1000 (95% CI -0.53-1.10). Antibody prevalence showed that cases arise in neighbourhoods with raised levels of previous exposure to malaria parasites.

Although evidence of non-inferiority of TIRS compared to GIRS was weak, TIRS appears to be a safe, more sustainable method of deploying IRS in areas of very low transmission. Since the neighbourhoods of passively detected cases are characterised by raised levels of past exposure to parasites, it is important that these areas are targeted with additional interventions such as focal IRS.



- Nearly 50% of recently infected index cases were antibody positive to either/both antigens
- Household members also had relatively high prevalence (36%) suggesting peri-domestic transmission.
- Neighbours of index cases were less likely to be positive (19%)
- Prevalence was significantly lower in randomly sampled neighbourhoods (10%)

Antibody prevalence in households and neighbours of index cases, compared to randomly selected households with no cases

Freedom from Infection: confirming the interruption of malaria transmission for elimination

Location of study: Cabo Verde, Indonesia, Haiti, Laos, Thailand, Vietnam

LSHTM Investigators: Lindsey Wu, Henry Surendra, Gillian Stresman, Chris Drakeley

External collaborators: Kim Linblade and Abdisalan Noor (WHO Global Malaria Programme), Arnaud Le Menach and Justin Cohen (Clinton Health Access Initiative), Pak Yono Supargiyono (Universitas Gadjah Mada, Indonesia), Adam Bennett and Andrew Lover (UC San Francisco Malaria Elimination Initiative), Adilson Jose de Pina (Cabo Verde Ministry of Health)

Funding Body: Bill & Melinda Gates Foundation

While a number of countries are targeting malaria elimination, epidemiologically confirming the absence of cases or infections is challenging. This project is adapting "Freedom from Infection" surveillance tools, used extensively in veterinary epidemiology, to guide national malaria control programmes in the design of optimal elimination surveillance strategies.

Freedom from Infection (FFI) surveillance tools have been used in veterinary epidemiology to demonstrate that infection in a population is below a defined threshold. These approaches characterise the absence of transmission within a degree of statistical certainty. This project is adapting FFI tools specifically for malaria in collaboration with national malaria control programmes (NMCPs) and research groups across a range of countries currently targeting elimination. Our analysis estimates the probability that regions are free from malaria transmission using routine health facility data or community surveys. Analysis also includes determining feasible time frames required to confirm elimination at national or subnational levels and quantifying the value-added of additional diagnostics or surveillance endpoints to more accurately estimate freedom from infection. Preliminary analysis, such as from the Kulon Progo Regency in central Java, Indonesia, has shown. that monthly surveillance data over the course of one year can be used to estimate the probability of freedom from malaria infection at the health facility level. Work is currently ongoing to demonstrate the use of these tools with surveillance data at national and subnational levels in Laos, Thailand, Vietnam, Haiti, and Cabo Verde. We are also partnering with NMCPs and the WHO to determine how these tools can be designed to contribute to programmatic decision-making for elimination certification.

Targeted parasite elimination in the human and mosquito to reduce malaria transmission: A randomised controlled trial evaluating reactive focal drug administration (rfMDA) versus reactive case detection (RACD), with and without reactive vector control (RAVC) in a low endemic setting of Namibia

Location of study: Zambezi, Namibia

LSHTM Investigators: Immo Kleinschmidt, Lindsey Wu, Chris Drakeley, Kevin Tetteh

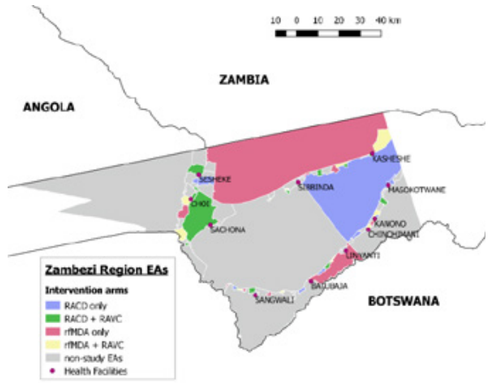
External collaborators: UC San Francisco Malaria Elimination Initiative; University of Namibia; University of the Witwatersrand, South Africa; Ministry of Health and Social Services, Namibia; Zambezi Ministry of Health and Social Services Namibia; UC San Francisco, Division of Experimental Medicine, USA

Funding Body: Bill & Melinda Gates Foundation, Novartis Foundation

A factorial design cluster-randomised controlled trial was conducted in Zambezi, Namibia to evaluate the effectiveness of reactive focal mass drug administration (rfMDA) and reactive vector control (RAVC) as targeted parasite elimination strategies used alone and in combination.

Reactive case detection (RACD) has been widely used as a malaria elimination strategy, but its effectiveness is not well validated due to the limited sensitivity of field diagnostics for asymptomatic infections. This study aimed to test the effectiveness of alternative elimination strategies targeting the parasite in both humans and mosquitoes, using a factorial design cluster randomised controlled trial.

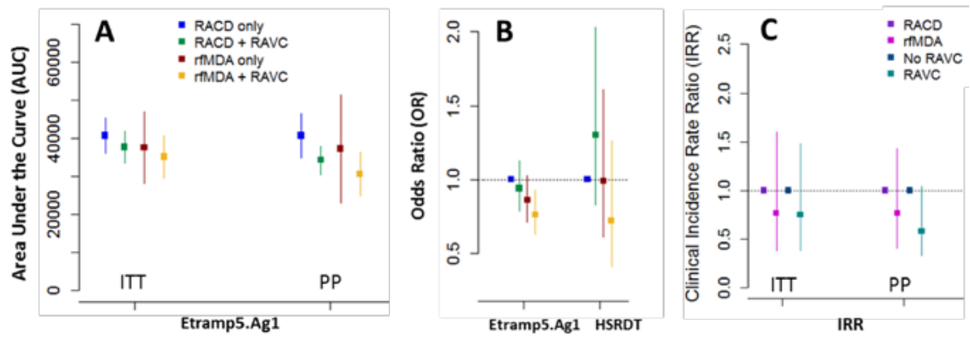
Between January and November 2017, 56 enumeration areas in Zambezi, Namibia were randomised to four study arms: reactive case detection only (RACD), reactive case detection with reactive vector control (RACD + RAVC), reactive focal mass drug administration only (rfMDA), or reactive focal mass drug administration with reactive vector control (rfMDA + RAVC). Factorial trial design enabled comparisons of test vs. control interventions targeting the human reservoir (rfMDA vs. RACD), the mosquito reservoir (RAVC vs. no RAVC) and in combination (rfMDA + RAVC vs. RACD only). The largest effect was observed in the combined intervention arm. Locally-acquired cumulative incidence in the rfMDA + RAVC intervention arm was 18.5 per 1000 individuals compared to 30.8 cases per 1000 in the reactive case detection control arm.



Fifty-six enumeration areas were randomised to the following study arms: reactive case detection only (RACD), reactive focal mass drug administration only (rfMDA), reactive case detection with reactive vector control (RACD + RAVC), or reactive focal mass drug administration with reactive vector control (rfMDA + RAVC)

The adjusted hazard ratio in the combined intervention compared to control was 0.56 (0.36 – 0.87, p=0.01).

A cross-sectional survey was conducted at the end of the malaria season (May – August 2017) to measure *P. falcparum* infection prevalence using two rapid diagnostic tests (CareStart Malaria HRP2/pLDH(Pf/PAN) and ultra-sensitive HRP2-based Alere™ Malaria Ag Pf RDT)) quantitative PCR, and a panel of over 20 serological markers on the Luminex quantitative suspension array (qSAT) immuno-assay. The serological analysis led by LSHTM researchers observed that several markers of recent malaria exposure, such as Etramp5.Ag1, were correlated with primary trial endpoints (clinical incidence rate ratio) and may have potential as secondary endpoints in future cluster randomised trials.



A. Area Under the Antibody Acquisition Curve (AUC) for Etramp5.Ag1 based on intention-to-treat (ITT) and per-protocol (PP) analysis
B. Odds of sero-positivity to Etramp5.Ag1 and highly-sensitive rapid diagnostic test (hsRDT) positivity by study arm (intention-to-treat only) C. Clinical Incidence Rate Ratio (IRR) by intervention

Proactive case detection and community participation for the elimination of drug-resistant malaria in Cambodia Study (PACES)

Location of study: Oddor Meachey Province, Cambodia

LSHTM Investigators: Shunmay Yeung, Nicola James

External collaborators: Soy Ty Kheang, Somphos Chhloeung, (Health and Social Development); Po Ly, Hu Rekol, Siv Sovannaroth, (National Center for Parasitology, Entomology & Malaria Control, Cambodia); Benoit Witkowski, Didier Ménard (Institut Pasteur du Cambodge, Cambodia); Koen Peeters (Institute of Tropical Medicine, Belgium); Iveth Gonzalez and Xavier Ding (Foundation for Innovative New Diagnostics, Switzerland)

Funding Body: UK DFID through the Tracking Resistance to Artemisinins Collaboration

The spread of artemisinin resistant P. falciparum malaria is one of the biggest threats to global malaria control and elimination. Cambodia, which is at the epicentre of multidrug resistant malaria, declared a goal of eliminating malaria by 2025. However it is unclear how to achieve this operationally, for example who to target for strategies such as active case detection, what screening tests to use and how acceptable these interventions are to the affected communities? The aim of our study was to address some of these questions and to contribute to the national and regional efforts to eliminate drug resistant malaria.

We carried out a mixed methods study at the core of which was a cluster randomised control trial of active case detection using village malaria workers in 130 villages in Oddor Meanchey province. We confirmed how focal and dynamic malaria is in this study area, and the importance of working very closely with the community. In most villages there was no local transmission, and little point in screening people who had not recently been to the forest.

Discussions with health centre staff on where the forest goers are getting malaria

For at-risk contacts of patients who had presented to the village malaria worker with symptomatic malaria, around one-fifth tested positive for malaria.

The intervention was generally very well accepted however the majority of the asymptomatic malaria that we uncovered was not due to *P. falciparum* but *P. vivax* and that the relapsing nature of *P. vivax* poses as much, if not more of a burden in affected communities. However, this is not related to drug resistance. Future plans include operational research focusing on providing radical cure for *P. vivax* with prior G6PD testing.

By understanding more about malaria and the at-risk population in these areas and sharing the lessons we have learnt in implementing active case detection, we hope to contribute to the national and regional efforts to eliminate drug resistant malaria.

Fig 1b Response team member getting instructions on where to find forest-going co-workers

Diagnostic performance of ultra-sensitive rapid diagnostic tests for detecting asymptomatic *P. falciparum* on the Cambodian-Thai border

Location of study: Oddor Meachey Province, Cambodia

LSHTM Investigators: Shunmay Yeung, Nicola James, David McGregor

External collaborators: Soy Ty Kheang (Health and Social Development); Po Ly, Siv Sovannaroth (National Center for Parasitology, Entomology & Malaria Control, Cambodia); Saorin Kim, Nimol Khim, Benoit Witkowski (Institut Pasteur du Cambodge, Cambodia)

Funding Body: UK DFID through the Tracking Resistance to Artemisinins Collaboration

Proposed interventions for eliminating drug resistant *P. falciparum* malaria, include the targeting of asymptomatic carriers through screening and treatment. We compared the diagnostic performance of the recently developed ultra-sensitive rapid diagnostic test (uRDT) under field conditions in Cambodia, compared to screening with conventional RDTs (cRDT) and polymerase chain reaction (PCR).

This study was nested within the PACES study, a cluster randomised control trial of active case detection for malaria which was carried out in Oddar Meanchey Province in Northwest of Cambodia. We carried out a comparison of uRDTs with conventional RDTs and PCR under two field conditions: active case detection and a cross-sectional survey. In total 2,729 tests were carried using all three diagnostic tests; 678 during active case detection and 2,051 during the cross-sectional survey. The positivity rate for *P. falciparum* infections by qPCR analysis was 3.8% (26/678) and 0.48% (10/2051) for active case detection and cross-sectional survey respectively. In both these contexts uRDT were shown to have similar sensitivity and specificity to cRDT. The results of this study therefore do not suggest an improved performance of uRDTs over conventional RDTs when used for either active screening or cross-sectional surveys in very low prevalence areas such as Northern Cambodia.

Conventional and ultra-sensitive Rapid Diagnostic Tests

Taking blood from forest goers on the back of timber truck

Surveillance

Effective malaria surveillance, and the monitoring and evaluation of control and elimination efforts, are essential to track progress in malaria control and to know where best to target resources to maximise their impact. The importance of malaria surveillance is highlighted in the WHO's Global Technical Strategy for Malaria 2016-2030 where it constitutes one of three pillars identified as vital for the continued success of malaria control. Emphasis is placed on surveillance in terms of a core intervention, but also as a way to help prevent re-establishment of transmission. Work within the Malaria Centre covers both these aspects, along with other important functions of surveillance.

A wealth of malaria surveillance data has been collected from nationally representative household surveys, such as Malaria Indicator Surveys and Demographic and Health Surveys. An example within the Malaria Centre of a long running community-wide survey is demonstrated by The Bioko Island Malaria Project where one aspect involves large annual cross-sectional surveys charting the longitudinal achievements of a control programme in an area of high to moderate transmission which has deployed extensive indoor residual spraying (IRS) and bed net distributions. The ongoing work has contributed data to enable evidence-informed deployment of intervention strategies. However, these types of surveys can be expensive to implement and their utility in areas of low malaria prevalence is questionable. In such settings, it is important to understand the performance of alternative methods of detecting infections, such as the use of highly sensitive RDTs – as has been done as part of the PACES project in Cambodia. The functional basis of the most commonly used RDTs is the identification of circulating HRP-2 antigen in infected (or recently infected) people; however, research has shown that HRP-2 and HRP-3 deletions in *Plasmodium* parasites are becoming more prevalent across the globe – potentially rendering RDTs less effective. Within the Malaria Centre, work is ongoing to compile global genomic data on pfhrp2/3 deletions and to establish the consequences of HRP-2 deletion for parasite fitness and virulence. Another potential way to detect infections is utilising serological markers. There is substantial ongoing work within the Malaria Centre to further the use of serological markers to assess malaria transmission dynamics, particularly in low transmission settings. This work includes optimising assays using multiplex technology such as luminex and microarray, as well as identifying novel *Plasmodium* antigens which elicit short-term antibody responses that can serve as a proxy for recent transmission.

An important aim of this work is to identify high-throughput methods to determine areas that are most susceptible to re-establishment of transmission. Work is also ongoing to identify appropriate sampling methods, as well as determining the distribution of emerging *Plasmodium* species, such as *P. knowlesi*.

Other key surveillance work within the Malaria Centre includes the identification of sub-standard antimalarial medicines across sub-Saharan Africa and South East Asia (an area where *Plasmodium* resistance to Artemisinin combination therapies has been reported), as well as the development of tools to quickly and simply identify key inherited blood disorders such as HbC, HbS and G6PD which are associated with severity of disease, or play an important role in determining treatment for disease.

Surveillance in very low transmission settings or post-elimination remains a key area of research and projects within the Malaria Centre are focussed on establishing methods to optimise data collection for confirmation of elimination, through identifying the relationship between passively collected data (which is often routine in pre-elimination countries) and community collected data (which can be logistically difficult to conduct), as well as using methods established in veterinary science to establish certainty around measures of zero to help inform whether elimination has been achieved (see elimination section for details). In addition, projects utilising alternative sampling strategies, such as easy access groups i.e. school children, or health facility attendees- can provide evidence for whether these surveys can contribute useful information on malaria risk in the community without the need for logistically complicated surveys. In addition, integrating novel ways to map where cases are occurring within the community can serve as a proxy for more logistically complicated surveys.

Antimalarial antibody detection assays: in search of a standardised tool to detect the absence of transmission

Location of study: Bataan, The Philippines; Praia, Cape Verde & LSHTM, UK

LSHTM Investigators: Lotus van den Hoogen, Nuno Sépulveda, Gillian Stresman, Kimberley Fornace, Kevin Tetteh & Chris Drakeley

External collaborators: Paolo Bareng, Ralph Reyes, Jennifer Luchavez, Malou Macalinao and Fe E. Espino (Research Institute for Tropical Medicine, Department of Health, the Philippines); Julio Rodrigues and Joana Alves (National Institute of Public Health, Cabo Verde); Alan Kitchen, (NHS Blood and Transplant, UK); Peter Chiodini, (Hospital for Tropical Diseases, UK)

Funding Body: Bill & Melinda Gates Foundation

The lack of antibody responses to malaria in young children signifies the absence of exposure to infections. As such, antibody detection may help in declaring an area malaria-free. At present there are no standardised assays to measure malaria antibodies for epidemiological use. There are several commercial assays but these have been developed to screen blood donations prior to transfusion. We aimed to evaluate the performance of commercially available assays for epidemiological use.

We compared five commercially available ELISA antibody detection assays for malaria. For Phase I, we assessed the ease-of-use, cost, sample volume needed and specificity (using malaria-naïve; n=223 as well as toxoplasma positive, malaria-naïve samples; n=191). Based on these results, one assay was taken forward for epidemiological evaluation.

For Phase II, samples from Bataan, the Philippines were assayed (n=1824; an area without clinically reported cases at local health facilities since the early 90s) as well as Praia, Cape Verde (n=1396; unstable, low transmission over the past decades). Preliminary results from Bataan show a change-point in the force of infection (SCR: seroconversion rate), which overlapped with the recorded drop in malaria cases. When a more stringent threshold for seropositivity was used, only four children under the age of 16 were seropositive while the recent SCR was 0.0001 (95% confidence interval, 0.0000-0.0004), Figure 1.

Whether the four detected seropositive children indicate responses from asymptomatic infections, outliers of seronegative responses, or cross-reactive responses to antigens from other pathogens is unknown. These results are promising in the development of standardised serological assays as adjunct measures to confirm claims of cessation of malaria transmission at a sub-regional level.

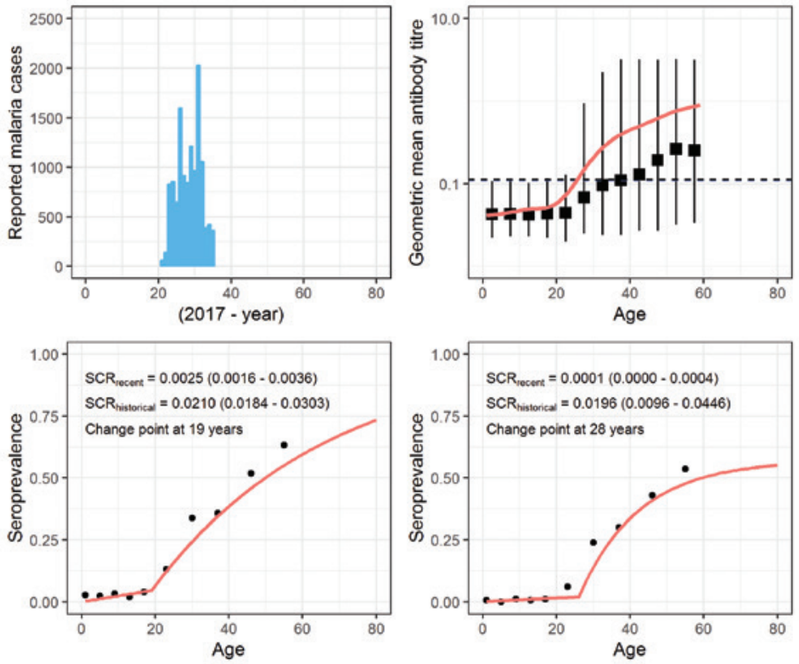


Figure 1: Malaria case counts and serological results from Bataan, the Philippines. Top left: reported malaria cases at local health facilities in Bataan, data available from 1982-1996, subsequently, unpublished observations suggest no cases detected. Top right: geometric mean antibody titre (optical density) over age groups per 5th percentile for ages 0 to 60 years old; the red line indicates the mean antibody response by age as described by Arnold et al. in PLoS NTD (2017). Bottom plots represent seroconversion curves of seroprevalence by age; the red line represents the fit of the reversible catalytic model (Corran et al. Trends in Parasitology, 2007) while black dots represent observed seroprevalence estimates. Thresholds for seropositivity were calculated from the mean of the lower distribution of antibody responses plus 3 (left) or 5 (right) standard deviations using a finite mixture model. SCR: seroconversion rate.

Malaria Zero: The Alliance for A Malaria-Free Haiti. Convenience sampling vs. household survey gold standard: assessment of spatial bias in predicted malaria exposure

Location of study: Haiti

LSHTM Investigators: Chris Drakeley, Gillian Stresman, Lotus van den Hoogen, Kevin Tetteh, Nuno Sépulveda

External collaborators: Katherine Battle and Ewan Cameron, (Malaria Atlas Project, University of Oxford, UK); Thomas Eisele, Ruth Ashton and Thomas Druetz, (Center for Applied Malaria Research and Evaluation, Tulane University, US); Michelle Chang and Karen Hamre, (Centers for Disease Control and Prevention, Atlanta, US); Bernadette Fouche, (CDC Foundation, Port-au-Prince, Haiti); Jean Frantz Lemoine, (Programme National de Controle de la Malaria, Port-au-Prince, Haiti)

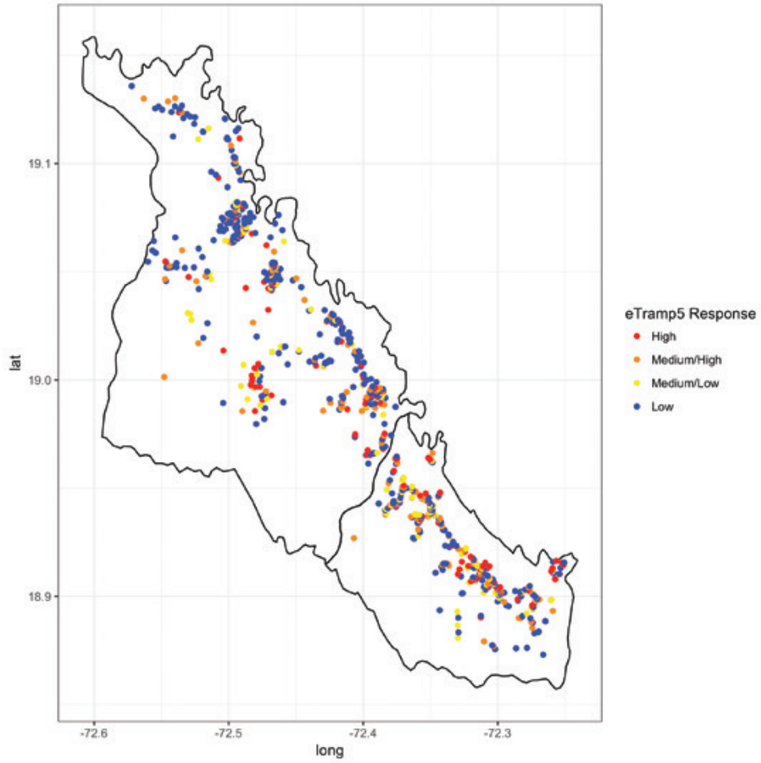
Funding Body: Bill & Melinda Gates Foundation

Maps showing the distribution of malaria are becoming an important tool for control and elimination activities. Here, we are determining if maps generated from data collected using convenient sampling methods can identify the same areas of high malaria exposure compared to the more accurate but labour intensive community-based data collection.

Convenience sampling in easy access groups (EAG) to measure malaria burden provides an attractive alternative to household (HH) surveys. Malaria seroprevalence estimates generated by sampling school-children as well as all health facility attendees have both been shown to provide reasonable estimates of malaria transmission in the community. Here, we assess the degree of spatial bias when generating maps of malaria seroprevalence according to the EAG sample compared to the gold standard HH survey conducted in the Artibonite Valley, Haiti.

For the EAG study, 2126 children in 22 schools and 2108 individuals attending 9 health facilities were sampled with spatial coordinates of the household available for 1092 participants. EAG seroprevalence were compared to fitted estimates according to a geostatistical model according to data from a HH survey of 21620 individuals residing in 6847 households.

Preliminary results suggest that estimates from the EAG study overall underestimated seroprevalence, with a smaller degree of bias in the health facility sample. However, the maps generated from the EAG data were able to identify similar areas of high burden as the HH survey in areas where the EAG and HH survey data overlapped spatially.



Map of intensity of responses to eTramp5, a marker associated with recent exposure to malaria. Each point is the location of participants sampled as part of the Easy Access Group surveys in primary school and health facilities in the Artibonite Valley, Haiti

Bias in routinely collected *Plasmodium falciparum* malaria surveillance data due to asymptomatic infections according to transmission intensity: A pooled analysis of paired health system and community cross-sectional survey data

Location of study: Worldwide (Brazil, Cambodia, Ethiopia, The Gambia, Haiti, Kenya, Malaysia, Myanmar, Peru, Philippines, Tanzania, Zambia)

LSHTM Investigators: Gillian Stresman, Nuno Sepulveda, Chris Drakeley, Kimberly Fornace, Shunmay Yeung, Lynn Grignard (LSHTM, UK); Umberto D'Alessandro, Julia Mwesigwa, Jane Achan (MRC Unit The Gambia)

External collaborators: Katherine Battle , Ewan Cameron and Peter Gething, (Malaria Atlas Project, University of Oxford, UK); Andre Siqueira, (Fundacao Oswaldo Cruz, Rio de Janeiro, Brazil); Emilie Poithin and Joanna Gallay, (Swiss Tropical and Public Health Institute, Basel, Switzerland); Siv Sovannaroth, (NMCP, Phnom Penh, Cambodia); Jennifer Stevenson and Antonio Quispe, (Johns Hopkins Bloomberg School of Public Health, Baltimore, US); Teun Bousema and Fitsum Tadesse, (Radboud University Medical Center, Nijmegen, The Netherlands); Effie Espino, Joy Lorenzo and Malou Macalinao, (Research Institute for Tropical Medicine, Manilla, Philippines); Jordi Landier, Gilles Delmas and Francois Nosten, (MAHIDOL Oxford Tropical Medicine Research Unit, Bangkok, Thailand); Jacklin Mosha, (NIMR, Mwanza, Tanzania); Thomas Eisele, (Center for Applied Malaria Research and Evaluation, Tulane University, US); John Miller and Daniel Bridges, (PATH, Lusaka, Zambia); Michelle Chang and Karen Hamre, (Centers for Disease Control and Prevention, Atlanta, US); Alyssa Young, (Clinton Health Access Initiative, Port-au-Prince, Haiti); Jean Frantz Lemoine, (Programme National de Controle de la Malaria, Port-au-Prince, Haiti)

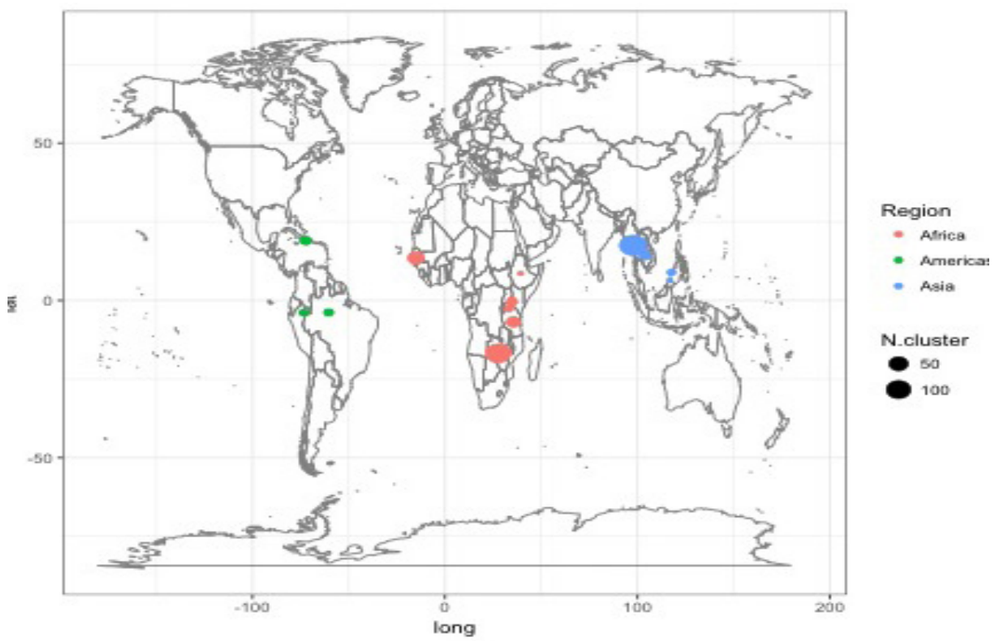
Funding Body: Wellcome Trust

Health systems data is the foundation information that malaria programs use to estimate malaria burden and inform control/elimination programming. However, the proportion of infected individuals that do not seek care, and thus not included in these statistics, can be significant. This work seeks to understand how well health systems data can mirror the total burden of infection, and how this changes according to transmission intensity.

The aim was to determine the relationship between the proportion of all infections in the community that are identified within the health system and transmission intensity, as a proxy for levels of protective immunity in the community.

Paired community and health system data, concordant in both time and space, were collected from 435 clusters in 12 countries. The proportion of infections detected in the health system was estimated according to the number of infections detected at the facility using a binomial distribution and the total number of infections in the community according to a hypergeometric distribution. Uncertainty was determined by bootstrapping. The proportion detected was modelled according to transmission intensity, measured by overall population PCR prevalence.

Preliminary results indicate the proportion of infections detected by the health system starts to increase when overall malaria prevalence in the community is less than ~10%. Facilities detect up to 100% of infections in non-African settings when the probability of seeking care and bednet use are adjusted for. In African settings, the proportion of infections detected gradually increases linearly. These findings suggest that once transmission is sufficiently low, health systems can be relied upon to detect most malaria infections in a community.



Map showing the distribution of study sites included in the analysis, coloured by geographical region. The size of the point reflects how many clusters were available from that site.

Evaluating the effectiveness of mass drug administration in The Gambia using novel serological markers of malaria exposure as a measure of transmission intensity

Location of study: The Gambia

LSHTM Investigators: Lindsey Wu, Kevin Tetteh, Immo Kleinschmidt, Chris Drakeley, Julia Mwesigwa, Jane Achan, Davis Nwakanma, Simon Correa, Mamadou Bah, Ahmad Abdullahi, Umberto D'Alessandro (Medical Research Council The Gambia at LSHTM)

External collaborators: Muna Affara (Bernhard Nocht Institute for Tropical Medicine, Tanzania)

Funding Body: UK Medical Research Council

While studies have identified novel serological markers highly correlated with recent malaria infection as potential measures of community-level changes in malaria transmission, limited studies have assessed their application in surveillance or cluster trial evaluation. This study compared antibody responses in villages receiving all-age mass drug administration (MDA) compared to villages receiving under-5 seasonal malaria chemoprevention (SMC) as part of the Malaria Transmission Dynamics Study in The Gambia.

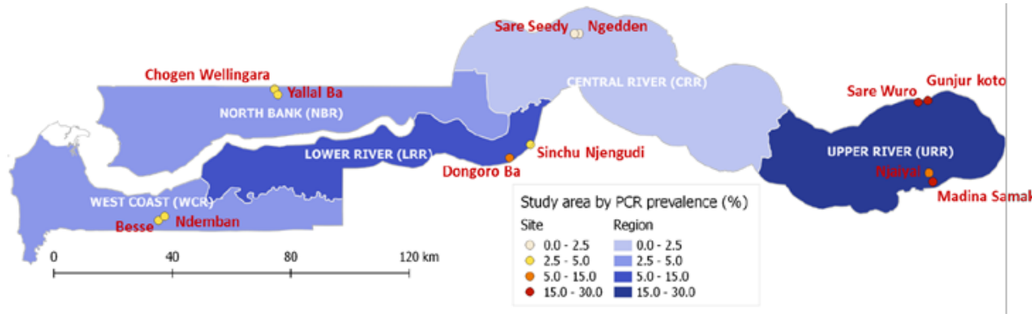
Antibody responses to a panel of over 20 *Plasmodium falciparum* antigens were quantified in two villages before (April-June) and after (December – January) receiving one round of all-age mass drug administration in 2014 in the Upper River Region South, The Gambia.

This was compared to antibody responses in control villages receiving only under-5 seasonal malaria chemoprevention (SMC) during the same period.

Compared to pre-transmission season (April – June), increases in antibody responses post-transmission season (December – January) were smaller in villages receiving MDA compared to villages receiving SMC alone. Change in under-15 seroprevalence was greater than 5% in SMC villages compared to MDA villages for Etramp5.Ag1, Rh2.2030, EBA175, GEXP18 and HSP40.Ag1, but remained below 5% in villages receiving MDA. Change in under-15 seroprevalence to longer-lived Abs to PfMSP119 and PfAMA1 was less than 5% in both MDA and SMC villages.

Villages receiving SMC alone experienced significant increases in post-transmission season age-adjusted Ab acquisition to several antigens, including Etramp5.Ag1 (p=0.039) and GEXP18 (p=0.048), while MDA villages did not experience significant increases over the same period.

New serological markers may have strong application in surveillance or cluster trials to measure short-term changes in malaria transmission. Future research will need to investigate the dynamics of village-level antibody responses for a greater duration to determine the long-term impact of MDA and quantify the relationship between serological, parasitological, and clinical study outcomes.



Study areas in the Malaria Transmission Dynamics Study by PCR prevalence (top) and sero-prevalence (bottom) comparing pre- and post-transmission season change in under-15 seroprevalence in villages receiving all-age mass drug administration vs. under-5 seasonal malaria chemoprevention in the Upper River Region South, The Gambia.

OPSIN: Optimising serological surveillance for malaria in Indonesia

Location of study: Indonesia

LSHTM Investigators: Henry Surendra, Chris Drakeley, Jackie Cook

External collaborators: Supargiyono & Riris Ahmad (Centre for Tropical Medicine, Faculty of Medicine, Public Health and Nursing, Gadjah Mada University, Indonesia)

Funding Body: Indonesia Endowment Fund for Education

This project explores the feasibility of optimising use of serological surveillance for estimating the magnitude and heterogeneity of malaria transmission in a pre-elimination setting in Indonesia. We integrate novel serological and geographical information system techniques with the existing public health surveillance system to better understand malaria transmission dynamics in this setting.

Sample collection

We found that rolling health facility-based cross-sectional surveys involving all facility attendees and their companions provide an alternative approach for quickly obtaining parasitological, serological, geolocation and epidemiological data. Simple addition of the use of mobile technology-based participatory mapping approaches into the surveys could provide attractive approach for remotely collecting accurate fine-scale geolocation data to study spatial pattern of disease in low resource areas without formal addresses and poor internet connection. Although the parasite

Data collection and participatory mapping

prevalence was very low (5/10,000), analysis of serological samples against a panel of novel antigens could facilitate the measurement of recent and historical exposure to *P. falciparum, P. vivax* and *P. knowlesi* as well as the characterisation of the spatial patterns and risk factors of malaria exposure in a pre-elimination setting in Indonesia. In conclusion, use of serological surveillance could be optimised by utilising existing surveillance system to monitor disease transmission dynamics for control and elimination programmes planning. However, further implementation research is needed to enable integration of these methods with existing surveillance systems.

Luminex machine established at collaborator lab at Gadjah Mada University, Indonesia

Using mobile technology-based participatory mapping approaches to geolocate health facility attendees

Location of study: Philippines and Indonesia

LSHTM Investigators: Kimberly Fornace, Henry Surendra, Gillian Stresman, Jackie Cook, Chris Drakeley

External collaborators: Tommy Abidin (Universiti Sabah Malaysia, Malaysia); Ralph Reyes, Maria Macalinao, Jennifer Luchavez, Fe Espino (Research Institute for Tropical Medicine, Philippines); Riris Ahmad, Supargiyono (Universitas Gadjah Mada, Indonesia)

Funding Body: Newton Fund, Philippine Council for Health Research and Development and UK Medical Research Council. Indonesia Endowment Fund for Education

Understanding the spatial distribution of disease is critical for planning disease control and elimination programmes. However, collecting spatial information is challenging in areas with no addresses or internet access. We piloted the use of tablet-based applications with offline maps to geolocate malaria patients and health facility attendees in rural areas of the Philippines and Indonesia.

Using the Open Data Kit GeoODK, we collected information for health facility attendees in 603 households. Local health facility workers were trained on the use of tablets and maps in addition to sample collection procedures to screen for malaria infection and exposure. Most health workers were able to use the tablets effectively to collect routine questionnaire data in addition to spatial data on household locations. Accuracy of the reported locations varied between health facilities and decreased in less populated areas with fewer landmarks. This tablet-based application is currently being used in rolling cross sectional surveys to collect data on health facility attendees in the Philippines and Indonesia to develop risk maps for malaria infection and exposure and identify environmental risk factors for disease.

Using mobile technology-based participatory mapping approaches to geolocate health facility attendees

Vector mapping on the Bijagós archipelago of Guinea-Bissau

Location of study: Bijagos Islands, Guinea-Bissau (study site), MRC The Gambia, LSHTM

LSHTM Investigators: Anna Last, James Logan, Thomas Ant, David Mabey (LSHTM, UK); Umberto D'Alessandro (MRC Unit The Gambia)

Funding Body: UK Medical Research Council

The islands of the remote Bijagós archipelago off the coast of Guinea-Bissau are endemic for a number of infectious diseases, including malaria. However, the vector species composition, bionomics and insecticide resistance status of the islands are poorly characterised. We have performed a baseline vector mapping and insecticide resistance survey across the major inhabited islands of the archipelago, as part of a broader project: Mapping NTDs and Malaria on the Bijagos archipelago of Guinea-Bissau.

The islands maintained majority *An. melas* populations in the dry season, with a strong shift towards *An. gambiae s.s., An. coluzzii,* and hybrids during the wet season. The high seasonality of *An. gambiae* and *An. coluzzii* is consistent with a dependence on fresh-water breeding sites, whereas the salt-tolerant *An. melas* can be maintained throughout the dry season in the flooded alluvial plains surrounding the many mangrove swamps.

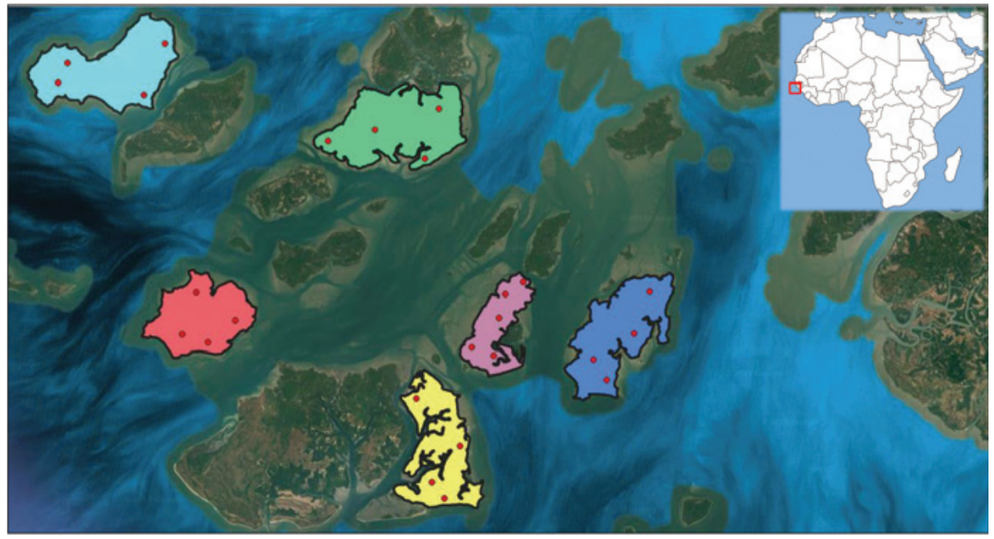
Sporozoites were found infecting *An. melas* during the dry season, suggesting a degree of perennial malaria transmission across the archipelago. In the wet season *An. gambiae s.s.* was found to be the major malaria vector.

The West African variant of the *kdr* allele was found in both *An. gambiae s.s* and *An. coluzzii.* Field collected *An. gambiae s.l.* were fully susceptible to a discriminating dose of permethrin, but we found low levels of resistance to α-cypermethrin. Susceptibility was restored upon pre-exposure to the synergist piperonyl butoxide.

The work described here is a prelude to an island-randomised ivermectin systemic insecticide MDA trial trial that started in September 2019.

Hanging of a CDC light trap used to collect Anopheles mosquitoes.

Sampling of mosquito larvae in a water pool.



Map showing Bijagos islands mapped during survey. Red dots show main sampling sites.

Can dogs identify people with malaria parasites? A proof-of-principle study

Location of study: United Kingdom, The Gambia

LSHTM Investigators: James Logan, Sarah Dewhirst, Chelci Squires

External collaborators: Steven Lindsay (Durham University, UK); Umberto D'Alessandro & Margaret Pinder (MRC Unit The Gambia at the LSHTM); B. Kandeh (National Malaria Control Programme, Banjul, The Gambia); Jenny Corish, Clare Guest & Mark Doggett, (Medical Detection Dogs, UK); S. Morant, (Medicines Monitoring Unit, University of Dundee, UK)

Funding Body: Bill & Melinda Gates Foundation

The task of malaria elimination would be simpler if a non-invasive method was available for detecting infected individuals in populations where the number of malaria cases is low; infected individuals could then be treated with antimalarials. Dogs have a highly developed sense of smell and may be able to detect volatiles released from people carrying malaria parasites. We carried out a proof-of-principle study to determine whether trained dogs could detect malaria infections in Gambian children aged 5-13 years old.

Why was this study done?

- The World Health Organization has a vision of a malaria-free world. Since 2000 *eight countries have been certified as free from malaria, with a further 12 reporting no indigenous cases. Once a country or region has been declared malaria-free detecting individuals with asymptomatic infections is critical so they can be treated with antimalarials and the transmission of the disease prevented.
- Ideally a non-invasive diagnostic device that can detect malaria-infected subjects would be an advance, helping to keep the disease out of malaria-free countries.
- In The Gambia, West Africa, the number of malaria infections has declined substantially since 2000 and provides an ideal site for detecting malaria at low frequencies.
- We tested the hypothesis that dogs, with their powerful sense of smell, could be trained to distinguish between individuals with and without *Plasmodium falciparum* malaria.

What did the researchers do and find?

- Gambian schoolchildren were screened to detect malaria parasites in their blood to determine those carrying parasites and those without.
- We provided socks for the schoolchildren to wear overnight in order to collect samples of their foot odours.
- Two dogs were trained to distinguish between sock samples from malaria-infected and uninfected individuals tested in a double blind fashion.
- Two dogs were able to identify strips of socks from malaria-infected and uninfected individuals with a sensitivity of 70% and 73% and a specificity of 90% and 91%.

What do these findings mean?

- Dogs can identify malaria-infected individuals by their odour with a credible degree of accuracy.
- In the future artificial odour sensors may be able to detect malaria parasites.
- Until then, trained dogs may be useful at ports of entry to detect asymptomatic malaria carriers entering malaria-free countries.
- Further studies are needed to detect *falciparum* malaria in people from different parts of the world before medical detection dogs can be used in the field.

Surveillance approaches to detect the quality of medicines in low-middle income countries with a focus on artemisinin combination therapies for malaria

Location of study: Senegal

LSHTM Investigators: Mirza Lalani, Sian Clarke, Jayne Webster and Harparkash Kaur

External collaborators: Aminata Dior Ndiaye, UCAD; Alioune Badara Ly, (Ministry of Health; Adama Diedhou, Laboratoire Mational de Contrôle des Médicaments; Badara Cisse, UCAD)

Funding Body: John Henry Memorial Fund, Leverhulme Charities Trade Trust

Several risk factors may contribute to the circulation of poor quality medicines. This study explored these risk factors with an overall aim of providing evidence to strengthen medicines quality surveillance systems (MQSS) in low-middle income countries (LMICs) using Senegal as a case study for transferable learning.

Data collection was conducted in two phases in Senegal. The first phase involved interviews with key stakeholders of the MQSS to explore the system's vulnerability to risk factors for poor quality medicines and their perceptions of the quality of medicines available in Senegal. The second phase comprised a series of laboratory-based studies with technicians at the national medicine quality control laboratory (MQCL) including an assessment ofa new rapid chromatographic test to check the quality of artemisinin based medicines - the artemisinin-derivative test (ADT).

We also carried out a systematic literature review to assess the study design and reporting of quality surveys to date, and evaluated the ability of these studies to generate reliable data against our newly proposed list of criteria.

Overall, interviewees expressed confidence in the quality of medicines available in the public and regulated private sectors in Senegal, which was attributed to effective national medicines regulation and adequate technical capacity at the MQCL. In contrast, poor quality medicines were thought to be available in the unregulated (informal) sector as medicines sold in informal markets were not subjected to national regulatory processes or stored appropriately. The ADT demonstrated a promising level of accuracy to detect fake or grossly substandard artemisinin based medicines and laboratory technicians favoured its simplicity of use. The literature review found that there is much heterogeneity in study design and inconsistency in reporting in past studies, which has impacted on the generalisability of findings for antimalarial medicine quality studies.

National governments need to invest in regulatory and technical capacity to strengthen MQSS to minimise the likelihood of poor quality medicines circulating in a country. Utilising simple, and portable (preferably handheld) tests like the ADT, in non-laboratory settings may enhance post-marketing surveillance, especially in resource constrained contexts. Medicine quality studies require standardisation of study design and reporting, thereby increasing the reliability of findings and allowing comparison between studies.

Medical Detection Dog, Lexi, sniffing skin odour loaded socks during testing

Social, Economic and Policy Research

Economic and social research helps inform evidence-based decision making and allows research methods to be adapted, based on epidemiological settings. This approach is utilised by researchers at the Malaria Centre to reduce both the burden and rise in antimalarial and insecticide resistance around the world. The evidence-base is also useful from a political and health care context, whereby researchers have recognised the role of the private sector in the strive towards malaria elimination. Finally, looking through economic and social lens has birthed novel technologies (PBO nets) and novel approaches, discussed further in this section.

To help address questions on how best to achieve malaria elimination in low transmission areas in Africa and in Southeast Asia, different strategies are being deployed. For example, Malaria Centre researchers are conducting economic evaluations, alongside trials of targeted community-based antimalarial drug administration, in The Gambia. Further, on the Cambodia-Thai border, sensitive diagnostic tests are being used for active case detection whilst concurrently, an ethnographic study is implemented to try to get a better understanding of the hard-to-reach at-risk population – adults who go to work deep in the forest. An ethnographic approach is also being used in Uganda, to explore the 'how' and 'why' of antimalarial and other antimicrobial usage in everyday life.

With a focus on vector control, researchers at the Centre have carried out economic evaluations of targeted reactive indoor residual spraying (IRS), compared to generalised IRS in South Africa. Further research is underway looking at the addition of IRS to case management in refugee settlements in Pakistan and as part of a private sector malaria prevention programme in Ghana. Likewise, economic evaluation of PBO long-lasting insecticide treated nets in Tanzania has been crucial to influencing WHO policy. The acceptability and feasibility of spatial repellents in Cambodia is also covered in this section.

Finally, in terms of case management, there is increasing recognition of the private for-profit sector as a source of antimalarial treatment. Centre members work on a range of health systems and economic studies, including exploring the role they play in Uganda and modelling cost-effectiveness of introducing rapid diagnostic tests (RDTs) in the private sector in Sub-Saharan Africa.

It is crucial that economic evaluation informs malaria research to ensure the market is functioning optimally, thus remaining as affordable as possible for the affected population and local governments. This will in turn prevent exploitative practices from developing during administering of treatment and practical supplies, such as nets and insecticide.

Effectiveness and cost-effectiveness of targeted reactive IRS compared with generalised routine IRS – results from a cluster randomised trial

Location of study: South Africa

LSHTM Investigators: Immo Kleinschmidt, Jackie Cook, David Bath, Catherine Pitt, Joseph Biggs

External collaborators: Maureen Coetzee (Wits Research Institute for Malaria, University of the Witwatersrand, South Africa); Natashia Morris, Rajendra Maharaj (South African Medical Research Council, South Africa); Aaron Mabuza (Mpumalanga Provincial Malaria Control Programme, South Africa)

Funding Body: Joint Global Health Trials (MRC/DFID/Wellcome)

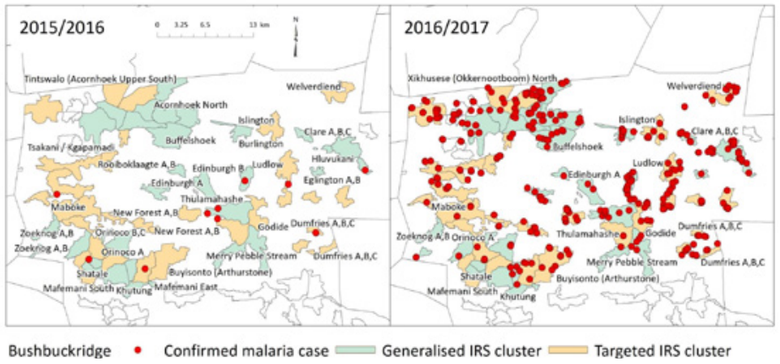
In areas of very low, pre-elimination malaria transmission the low number of incident cases may call into question the resources needed for spraying all houses annually with insecticide (generalised indoor residual spraying (GIRS)). An alternative that may be more cost-effective is to target IRS, reactively spraying only houses in the immediate neighbourhood of incident cases as they arise.

62 clusters consisting of settlements of approximately 5000 persons each in the provinces of Mpumalanga and Limpopo in South Africa, were randomly allocated to receive the currently practised GIRS whilst the other half received reactive targeted IRS (TIRS) in the immediate neighbourhood (about 8 houses) of every passively reported local case. Non-inferiority of TIRS was assessed as passive incidence of no more than 1 case/1000 per year above that of the GIRS arm. Financial and economic costs of IRS implementation, treatment costs of malaria cases, and associated patient costs were calculated using data from the trial, provincial MCPs, and secondary sources.

Incidence in the targeted IRS was higher than in the generalised IRS arm by 0.29 cases per 1000 (95% CI -0.53-1.10). The total cost of TIRS was around 40% of the cost of GIRS, due to reductions in insecticide and contracted sprayers. The mean incremental cost of GIRS relative to TIRS was $75,000 per local malaria case averted in the lower transmission season and $5,000 per local malaria case averted in the higher transmission season, neither of which are cost-effective.

Although evidence of non-inferiority of TIRS compared to GIRS was weak, TIRS appears to be a safe, more sustainable method of deploying IRS in areas of very low transmission.

The resources saved could be redeployed more effectively and cost-effectively, for example, in improved surveillance, promotion of care seeking, and improved case management.



Map of study clusters and confirmed local malaria cases by year (Bushbuckridge)

Understanding how and why antimicrobials are deployed in everyday life in Uganda: an ethnographic study of lives, livestock and livelihoods in Tororo, Uganda

Location of study: Tororo, Uganda, East Africa

LSHTM Investigators: Susan Nayiga, Dr. Clare Chandler, Dr. Laurie Denyer Willis, Prof. Sarah Staedke

Funding Body: The Antimicrobials in Society (AMIS) Project Uganda funded by the Antimicrobial Resistance Cross Council Initiative supported by the seven research councils in partnership with other funders.

This research is investigating the role of antimicrobials including antimalarials in everyday day life with a focus on a rural setting in comparison with data from urban and peri-urban settings. The research is focusing on societal rather than individual behavioural factors that shape the ways antimicrobials are deployed in Ugandan society today.

Antimicrobial resistance (AMR) is a threat to public health worldwide. To minimize the spread of AMR, policy makers have emphasized the need to reduce the use of antimalarials and antibiotics, but this is a challenging task.

In Uganda, antimicrobial use including use of antimalarials appears to be increasing, yet there is limited evidence on this phenomenon in the rural settings. This research is investigating the role of antimicrobials in everyday day life with a focus on a rural setting. Results will be compared with data from urban and peri-urban settings that is currently ongoing. The research is examining the wider social, economic and political factors that shape the ways antimicrobials are deployed in Ugandan society today. The research is employing ethnographic methods, involving extended fieldwork with participant observations and interviews conducted in Tororo district, eastern Uganda over a period of 15 months. It is primarily focusing on residents of the area and their health care providers in their everyday life, and the ways in which they use these medicines to care for households including humans, animals and crops. By moving beyond looking at individual behavior, this research will provide new insights to guide approaches to reduce reliance on these medicines at the societal level.

Economic Evaluation of the Private Sector Malaria Prevention (PSMP) Project in Ghana

Location of study: Ghana

LSHTM Investigators: Lucy Paintain, Jayne Webster

External collaborators: Matt Lynch, Kathryn Bertram, Danielle Piccinini (Johns Hopkins Center for Communications Programs, Baltimore, US); Felix Nyanor-Fosu (Johns Hopkins Center for Communications Programs, Accra, Ghana)

Funding Body: DfID Ghana

PSMP is a three-year pilot project that catalyses the private sector in Ghana in support of increased domestic resources for malaria prevention and the development of a long lasting insecticidal net (LLIN) market. LSHTM is conducting an economic evaluation of PSMP to understand intervention costs and broader lessons for other settings.

The PSMP project in Ghana catalyses the private sector through three project components, all of which link, in support of increased resources for malaria and the development of an LLIN market: (i) retail sales of LLINs; (ii) workplace programs that support institutional purchases of LLINs; and (iii) advocacy, which cuts across both (i) and (ii) and includes resource mobilization support to the national malaria control programme.

After years of global free distribution of LLINs, ownership of LLINs has rapidly increased, bringing major benefits to millions of families now protected from malaria. However, with uncertainties in the future funding landscape, there is strong international interest in finding effective methods to mobilise domestic resources for malaria control. Thus evaluation of the PSMP project, including a detailed costing analysis, will be valuable.

LSHTM is conducting a thorough financial and economic costing analysis of PSMP, producing unit costs per LLIN delivered as a result of the project and exploring the main cost drivers. Interviews with stakeholders in the private and public sectors will provide further understanding of the sustainability of the approach and opportunities for lessons learned to be applied elsewhere.

Cost-effectiveness of a novel long-lasting insecticidal net against malaria in the context of emerging pyrethroid-resistant mosquitos

Location of study: Muleba district, Kagera Region, Tanzania

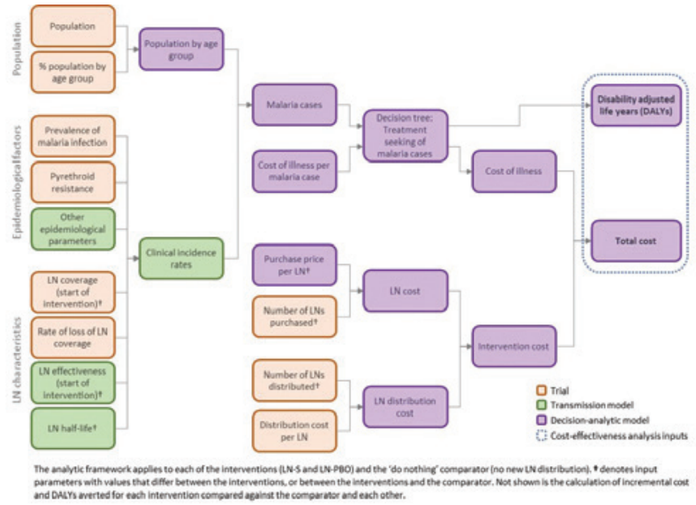
LSHTM Investigators: David Bath, Tom Drake, Catherine Pitt, Stacie Gobin, Andreia Santos, Natacha Protopopoff, Mark Rowland

External collaborators: Tom Churcher and Ellie Sherrard-Smith, (Imperial College London, UK); Franklin W Mosha, Jacklin Mosha and Alphaxard Manjurano, (Kilimanjaro Christian Medical University College, Tanzania)

Funding Body: Global Health trial DFID/MRC/NIHR/Wellcome Trust

We conducted an economic evaluation alongside a cluster randomised controlled trial in Muleba district, Tanzania, to assess the cost and cost-effectiveness of a new type of long-lasting insecticidal net, compared against either standard net or no new net distribution.

We conducted an economic evaluation alongside a cluster-randomised controlled trial of a new type of long-lasting insecticidal net, which incorporates the synergist PBO (Olyset Plus), compared against a standard net treated with pyrethroid only (Olyset Net). The trial was conducted in Muleba district, Tanzania – an area of high resistance to pyrethroids– and provided evidence of the effectiveness of the PBO net in reducing malaria infection prevalence.



Analytic framework for cost-effectiveness analysis of a novel long-lasting insecticidal net in a context of pyrethroid resistance

We estimated the costs and cost-effectiveness of the PBO nets, standard nets, and a hypothetical 'no net distribution' comparator over a three-year time horizon. To estimate clinical malaria incidence associated with each of the three alternative interventions, we used a pre-validated mathematical model of P. falciparum transmission. The transmission model was fitted to trial estimates of baseline malaria prevalence, baseline pyrethroid resistance, and net coverage. These outputs were combined with cost estimates in a decision-analytic model.

Preliminary results indicate that PBO nets are highly cost-effective in the Muleba context, due to lower health care costs associated with a reduction in clinical malaria cases. Results appear robust in both deterministic and probabilistic sensitivity analyses. This analysis will provide an important contribution to decision-making around the adoption of next generation nets, particularly in areas of high insecticide resistance.

Modelling the cost-effectiveness of introducing malaria rapid diagnostic tests in the private retail sector in sub-Saharan Africa

Location of study: Africa

LSHTM Investigators: David Bath, Catherine Goodman, Shunmay Yeung

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

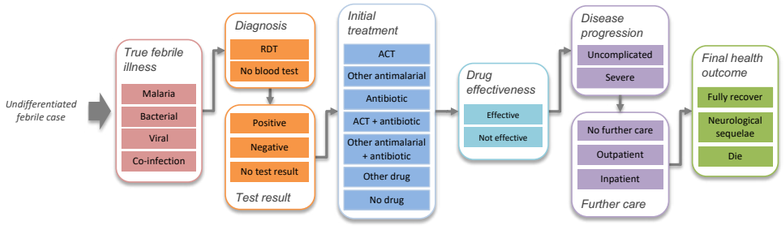
There are increasing calls for rapid diagnostic tests (RDTs) to be made available in the private retail sector where many people with suspected malaria seek care. We developed a decision tree model to identify the conditions under which RDTs would be cost-effective in a sub-Saharan African private retail setting.

Over the last five to ten years, major changes have occurred in the diagnosis of malaria in public health facilities in endemic countries, due in part to the increased availability and affordability of rapid diagnostic tests (RDTs). There are increasing calls for RDTs to be made available at-scale in the private sector as well –particularly in the private retail sector where a high proportion of people with suspected malaria seek care. Private sector RDT introduction has already begun in several sub-Saharan African (SSA) countries, although robust evidence on value for money is not yet available.

Drawing on recent data, we developed a cost-effectiveness decision tree model of management of febrile illness in SSA, which follows febrile patients from initial presentation at a private retail outlet to their final health outcome. We modelled three different 'treatment scenarios' using empirical data from three different private retail sector studies, which reported very different findings in terms of the impact that RDT introduction had on the likelihood of receiving antimalarial or antibiotic treatment.

The cost-effectiveness of introducing RDTs differed greatly across the three scenarios, and was particularly influenced by the likelihood that a case with a positive test result received an antimalarial. RDTs were also more cost-effective in higher transmission settings where a greater proportion of cases have malaria, as they led to an overall increase in the proportion of cases receiving antimalarial treatment. This is in contrast to previous evidence of public sector RDT introduction, where antimalarial use was already high and cost-effectiveness was driven instead by reducing antimalarial over-prescription in lower transmission settings.

This analysis contributes to our understanding of the epidemiological and health system settings in which private sector RDT introduction should be considered, and factors likely to influence cost-effectiveness.



Modelling the cost-effectiveness of introducing malaria rapid diagnostic tests in the private retail sector in sub-Saharan Africa

Forest goers and drug resistant malaria in Cambodia: An ethnographic study

Location of study: Oddor Meanchey Province, Cambodia

LSHTM Investigators: Shunmay Yeung , Nicola James

External collaborators: Melanie Bannister-Tyrrell, Charlotte Gryseels and Koen Peeters Grietens, (Institute of Tropical Medicine, Antwerp, Belgium); Suon Sokha, Lim Dara, Noan Sereiboth and Kheang Soy Ty, (Health and Social Development, Phnom Penh, Cambodia); Boukheng Thavrin, Po Ly and Siv Sovannaroth, (National Center for Parasitology, Entomology and Malaria Control, Phnom Penh, Cambodia)

Funding Body: UK Department for International Development (DfID) through the Tracking Resistance to Artemisinins Collaboration

Multidrug resistant *Plasmodium falciparum* malaria on the Cambodia-Thailand border is associated working in forested areas. Beyond broad recognition of 'forest-going' as a risk factor for malaria, little is known about different forest-going populations in this region. In Oddar Meanchey province, north-western Cambodia, qualitative ethnographic research was conducted to gain an in-depth understanding of how different populations, mobility and livelihood patterns and activities within the forest intersect. This led us to understand malaria risk and the effectiveness of malaria control and elimination strategies.

We found that most forest-going in this area is associated with obtaining precious woods, particularly Siamese Rosewood. In the past, at-risk populations included large groups of temporary migrants. As timber supplies have declined, so have these large migrant groups. However, groups of local residents continue to go to the forest, and are staying for longer. Most forest-goers had experienced multiple episodes of malaria and were well-informed about malaria risk. However, economic realities mean that local residents continue to pursue forest-based livelihoods. Severe constraints of available vector control methods mean that forest goers have limited capacity to prevent vector exposure. As forest-goers access the forest using many different entry and exit points, border screening and treatment interventions will not be feasible. Once in the forest, groups often converge in the same areas, therefore interventions targeting the mosquito population may have a potential role. Ultimately, a multi-sectoral approach as well as innovative and flexible malaria control strategies will be required if malaria elimination efforts are to be successful.

Going towards the forested Cambodia-Thai border

The cost of active case detection for malaria elimination: findings from the PACES trial in Oddar Meanchey province, Cambodia

Location of study: Oddar Meanchey Province, Cambodia

LSHTM Investigators: Blandine Binachon, Shunmay Yeung, Nicola James, David Bath

External collaborators: Po Ly & Siv Sovannaroth (Cambodia National Center for Malaria, Parasitology and Entomology, Phnom Penh, Cambodia); Sok Panhary & Soy Ti (Health and Social Development, Phnom Penh, Cambodia)

Funding Body: UK Department for International Development through the Tracking Resistance to Artemisinins Collaboration

We analysed the costs of implementing an active case detection approach targeting malaria asymptomatic cases (human reservoir) in a low transmission setting. This intervention was delivered as part of the PACES trial, which aimed to generate evidence for the feasibility and implementation of this approach.

We analysed the total and unit costs of implementing different approaches to active case detection of asymptomatic malaria cases in Oddar Meanchey province, Cambodia – an area of low transmission. The analysis was part of the PACES trial, conducted in 2016-17, which implemented both proactive and reactive case detection methods.

Active case detection activities targeted high-risk forest goers, their households and neighbours, according to the predicted pathways of malaria transmission in the area. Reactive case detection was done upon reporting of a *P. falciparum* malaria 'index case' by a village malaria worker, as well as proactive case detection in villages suspected to have high *P. falciparum* incidence. High-risk individuals were screened for malaria with rapid diagnostic tests and PCR.

A 'top down' costing analysis was conducted, based on project accounts. Research costs were excluded, and costs allocated to reactive and proactive case detection activities. Part of the intervention preparation and delivery costs were adjusted to reflect actual costs of implementation in an operational setting. The main cost drivers were employment and laboratory costs (incurred by PCR analysis), comprising 46% and 14% of total costs, respectively. The average cost per investigation was higher for proactive compared with reactive investigations ($1,021 vs. $552), due to the more resource-intensive nature of proactive investigations. However, the average cost per positive malaria case detected was lower for proactive compared with reactive investigations ($243 vs. $634). The results of this analysis will help inform future case detection activities, to more effectively and efficiently target high-risk individuals.

PACES team member putting up posters encouraging people coming back from the forest to get tested for malaria

Field assessment of a novel spatial repellent for malaria control: a feasibility and acceptability study in Mondulkiri, Cambodia

Location of study: Mondulkiri, Cambodia

LSHTM Investigators: Shunmay Yeung, Marco Liverani, Harriet Lawford

External collaborators: Jacques Derek Charlwood, (Liverpool School of Tropical Medicine)

Funding Body: Department for International Development through the Tracking Resistance to Artemisinins Collaboration

In Southeast Asia malaria is transmitted by forest dwelling mosquitoes that prefer to rest and bite outdoors. Insecticide-treated nets and indoor residual spraying are therefore less effective than in Africa, and there is a need for new approaches such as spatial repellents. This study explored the explored the social and contextual factors that may influence the uptake and sustainable use of spatial repellents.

The cross-sectional study was conducted before and after the introduction of a spatial repellent in two villages. The repellent consisted of polyethylene emanators, held in an open plastic frame and impregnated with 10% metofluthrin.

In a baseline survey, data were collected for all household occupants in the two villages (n = 448) . Prior to the installation of the repellent, 50.6 and 59.5% of respondents noted that bites occurred “very often” inside the house and in the outdoor area surrounding the house, respectively. Indoor biting was reported to occur more frequently in the evening, followed by at night, while outdoor biting occurred more frequently in the early morning. In the follow-up survey, spatial repellents were well received in both villages with most participants (96.6%) reporting a willingness to use the product again; the mean willingness to pay was US$ 0.3 per unit.

If their entomological efficacy of spatial repellents can be ascertained, outdoor application has the potential to enhance vector control strategies in Cambodia. However further research is required. Successful implementation would require subsidisation and integration with the existing national malaria control strategy.

Economic evaluation of a reactive household-based self-administered treatment against residual malaria transmission in The Gambia (RHOST)

Location of study: North Bank East region of The Gambia

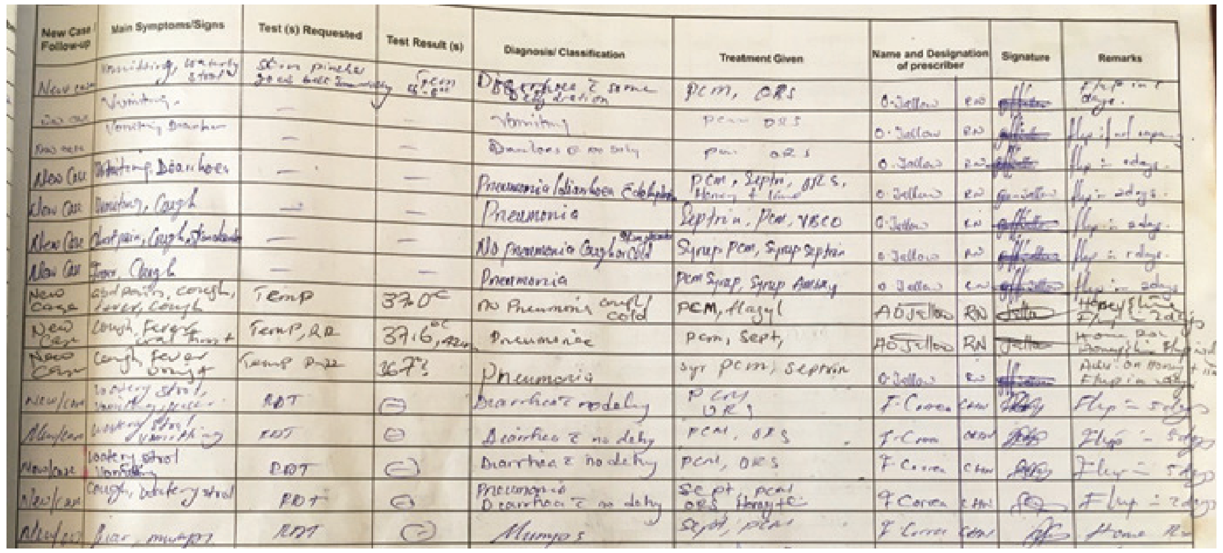
LSHTM Investigators: Shunmay Yeung, Marco Liverani, Harriet Lawford

External collaborators: Jacques Derek Charlwood, (Liverpool School of Tropical Medicine)

Funding Body: Joint Global Health Trials scheme (UK Department for International Development, Medical Research Council, Wellcome Trust)

As countries move towards malaria elimination, there is a need for sustainable interventions targeting the residual parasite transmission that is due to human reservoirs. We are conducting an economic evaluation of the he RHOST trial which aims to assess the effectiveness of treating all members of the compound of confirmed cases.

The RHOST cluster randomized trial has been implemented since 2016 in the North Bank East region of the country by the MRC unit The Gambia at the LSHTM. When a malaria case from one of the study intervention villages is detected, all individuals living in their compound are treated by the community health worker with dihydroartemisinin-piperaquine. The economic evaluation is conducted alongside the trial. We are collecting primary costs data from the trial. After analysing the financial and economic costs of delivering the intervention under trial conditions, we will estimate, through modelling, the resources needed for its implementation in operational circumstances in The Gambia. Preliminary results show that the main costs drivers of the intervention, are the field staff employment and transports costs. We are collecting more detailed data on personnel and transport time, in order to inform the allocation of resources to each set of activities. These data will be used to refine our assumptions regarding the exclusion of research costs and the integration of the RHOST approach in the Gambian national malaria elimination strategy.



Health centre register showing lots of negative RDT results

Cost-effectiveness of adding indoor residual spraying to case management in Afghan refugee settlements in Pakistan during a prolonged malaria epidemic

Location of study: Ghana

LSHTM Investigators: Natasha Howard, Mark Rowland

External collaborators: Naeem Durrani, (HNTPO Pakistan); Lorna Guinness, (Consultant, UK); Kristian Hansen, (U of Copenhagen, Denmark)

Funding Body: DFID

This retrospective study aimed to determine whether adding malaria prevention using targeted indoor spraying of insecticide to malaria case management using quality-assured microscopy and national first-line treatment was a better use of limited resources than case management alone during a five-year epidemic in Afghan refugee settlements in Pakistan. While our cost-effectiveness results were relatively high, when compared with internationally recognised cost-effectiveness thresholds both prevention and case management were highly cost-effective, indicating the relevance of an integrated approach for epidemic malaria control and global malaria elimination.

Financing of malaria control for displaced populations is limited in scope and duration, making cost-effectiveness analyses relevant but difficult. This study analyses cost-effectiveness of adding prevention through targeted indoor residual spraying (IRS) to case management in Afghan refugee settlements in Pakistan during a prolonged malaria epidemic.

Taking a societal perspective, provider and household costs of vector control and case management were collected from provider records and community survey. Health outcomes (e.g. cases and DALYs averted) were derived and incremental cost-effectiveness ratios (ICERs) for cases prevented and DALYs averted calculated. Population, treatment cost, women's time, days of productivity lost, case fatality rate, cases prevented, and DALY assumptions were tested in sensitivity analysis.

Malaria incidence peaked at 44/1,000 population in year 2, declining to 14/1,000 in year 5. In total, 370,000 malaria cases, 80% vivax, were diagnosed and treated and an estimated 67,988 vivax cases and 18,578 falciparum and mixed cases prevented. Mean annual programme cost per capita was US$0.56. The additional cost of including IRS over five years per case prevented was US$39; US$50 for *vivax* (US$43 in years 1-3, US$80 in years 4-5) and US$182 for *falciparum* (US$139 in years 1-3 and US$680 in years 4-5). Per DALY averted this was US$266 (US$220 in years 1-3 and US$486 in years 4-5) and thus ‘highly cost-effective’ or cost-effective using WHO and comparison thresholds.

Adding IRS was cost-effective in this moderate endemicity, low mortality setting. It was more cost-effective when transmission was highest, becoming less so as transmission reduced. Because *vivax* was three times more common than *falciparum* and the case fatality rate was low, cost-effectiveness estimations for cases prevented appear reliable and more definitive for *vivax* malaria.

Exploring the health system, regulation and drug shops in Uganda

Location of study: Luwero District, Uganda

LSHTM Investigators: Sian Clarke, Eleanor Hutchinson, Catherine Goodman, Heidi Hopkins

External collaborators: Anthony Mbonye, Esther Buregyeya, Elizeus Rutebemberwa & Phyllis Awor, (Makerere University School of Public Health, Uganda); Pascal Magnussen & Kristian Hansen, (University of Copenhagen, Denmark)

Funding Body: MRC/ESRC/DfID/Wellcome Trust: Development Grant (Health Systems Research)

Poor prescription practices by health workers, such as overuse of drugs, sale of partial doses, or non-adherence by patients to the full treatment course, all create situations which are conducive to the selection and spread of drug-resistant mutations. In order to develop effective strategies to combat resistance, good understanding of the factors that influence the prescribing practices of health workers is essential.

The private sector plays an important role in provision of health care in many countries and cannot be overlooked in strategies to control misuse of medicines. Neither can treatment practices and standards be addressed by focusing on one sector in isolation. The private sector interacts with, and is shaped by the organisation and performance of the public sector, demand from patients and regulatory controls. Poor practices in one sector can easily undermine or disincentivize behavioural change in another. Yet regulation of the private sector is an acknowledged weakness of the health system in many low income countries.

This study investigates the situations, norms, experiences, and motivations that affect health care practice in drug shops in rural Uganda, including the influence of interactions between private providers, government health workers and public health officials.

Malaria medicines on sale in a drug shop in Uganda

Data are collected through direct observations in drug shops, as well as interviews with drug shop vendors, local authorities and regulators.

Through understanding the factors that shape treatment practices among private providers, the study aims to generate novel insights on how health system levers could be used more effectively to combat irresponsible prescribing and use of medicines in drug shops, and improve the quality of care that patients receive.

LINK (LSHTM-INFORM-NMCP-Knowledge) Data for malaria decision-making

Location of study: Demographic Republic of the Congo (DRC), Ghana, Kenya, Malawi, Mali, Mozambique, Nigeria, Republic of South Sudan, Republic of Sudan, Senegal, Sierra Leone, Tanzania and Uganda.

LSHTM Investigators: Caroline Lynch, David Schellenberg

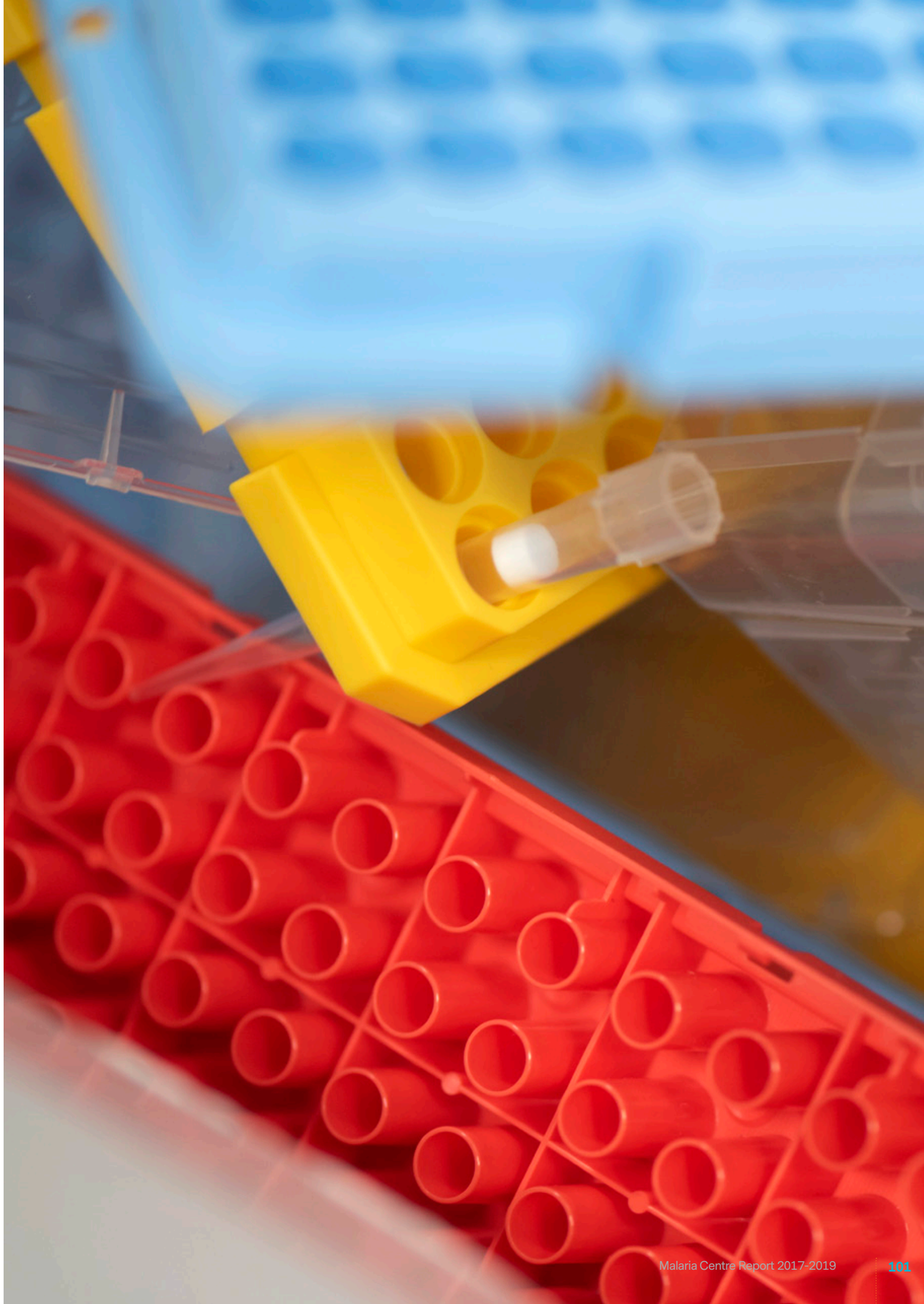
External collaborators: Robert Snow (KEMRI-Wellcome Trust Research Programme, Nairobi, Kenya); WHO Regional Office for Africa, Brazzaville, DRC

Funding Body: UK Department for International Development (DFID)

The LINK project aimed to; work with National Malaria Programmes (NMPs) in 13 countries to generate detailed epidemiological profiles of malaria risk and intervention coverage, work with WHO AFRO to assist national and regional decision-makers improve evidence-based decision making; and improve capacity of NMPs to update profiles in future

LINK produced 24 epidemiological profiles for the highest burden malaria countries in sub-Saharan Africa. Multiple requests for re-profiling were made by National Malaria Programmes (NMPs), indicating the value placed on LINK profiling and engagement. Our evaluation found that LINK epidemiological profiles were used for strategic and operational decisions by NMPs including; tailoring interventions to epidemiological strata, prioritising resource allocation, identifying where interventions should be implemented, and critically analysing trends over time to determine potential reasons for increases in risk associated with gaps in coverage.

We identified several challenges to using data for decision-making. These included; fragmented data making it difficult for NMPs to have a comprehensive view of programme issues, lack of availability of data in a timely enough way for strategic decision-making, a lack of understanding and subsequent mistrust of modelled estimates of malaria risk, challenges in interpreting discordant data from different sources, and tension between the global call for universal coverage and local needs to target resources because of insufficient funding. There is an emerging culture of data use at national level, which needs to be strengthened as NMPs begin to think about the most effective mix of interventions for different epidemiological strata.




ENTOMO

The Malaria Centre was established in 1998 to facilitate interdisciplinary research at London School of Hygiene & Tropical Medicine, to support links with research in malaria endemic countries, and to provide an authoritative academic voice on malaria. The Centre brings together around 300 researchers, postgraduate students and support staff with members working in around 40 countries at any one time.

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