TARGETING VIVAX MALARIA in the Asia Pacific
Asia Pacific Malaria Elimination Network Vivax Working Group Report
2009 - 2014
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This report has been prepared by the APMEN Vivax Working Group coordinating team based at the Menzies School of Health Research, Darwin, Australia following a consultative process with the APMEN Vivax Working Group members and participants and other stakeholders. The findings, interpretations and conclusions expressed in this report do not necessarily reflect the views of all APMEN partners. The designations employed and the presentation of material within this document does not imply the expression of any opinion of APMEN or its partners concerning the legal status of any country, territory, city or areas or of its authorities or concerning the delimitation of its frontiers or boundaries.

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FOREWORD

It is our pleasure as the Co-Chairs of the Asia Pacific Malaria Elimination Network’s Vivax Working Group to introduce this important publication. This report is a milestone of the last five years of the APMEN Vivax Working Group and highlights the coordinated efforts of many APMEN Country Partners and Partner Institutions, who have worked together to share the challenges and successes in *P. vivax* technical and research capacity with the common goal of ensuring that the Asia Pacific region can be free of malaria.

The Vivax Working Group has come a long way since the early discussions amongst malaria programme managers, scientists, development partners and advocates at the first meeting in Brisbane in February 2009 when APMEN was “born”. At this meeting the Network agreed that one of the greatest challenges in the Asia Pacific region for malaria elimination was *Plasmodium vivax*. The most prevalent species and specific to our Asia Pacific region, *P. vivax* demands novel strategies to eliminate the disease.

During the last five years the Vivax Working Group has reviewed the existing evidence, identified priorities and implemented a process to gather relevant evidence needed to inform national policy makers. It is our opinion that this process has been vital in generating the knowledge and experience that can be shared between partners, and where possible, translated into practice.

The unique nature of APMEN is an ability to bring together scientists and programme managers alongside partners from research institutions and other malaria stakeholders from the public and private sectors. This process has helped the Vivax Working Group to facilitate quality specific research and build individual and institutional capacity of the Country Partners, to help them achieve their elimination targets.

Significant challenges remain – including the well documented evolution of parasite and vector resistance to drugs which threaten to turn back the hard earned successes of the last 10 years. The Vivax Working Group is committed to working closely with all our partners, including the World Health Organization and Roll Back Malaria – to find efficient, effective, affordable and accessible solutions to country malaria programme challenges.

We hope you enjoy reading about the APMEN Vivax Working Group, its research projects, achievements, challenges and lessons learnt. APMEN welcomes your interest and feedback and looks forward to working with the global community to achieve the elimination of malaria.

*Professor Ric Price*  
*Co-Chair, APMEN Vivax Working Group*  

*Dr Chee Chong Kheong*  
*Co-Chair, APMEN Vivax Working Group*
ACKNOWLEDGEMENTS

This report “Targeting vivax malaria in the Asia Pacific 2009-2014” was prepared by the APMEN Vivax Working Group Coordinating Team (based at the Menzies School of Health Research in Darwin, Australia), the APMEN Country Partners National Malaria Control Programs and APMEN Partner Institutions.

The Vivax Working Group is supported by a large number of collaborating partners from academic institutions and government departments who provide expertise and operational support of the APMEN Research Projects. Their contributions have ensured that APMEN funded research is of high quality, sound methodology and relevant to the needs of the Country Partners. We also acknowledge the valued support from a range of other partners including policy makers, healthcare providers, researchers and funders for their participation in a range of activities including workshops and meetings. APMEN would like to acknowledge the photographs supplied by its Country Partners and Partner Institutions and the generosity of the Network for permission to use images taken during Network events and activities.

The Vivax Working Group and recipients of the research project grants thank the sponsors and donors of the Working Group including the Department of Foreign Affairs and Trade – Australian Aid Program (DFAT-AAP) and the Bill and Melinda Gates Foundation and the in-kind contribution of the Country Partners and Partner Institutions of the Vivax Working group and APMEN. The Working Group also thanks the APMEN Joint Secretariat at the University of Queensland, Brisbane, and the Global Health Group at the University of California, San Francisco for their tireless support of the programme.
EXECUTIVE SUMMARY

To date, 16 countries in the Asia Pacific region have committed to eliminating malaria within their borders. The Asia Pacific Malaria Elimination Network (APMEN) was created to help them achieve this goal. A fundamental prerequisite to any regional malaria elimination agenda is the existence of robust and efficient national malaria control programmes. However, malaria elimination poses challenges above and beyond standard control activities; these include addressing cross border issues, preventing the reintroduction of malaria, and tackling hidden reservoirs of infection that can sustain transmission long after the primary burden of clinical disease has fallen below that of other health priorities. To successfully address these challenges, two vital components are necessary: collaboration and innovation.

APMEN Country Partners are all at different stages of elimination, and each have varied challenges; and as such, the approaches to meet the needs of each differ. However, the threat posed by \textit{P. vivax} malaria is a common theme across all 16 APMEN Country Partners, with recurrent \textit{P. vivax} infections potentially undermining the goal of elimination.

Given the urgent need for specific action against \textit{P. vivax} infections, APMEN established the Vivax Working Group at its inaugural meeting in Brisbane in 2009.

Over the past four years, APMEN and the Vivax Working Group (henceforth referred to as ‘the Working Group’) have worked with National Malaria Control Programmes to identify and build the knowledge, tools and in-country technical expertise important to reducing the burden of malaria, and ultimately, achieving elimination. In pursuing this objective, the Working Group developed a four-stage strategy to identify knowledge gaps, build regional consensus on shared priorities, and generate evidence to inform National Malaria Control Programs (NMCP) activities and policies. The latter constitutes the fourth phase of the Working Group strategy.

This report documents activities within the first three stages of the Working Group strategy. The Working Group now represents a well-informed Group, within a strong, firmly-established Network. The Working Group has worked collaboratively to facilitate partnerships between national programs and key agencies, and to develop regional capacity and tools toward addressing the elimination challenges posed by \textit{P. vivax}. The Working Group is now well positioned to act on stage 4 of its strategy, Influencing Policy and Practice.

Stage one focused on \textbf{Identifying Knowledge Gaps} impeding the elimination of \textit{P. vivax}. Through a series of systematic literature reviews, meetings and workshops, the Working Group documented the available knowledge, and identified operational research gaps in several priority areas, including: the treatment of infection (both blood stage and radical cure of \textit{P. vivax}), methods for identifying patients with Glucose-6-Phosphate Dehydrogenase deficiency (G6PDd), surveillance strategies for assessing the transmission of \textit{P. vivax} malaria, identifying reservoirs of infection, and quantifying parasite diversity.

Stage two involved \textbf{Building Consensus} to identify common regional agendas to address the key gaps identified in stage one. A key component of this stage has been meetings and workshops that were informed by the operational experiences of National Malaria Control Programmes and the skills and expertise of Partner Institutions. Through these workshops the Working Group refined its focus and in 2011 three priority themes were agreed upon: \textit{Surveillance, Diagnostics and Therapeutics}.

Stage three focused on \textbf{Generating the Evidence} needed in each priority area. The Working Group provided technical assistance to National Malaria Control Programmes to build regional technical expertise and supported operational research projects which aimed to address key knowledge gaps, and ultimately, to \textit{Influence Policy and Practice}. The latter constitutes the fourth phase of the Working Group strategy.

\hspace{1cm}^{1}Hsiang et al. Malaria elimination in Asia-Pacific: an under-told story. \textit{Lancet} 2010; 375(9726):1586-7
1. WHAT IS APMEN?

APMEN is a network of countries and other stakeholders that are committed to working collaboratively to achieve malaria elimination in the Asia Pacific region. APMEN brings together country program managers, development agencies, non-governmental organizations, academic institutions, the private sector and global agencies involved in malaria elimination, including the World Health Organization (WHO). Together these partners collaboratively pursue regional malaria elimination through knowledge exchange, capacity building, building the evidence base and leadership and advocacy for malaria elimination.

APMEN was formed in 2009 in recognition of the dramatic successes in malaria control that countries across the Asia Pacific were achieving. Many regional countries had greatly reduced malaria, and elimination had once again become a feasible goal. This was a time of growing political and economic support for malaria elimination globally, through initiatives such as the Roll Back Malaria Partnership (RBM) and the Global Fund to Fight AIDS, Tuberculosis and Malaria. The Australian Government, the foundational donors of APMEN, were leaders in mobilising the regional elimination agenda in the Asia Pacific.

Despite the progress that is being made in the region, a range of technical and operational challenges continue to face countries pursuing malaria elimination. These challenges include \( P. \) vivax, control of the diverse range of vectors in the region, case detection, imported malaria, and the challenges of transitioning to a low transmission context. APMEN acts as a collegial forum for country-to-country knowledge exchange, capacity building and evidence building on these technical and operational issues, while also advocating for elimination as a long term regional goal. APMEN has grown steadily since its inception in 2009 to become a crucial element of the regional malaria elimination landscape. In 2014 APMEN consists of fifteen countries: Bhutan; Cambodia; China; the Democratic People’s Republic of Korea (DPRK); Indonesia; Lao People’s Democratic Republic; Malaysia; Nepal; the Philippines; the Republic of Korea (ROK); the Solomon Islands; Sri Lanka; Thailand; Vanuatu and Vietnam; together with 29 Partner Institutions. In addition to their country elimination targets, APMEN countries have a shared goal that by 2025, half of all the countries in the region with malaria today will have achieved their malaria elimination targets, and all have declared a commitment to collectively pursuing the long term goal of regional malaria elimination. As more countries in the Asia Pacific are shifting toward elimination, APMEN continues to grow and evolve to support countries to achieve these goals.
2. BACKGROUND TO THE FORMATION OF THE APMEN VIVAX WORKING GROUP

Malaria remains endemic to 20 countries in the Asia Pacific region where more than 2.2 billion people are at risk of infection. Infants and pregnant women carry the largest burden of the disease, and this is a key hurdle to achieving the 2015 Millennium Development Goals of improving maternal health and reducing child mortality. Moreover, malaria exerts a huge socioeconomic burden on the poorest communities in the region.2

Since 1960, only five countries in the Asia Pacific have been certified free of malaria: Taiwan (1965), Australia (1981), Singapore (1982), Maldives (1984) and Brunei (1987)3. In other locations significant progress has been made in the control of malaria and reduction of disease burden, a likely reflection of increasing political commitment and better access to diagnostics, treatment and bed nets. As a result several countries raised their goal from control to elimination.

In 2009, at a time when malaria elimination in the Asia Pacific region was seen as a feasible goal and with high level political support from numerous countries, APMEN was established to create an innovative, country-led platform to support malaria elimination in the Asia Pacific region. As a Network, APMEN brings together a wide range of stakeholders from across the region to support each other to achieve individual country targets and the long term collective goal of regional malaria elimination.

At the inaugural meeting held in Brisbane, representatives from National Malaria Control Programmes of 10 founding countries came together with representatives from the World Health Organisation, the Australian Government, and scientific and academic institutions supporting malaria elimination in the region. These founding Network members shared the long-term collective goal of regional malaria elimination. During this initial meeting, the Network recognised that elimination in the Asia Pacific required specific attention to the technical and operational challenges faced by many countries across this region.

Addressing the emerging challenge of P. vivax malaria was highlighted as being a major impediment to malaria elimination in the region. Historically, P. vivax malaria had not been a focus of attention for national malaria control programmes, nor as a research priority. In 2009, 91% of the global burden of P. vivax was concentrated in Central and South-East Asia and between 2006 and 2009, accounted for only 3.1% of expenditures on malaria research.

Malaria control leaders from almost all countries reported an increased proportion of P. vivax cases, compared to Plasmodium Falciparum (P. falciparum), as well as critical knowledge gaps in controlling the P. vivax parasite. In light of this, the essential need to increase attention and resources on P. vivax malaria was clear. Furthermore, the high mortality and morbidity attributed to P. vivax meant that a comprehensive strategy to address P. vivax malaria had become an urgent public health priority.

The Vivax Working Group was created to address the key challenges of controlling and eliminating P. vivax malaria. The aim of the Working Group is to extend the gains made against P. falciparum malaria to P. vivax malaria, thereby assisting the region to achieve and sustain malaria elimination.

This report documents the activities of the Working Group from 2009 to 2014, and looks ahead to the future needs of the region for elimination of P. vivax malaria.

KEY P. VIVAX CHALLENGES

• P. vivax has the ability to enter into a dormant liver stage (hypnozoite). Weeks to months after symptoms of the primary infection have ceased, the disease may relapse through this dormant stage. In endemic areas, P. vivax relapses are a major cause of malaria in young children and an important source of malaria transmission. Relapses can only be prevented by eliminating hypnozoites using 8-aminoquinoline anti-malarials, of which primaquine is currently the only licensed and available drug. However, primaquine can cause a significant haemolytic reaction among those with a deficiency in the enzyme G6PD, a genetic disorder present in a significant proportion of populations at risk for malaria.

• P. vivax is often difficult to detect, as the number of parasites within the blood of affected individuals may be very low. In addition, the transmission dynamics of P. vivax significantly differ from P. falciparum; lessons learned and models created cannot be applied to P. vivax malaria. This is reflected in the continuous decline of falciparum malaria incidence rates in many countries, whereas P. vivax infections remain at a steady level.

• P. vivax is difficult to eliminate using the same methods shown to be successful against P. falciparum. As the current malaria control and elimination programs disproportionately reduce the incidence of P. falciparum, the global fraction of malaria due to P. vivax is expected to rise. Importantly, there is increasing recognition that P. vivax can cause severe clinical manifestations and associated mortality. However, historical data from Europe and the US have demonstrated that complete eradication from large areas of the world is feasible.
3. ESTABLISHING THE VIVAX WORKING GROUP AND DEFINING RESEARCH PRIORITIES

3.1 ORGANISATION AND GOVERNANCE OF THE VIVAX WORKING GROUP

At the first APMEN meeting in Brisbane, 2009, the membership of the group was proposed to include representatives from the National Malaria Control Programmes of each of the APMEN Country Partners as well as \textit{P. vivax} experts and researchers from leading national, regional and international organisations (known as APMEN Partner Institutions).

The Menzies School of Health Research was requested to host a Coordinating Team to provide technical skills and capacity to mentor and support the development and implementation of a Vivax Work Plan. The Coordinating Team was asked to work with Country Partners and Partner Institutions to develop a comprehensive \textit{P. vivax} agenda, to build regional capacity and gather evidence for policy makers.

The Working Group provides a unique opportunity to bring together National Malaria Control Programmes and research institutions in a collaborative program. All of the APMEN Country Partners are represented within the Working Group and play a lead role in directing the focus of activities, and helping to ensure that results and reports are disseminated to their respective Programmes.

3.2 THE AIMS AND OBJECTIVES OF THE VIVAX WORKING GROUP

In 2010, during the first year of APMEN, the Working Group convened in Colombo, Sri Lanka, to draft their Terms of Reference and develop an operational research agenda. The Working Group agreed on the following primary objectives:

- To identify knowledge gaps and operational research priorities necessary for the optimal control and elimination of \textit{P. vivax} malaria.
- To exchange research and operational experience between APMEN countries to maximise synergistic activities and minimise unnecessary duplication, including:
  - Sharing (and where possible standardization) study designs, standard operating procedures and other research methodologies.

3.3 RESEARCH PRIORITIES OF THE VIVAX WORKING GROUP

A year later in 2011, the Working Group convened again in Kota Kinabalu, Malaysia. During this meeting, priority areas of the Working Group were defined by Country Partners and Partner Institutions. Based on criteria of critical importance to regional elimination, available resources and viability, three themes were selected \textbf{Surveillance, Diagnostics and Treatment}. These themes have since guided the activities of the Working Group, including research projects funded and undertaken, workshops and trainings (see more in section 6.3).
4. GENERATING KNOWLEDGE TO INFLUENCE POLICY AND PRACTICE

The Working Group has a four-phase strategy designed to build regional capacity, share operational experience, and ultimately enhance national malaria control programme activities.

In stage one, literature reviews bring together information and experiences relevant to the Asia Pacific region, and Identify Knowledge Gaps that needed to be addressed to enhance *Plasmodium vivax* control and elimination activities.

Stage two Building Consensus, involves setting common agendas for developing projects and partnerships to address the key gaps identified in stage one. Annual workshops and meetings are a critical part of this work, providing the Working Group with the opportunity to discuss ideas and to consult with other Working Group members and invited experts.

The third phase aims Generate the Evidence needed to inform policy and practice. The Working Group Coordinating Team supports National Malaria Control Programmes and Partner Institutions to undertake research and field activities to address the knowledge gaps and elimination strategies highlighted in the first two phases. Projects funded through APMEN have also contributed to building local research and technical skills.

The fourth phase is the translation of evidence into recommendations that can Influence Policy and Practice Change towards *P. vivax* malaria elimination.

The Working Group strategy has taken on cyclical process, informed by the changing burden of *P. vivax* and the changing needs of the APMEN Country Partners. As countries move through the various stages of elimination and as our understanding of *P. vivax* improves, a dynamic program of operational research is needed that can adapt to an evolving agenda.
4.1 IDENTIFYING THE KNOWLEDGE GAPS (2009-2014)

Four systematic reviews were carried out by identifying, appraising and examining existing data related to the following questions:

- What is the status of malaria research in the member countries?
- What is the available evidence of clinical efficacy of primaquine treatment regimens?
- What are the key knowledge gaps in G6PD diagnostics?
- What is the level and geographic range of drug resistance against *P. vivax* malaria?

### Key findings of the systematic reviews

The reviews provided a comprehensive summary and analysis of the literature pertinent to each specific question. The conclusions were:

- There has been a decline in the proportion of malaria-related literature amongst all biomedical publications.
- Treatment with low dose primaquine is not consistently effective in all areas. A higher dose of primaquine appears to offer significant benefits; however, these need to be confirmed in a range of endemic settings, and amongst high-risk patients. Multi-site trials to assess higher doses of primaquine with a control arm, and careful and long-term patient follow up, are needed.
- Improved diagnostics for G6PDd are required to facilitate the broader and safer use of primaquine. Current methods are impractical in areas with limited resources, and where most malaria patients live.
- Enhanced monitoring and better surveillance tools are needed to better assess the burden of *P. vivax* malaria, identify areas of infection and drug resistance, and quantify changes in drug resistance patterns. Up to date information is critical to ensure optimal treatment recommendations.

4.1.1 TRENDS IN MALARIA RESEARCH IN ASIA PACIFIC COUNTRIES

*Trends in malaria research in 11 Asia Pacific countries: an analysis of peer-reviewed publications over two decades*[^4^]

The first literature review was a joint effort involving Country Partners from Sri Lanka, China, Indonesia and Vanuatu, and the World Health Organization, as well as the APMEN Secretariat and the Working Group Coordinating Team.

The review assessed the spectrum of malaria-related research in the region over the last two decades. The MEDLINE database was searched through PubMed from January 1990 to December 2009 for all biomedical and malaria-specific literature from countries who were APMEN members at that time. The number of malaria-related articles was compared with all biomedical publications over the study period (Figure 2).

The number of biomedical publications from the target countries increased exponentially, whereas the rise in malaria-related publications was more modest. The proportion of all biomedical publications addressing malaria fell from 3% in 1992 to less than 1% by 2005, and stayed below 1% thereafter. Articles on *P. vivax* represented only 22-38% of malaria related publications. This bias was particularly apparent for articles describing clinical trials, only 15% of which were related to the treatment of *P. vivax*.

In subsequent discussions, Country Partners expressed concern that the decrease could reflect a decline in national expertise and support for malaria research, particularly that of clinical trials. The Working Group concluded that operational research should be encouraged to increase and inform clinically related activities.


enrolled 59,735 patients into 156 treatment arms, in 20 countries. Primaquine regimens were categorized according to the total dose administered: very low (≤ 2.5 mg/kg), low (>2.5 mg/kg- < 5.0 mg/kg) and high (≥ 5.0 mg/kg).

There was marked heterogeneity in study design, particularly for primaquine dosing and duration of follow up. The median rate of recurrence following very low dose of primaquine and low dose primaquine was 25% and 6.7%, respectively, at 4-6 months. High dose primaquine regimens were assessed in 28 treatment arms and were associated with very low rates of recurrence at one month. In 18 studies with control arms, the effectiveness of a very low dose primaquine regimen was not different from patients who did not receive primaquine, whereas for the low dose regimens, a significant difference was reported in 6 of 12 studies. Two studies, enrolling 171 patients, demonstrated high effectiveness of high dose primaquine compared to a control arm.

The report was published in the Malaria Journal in 2012. The review concludes that low dose regimens retain adequate efficacy in some areas; however, this is not uniform. Higher dose primaquine regimens appear to offer significant benefits in efficacy, though this needs to be confirmed in a range of endemic settings and in high-risk patient groups, such as young children and partially G6PDd females.

The review also proposed several key elements of antirelapse clinical trials that need to be addressed to maximise their quality and clinical relevance. These key elements informed the Working Group’s discussions and design of primaquine studies (see sections 5.3 to 4.2.2).

To contribute to building national expertise and addressing knowledge gaps, Country Partners were invited to participate in the APMEN Country Partner Technical Development Program (see Section 6: Building the Evidence).

This program funds projects that contribute to filling the identified knowledge gaps, and targets junior to mid-level researchers. These researchers are supported by Partner Institutions and the Coordinating Team to design and deliver research that address national and Working Group priorities.

http://apmen.org/research-grants/

4.1.2 PRIMAQUINE RADICAL CURE OF PLASMODIUM VIVAX

The evidence for the antirelapse efficacy of primaquine for P. vivax was investigated using a reference library of antimalarial clinical trials generated by APMEN Partner Institution, WorldWide Antimalarial Resistance Network (WWARN, http:// www.wwarn.org/resistance/malaria/literature/overview).

All clinical trials published since 1950 involving the use of primaquine regimens for the radical cure of P. vivax were identified. Data could be retrieved from 87 clinical trials that enrolled 59,735 patients into 156 treatment arms, in 20 countries. Primaquine regimens were categorized according to the total dose administered: very low (≤ 2.5 mg/kg), low (>2.5 mg/kg- < 5.0 mg/kg) and high (≥ 5.0 mg/kg).

There was marked heterogeneity in study design, particularly for primaquine dosing and duration of follow up. The median rate of recurrence following very low dose of primaquine and low dose primaquine was 25% and 6.7%, respectively, at 4-6 months. High dose primaquine regimens were assessed in 28 treatment arms and were associated with very low rates of recurrence at one month. In 18 studies with control arms, the effectiveness of a very low dose primaquine regimen was not different from patients who did not receive primaquine, whereas for the low dose regimens, a significant difference was reported in 6 of 12 studies. Two studies, enrolling 171 patients, demonstrated high effectiveness of high dose primaquine compared to a control arm.

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The review also proposed several key elements of antirelapse clinical trials that need to be addressed to maximise their quality and clinical relevance. These key elements informed the Working Group’s discussions and design of primaquine studies (see sections 5.3 to 4.2.2).
Essential and desirable elements of antirelapse clinical trials

Essential elements:

• Combination of primaquine with a blood schizontocide known to have high efficacy in the study population. This will minimise the risk of recrudescent infections. Primaquine efficacy may vary with partner drug; this needs to be confirmed.

• Prolonged duration of follow up (12 months) – since relapses can occur over a 12 month period, it is important to conduct studies with sufficient follow up periods.

• Inclusion of a control arm. The risk and timing of \( P.\ vivax \) relapse varies with the location of the study site; hence, the efficacy, safety and risk-benefit ratio of primaquine needs to be gauged, where ethically acceptable, by comparison with a control arm (no antirelapse medication provided).

• Assessment of adverse events, with particular attention to haemolysis. Primaquine can cause haemolysis, particularly in patients with G6PD deficiency, and this varies with the study population. This requires assessment throughout any clinical trial.

• Multicentre assessment. The risk of relapse varies between \( P.\ vivax \) strains, and the intensity of sporozoite inoculation. The risk of haemolysis is related to the G6PD deficiency genotypes present within the study population. Both factors vary regionally. Risk and benefit therefore need to be assessed across multiple heterogeneous sites.

Desirable elements:

• Re-treatment of relapsing patients with same treatment regimen. The clinical consequences of relapsing infections, and the benefits of their prevention, require characterisation of the total number of relapses following an initial infection.

• Quantification of haematological recovery. Both malaria and primaquine may cause haemolysis. The risks and benefits of primaquine treatment need to be confirmed through close monitoring of effectiveness and adverse events.

• Economic, and knowledge, attitudes and practice studies, on the perception and acceptance of antirelapse therapies within local populations.

4.1.3 REVIEW OF KEY KNOWLEDGE GAPS IN GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY DETECTION WITH REGARD TO THE SAFE CLINICAL DEPLOYMENT OF 8-AMINOQUINOLINE TREATMENT REGIMENS

The diagnosis and management of Glucose-6-Phosphate Dehydrogenase deficiency (G6PDd) is a crucial aspect in the current phases of \( P.\ vivax \) control and elimination. In May 2012, the Working Group convened a workshop in Incheon, Korea, bringing together National Malaria Control Programmes, researchers and industry representatives to review key knowledge gaps of G6PDd.

Glucose-6-Phosphate Dehydrogenase (G6PD) is an essential enzyme in the pentose phosphate pathway (PPP), the only pathway for human red blood cells (RBC) to maintain the cells’ redox power by reducing NADP⁺ to NADPH. The enzyme consists of two dimers, and is encoded on the long arm of the X-chromosome (q28). Hemizygous men and homozygous women with mutations of the G6PD gene manifest different degrees of enzyme deficiency, dependent upon the genetic variant carried, and the age of the red blood cell population at time point of measurement. Since the inheritance is X-linked, heterozygous women can manifest different patterns of G6PD deficiency, and the degree of deficiency is further confounded by the process of lyonization.

The WHO defines five categories of G6PDd (I-V), dependent upon the level of enzyme activity. However, these categories do not relate to quantitative measures of enzyme activity, but to the population average, which varies according to prevalence and local genetic variants present.

G6PDd is among the most common enzymopathies, with an estimated prevalence of at least 400 million individuals worldwide. More than 160 different variants of G6PDd have been reported, with a spectrum of associated enzyme deficiencies. The host selection and high prevalence of these mutants is likely to have been driven by their ability to provide some degree of protection from \( P.\ vivax \) malaria infections. Hence, G6PDd is less frequently encountered among malaria patients than in the rest of the population.

While G6PDd offers some protection against malaria, it complicates the radical cure of the dormant hypnozoite stages of \( P.\ vivax \). The only currently available drug to eliminate hypnozoites, primaquine, causes haemolysis, the risk of which increases with the dose of the drug and degree of enzyme deficiency. Prior to administration of primaquine, recipients should be tested for G6PD deficiency as well as having their haematological status assessed. Haemolysis in severely G6PDd individuals can be potentially fatal, and the fear of this complication is one of the most important barriers to the safe and effective delivery of primaquine for \( P.\ vivax \).
radical cure. There are a number of other 8-aminoquinolines under development, among which Tafenoquine is the most promising2.

At the workshop (Section 4.2.4), key knowledge gaps in G6PDd and the tests available for assessing an individual’s G6PD activity status were reviewed. Participants at the workshop concluded that the National Malaria Control Programmes face two key challenges in diagnosing and managing G6PDd:

1. Genetic tests are not useful for informing case management, given the diverse nature of G6PD variants and the complexity and cost of the tests.

2. A number of enzyme activity assays can diagnose G6PDd, but they require a cold chain, specialized equipment and laboratory skills. These assays, mainly applied in clinical trials, are impractical for use during delivery of care in areas with limited resources, where most malaria patients live.

3. One point-of-care test for G6PD deficiency is in the late stages of development, but at the time of this meeting, the data available indicated concerns about the performance of the test10.

Improvements to the diagnosis of G6PDd are required for the broader and safer use of primaquine, and will also be needed for the safe investigation of tafenoquine. To address these issues, APMEN has implemented a number of studies, in multiple sites, to evaluate the performance and field applicability of the G6PD assays currently available on the market.

The proceedings of the workshop were published in a joint paper co-authored by APMEN Partner Institutions in Malaria Journal in 201311.

4.1.4 DEFINING DRUG RESISTANT PLASMODIUM VIVAX: A SYSTEMATIC REVIEW OF THE LITERATURE

Up to date information on antimalarial efficacy is essential to inform rational P. vivax treatment policy. Whereas clinical trials for P. falciparum are generally standardised, defining antimalarial efficacy against P. vivax is significantly more difficult. A systematic search of Medline, Web of Science, Embase and the Cochrane Database of Systematic Reviews was undertaken to identify P. vivax clinical trials published in English between January, 1960 and April, 2014. A total of 130 eligible clinical trials involving 22,382 patients and 26 case reports of 54 patients were identified. Chloroquine efficacy was classified into four categories, according to the risk of recurrence at day 28, whole blood concentrations and enrolment criteria.

There were marked variations in the study designs of the trials, preventing the interpretation of more than a third of the study site estimates of chloroquine efficacy. Overall P. vivax was defined as chloroquine resistant for more than half of the site estimates and delayed parasite clearance was found to be predictive for early recurrence. Patients receiving chloroquine plus primaquine had an equivalent or lower risk of P. vivax recurrence by day 28 compared with patients receiving chloroquine alone. The overall results suggest that chloroquine resistance has emerged or spread over much of the P. vivax endemic world (Figure 4a and b).

The review concludes that information on emerging chloroquine resistance is likely to be missing or overlooked in many areas12. Enhanced monitoring and better surveillance tools are needed to quantify the level of resistance and identify alternative treatment options.

4.2 BUILDING CONSENSUS ACROSS THE NETWORK

The identified knowledge gaps and literature reviews provided a guide to understanding the available evidence for P. vivax control and elimination. The next important phase is an ongoing dialogue between Country Partners and Partner Institutions to make all parties aware of the research outcomes, and build a consensus on how to move forward and fund appropriate activities to address these priorities.

Critical to the success of the Working Group is a common decision making process that seeks the participation of all members. Consensus building through exchanging ideas and experience contributes to better planning, improves the implementation of projects, and fosters continued collaborations. This is carried out through regular discussions between partners, coordination of activities, and sharing of methodologies and experience. The Working Group facilitates this process through the following activities:

- Annual workshops and Business Meetings
- Site visits to discuss local issues and priorities
- Regular liaison with Working Group representatives to assess progress, challenges, and emerging regional priorities
- Opportunistic meetings at major international conferences; the Working Group has endeavoured to help stakeholders attend important meetings.

The priorities identified by the Working Group (addressing a decline in operational research, improving treatment options, improving diagnostics for G6PDd and enhancing monitoring and surveillance tools to identify areas of infection and drug resistance) are prioritised within the broader APMEN agenda. Country Partners remain committed to a strong strategic focus on P. vivax within APMEN.
4.2.1 PLASMODIUM VIVAX GENOTYPING WORKSHOPS – 2011 AND 2012

What was the rationale for the workshop?

The first round of research projects funded by APMEN generated a large number of applications seeking to genotype their local *P. vivax* parasite populations. Projects with a *P. vivax* genotyping component have been supported in China, Indonesia, Malaysia, the Republic of Korea, Sri Lanka and Bhutan. As detailed below, Country Partners had specific questions which they wished to address using genotyping, and many of these questions could be best answered by comparing genotyping datasets between the partner sites. Consensus genotyping methods, and a platform for data sharing were needed to achieve this. However, prior to the first workshop, no such consensus had been established for *P. vivax* genotyping. The rationale for the workshops was therefore to provide a forum for Country Partners to review ways in which they could work more closely together, with a common agenda.

How is *P. vivax* genotyping informative for elimination efforts?

Genotyping allows parasites to be compared, to see how different they are from one another. For example, parasite genotyping is used routinely in clinical drug surveillance to determine whether parasites present in an infection before treatment are the same as those in recurrent infections, and therefore, whether the recurrence was likely to have resulted from a true drug failure, or a new infection. The Country Partners wanted to use parasite genotyping to answer the following questions:

1. **Have the local intervention strategies at my site made containment of the parasites more manageable?**
   
   Parasite populations with many different parasite genotypes (diverse populations) may be more difficult to contain, as they are more likely to carry parasite strains which are able to escape host immune pressures or drug treatment. The presence of different parasite strains in a single infection (polyclonal infection) may also promote parasite growth and disease severity. In general, reduced transmission leads to lower parasite diversity. By genotyping parasite populations over time, we can assess how ongoing interventions are affecting the parasite diversity.

2. **How great is the risk of malaria resurgence at my site?**
   
   Malaria has been successfully eliminated from several island populations including Cyprus, the Maldives, Mauritius, Singapore and Taiwan. In general, island parasite populations are easier to eliminate than mainland populations because importation is generally low and easier to contain. However, as seen in Sri Lanka, resurgence still remains a risk. Genotyping can help to assess the general movements and spread of parasites within and across borders. In addition, genotyping can be used to identify clusters of identical parasite strains indicating unstable, epidemic transmission. This knowledge may facilitate early warning of resurgences. The success of this approach depends in part on a collaborative effort, with consensus methods applied across sites, to enable assessment of parasite movements between different locations.

3. **Can I distinguish the local parasites at my site from imported cases?**
   
   Parasites from different locations may exhibit some genetic differences. Some of these differences may even be observable by genotyping a few markers, potentially enabling imported malaria cases to be distinguished from local cases. Genotyping data from parasites from a range of different countries is required to assess how well certain marker sets are able to distinguish parasites by country. Information on the scale of imported cases may help to decide whether to focus efforts on reducing local transmission or importation of cases. As with the assessment of resurgence risk, effective assessment of markers for identifying imported cases relies on a collaborative approach, with consensus methods, between partners from different countries.

In response to the interest across the Network in addressing these questions, *P. vivax* genotyping workshops were held in Sabah, Malaysia, in 2011 and Incheon, Republic of Korea, in 2012.
WORKSHOP OBJECTIVES

The objectives of the genotyping workshops were to 1) coordinate parasite genotyping research activities amongst the Country Partners, 2) develop consensus methods for genotyping, 3) facilitate data sharing across the Asia Pacific region, and 4) strengthen local capacity. These objectives were achieved across two workshops, separated to first allow activities to start, and then to review progress, and identify further lessons and capacity requirements.

GENOTYPING WORKSHOP 1 - SABAH, MALAYSIA, 2011: OVERVIEW AND OUTCOMES

The first two objectives were addressed at the Sabah genotyping workshop. The Country Partners who had been supported to conduct *P. vivax* genotyping each gave an overview of their study plans. A panel of researchers with expertise in parasite genotyping provided advice for each of the studies. A few common themes (summarised above) were shared by all of the Partners, and the importance of a collaborative approach to address the challenge of imported cases was recognized. The main outcome of the workshop was the development of a consensus genotyping methodology.

This methodology is currently being used by Country Partners and Partner Institutions in Bhutan, China, Indonesia, Malaysia, Republic of Korea and Sri Lanka.

GENOTYPING WORKSHOP 2 - INCHEON, KOREA 2012: OVERVIEW AND OUTCOMES

Objectives 3 and 4 were addressed in the Incheon genotyping workshop. A year on from the Sabah workshop, the Country Partners were able to provide a progress update on their APMEN-funded projects, identifying any challenges they had faced and highlighting areas where further capacity building support was required.

Common challenges raised were the need for 1) support for data analysis and 2) a standardised analytical framework to enable country members to share their data effectively. Drs Sarah Auburn and Hidayat Trimarsanto (Eijkman Institute for Molecular Biology, Indonesia) described a Menzies-Eijkman database framework (vivaxgen) under development (http://vivaxgen.menzies.edu.au) which aims to address these two challenges. The database is designed to have user friendly data analysis tools for standard *P. vivax* genotyping analyses, including those required to address the Country Partners’ three key questions. The database also includes features to improve standardisation of data analysis across study sites, despite some inevitable differences in how the data was generated across sites. This feature is particularly important so that comparative analyses can be conducted between populations. Assessment of the ability of specific markers to distinguish infections from different countries will allow methods to be refined so that parasite movement between countries can be assessed.

The main outcomes of the workshop were the consensus agreements reached on data analysis and sharing methods (using the vivaxgen framework) amongst the Country Partners.
### APMEN GENOTYPING RESEARCH PROJECTS – PROGRESS IN 2014

#### Stage Field work and data collection still active

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<thead>
<tr>
<th>Location</th>
<th>Project</th>
<th>Questions that it helps addresses</th>
<th>Implications and Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bhutan</td>
<td>Dr Sonam Wangchuck, Ministry of Health Molecular Assessment of *P. vivax transmission dynamics in Bhutan</td>
<td>Can I distinguish the local parasite from imported cases? Have the local intervention strategies at my site made the parasites more manageable to contain?</td>
<td>The preliminary data suggests that the *P. vivax population is diverse. Given that these features are normally observed in high transmission settings it suggests that Bhutanese *P. vivax population may be largely sustained by imported cases. Further analysis of non-national samples from bordering India will enable assessment of whether this is the source of importation.</td>
</tr>
<tr>
<td>China</td>
<td>Dr Cao Jun, Jiangsu Institute for Parasitic Disease Sero-epidemiological analysis for monitoring malaria elimination in China</td>
<td>Have the local intervention strategies at my site made the parasites more manageable to contain?</td>
<td>This project is exploring the utility of ELISA as a surveillance tool to support intensive malaria control, foci identification and elimination evaluation. If ELISA is deemed successful in these three areas it will be incorporated into China’s standard surveillance program. Data has been collected and preliminary analysis conducted. Concluding results are pending.</td>
</tr>
<tr>
<td>Indonesia</td>
<td>Dr Rintis Noviyanti, Eijkman Institute for Molecular Biology Genetic diversity of *P. vivax in Indonesia</td>
<td>Have the local intervention strategies at my site made the parasites more manageable to contain?</td>
<td>The diversity of malaria epidemiology across Indonesia suggests that interventions which have been successful in one setting may not have the same impact in another. This study established *P. vivax genotyping for parasite surveillance in Indonesia and mapped the genetic diversity of *P. vivax in Bangka-Belitung and Sumba. With additional funding from the Malaria Transmission Consortium, a comparative assessment of *P. falciparum is underway, highlighting the impact of the hypnozoite reservoir on malaria transmission.</td>
</tr>
<tr>
<td>Sri Lanka</td>
<td>Dr Preethi Udagama-Randeniya, University of Colombo Dynamics of Plasmodium *vivax parasite populations during malaria elimination efforts in Sri Lanka, with low transmission and unstable malaria</td>
<td>Have the local intervention strategies at my site made the parasites more manageable to contain?</td>
<td>As Sri Lanka approaches elimination, the country faces the challenges of declining host immunity, imported cases, outbreaks and associated risks of resurgence. This study aimed to assess the impact of the declining incidence on the local parasite population diversity and transmission dynamics. A parasite vaccine candidate, the circumsporozoite protein, was shown to be very diverse in the population despite the low transmission.</td>
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#### Stage Analysis and translation for National Malaria Control Programs

<table>
<thead>
<tr>
<th>Location</th>
<th>Project</th>
<th>Questions that it helps addresses</th>
<th>Implications and Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>China*</td>
<td>Prof Gao Qi, Jiangsu institute for Parasitic Disease Improving the accuracy of *P. vivax case reporting using molecular methods</td>
<td>Can I distinguish the local parasite from imported cases?</td>
<td>The *P. vivax genotyping component of this research was conducted in Central China. Analysis highlighted unstable transmission with limited barriers to gene flow between the central provinces. The challenge of imported cases and risks of resurgence emphasize the need for continued surveillance to detect outbreaks early within this region.</td>
</tr>
<tr>
<td>Republic of Korea</td>
<td>Dr Jung- Yeon Kim, Center for Disease Control Evaluation of sensitivity and specificity of RDTs using microscopy and PCR in vivax malaria detection</td>
<td>Can I distinguish the local parasite from imported cases? Have the local intervention strategies at my site made the parasites more manageable to contain?</td>
<td>This project had both a diagnostics and surveillance focus. Preliminary genotyping data generated in the surveillance component demonstrated that a selection of imported *P. vivax cases had very different genotype profiles from the local South Korean isolates. This finding highlights the potential utility of genotyping to inform the CDC on imported cases of *P. vivax. A manuscript is in preparation.</td>
</tr>
<tr>
<td>Malaysia</td>
<td>Study of Drug sensitivity profile and Molecular Genotyping of Plasmodium *vivax isolates in Sabah, Malaysia</td>
<td>Have the local intervention strategies at my site made the parasites more manageable to contain?</td>
<td>By genotyping the parasite, this study assessed the impact of on-going intervention efforts on *P. vivax transmission dynamics in two low endemic settings in Sabah. The shrinking *P. vivax population in the regions investigated appeared to have left the populations vulnerable to outbreaks. Despite low transmission, the parasites remained moderately diverse, but the reservoirs maintaining this remain unclear. Imported cases may play an important role in sustaining *P. vivax infections in Sabah, which has important implications for malaria elimination strategies.</td>
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</table>
In addition to these APMEN-funded projects, Dr Qin Cheng from APMEN Partner Institution, the Australian Army Malaria Institute, has undertaken genotyping studies in the Solomon Islands and Vanuatu for the Australian Initiative for the Control and Elimination of Malaria (AICEM) (formerly known as the Pacific Malaria Initiative Support Centre), funded by the Australian Government. Dr Qin Cheng used the consensus genotyping methodology from the first APMEN genotyping workshop for her studies, making her results comparable to data generated by the research grant recipients.

WHAT STILL NEEDS TO BE DONE?

Mapping parasite transmission across the Asia Pacific region

Once each of the Country Partners has published the results of their individual site data, the data can be pooled across the sites and national boundaries to allow highly informative comparative analyses between study sites. The results of the cross-country data analysis will provide Country Partners with additional information on parasite spread between countries, helping to optimise on suitable interventions and where these should be prioritised. A targeted approach will enable greater efficiency and appropriate use of resources. In resource-limited settings, where interventions cannot be feasibly applied across large areas or populations, targeting the major reservoirs of infection is critical to reduce transmission to neighbouring areas. For example, in the Indonesian archipelago, which consists of thousands of islands, with varying malaria endemicity and epidemiology, a targeted intervention approach is essential. In addition, the results will help researchers to determine whether the current markers can be used to distinguish isolates from different countries and therefore aid confirmation of imported cases. Information on the relative burden of local versus imported cases will enable health officials to make informed decisions on how intervention efforts need to be targeted.

New genotyping markers for geographic location and drug resistance

In parallel to the genotyping studies, new markers need to be identified to optimise the distinction of local from imported cases and to assign geographic origins to infections. Molecular markers of \textit{P. vivax} chloroquine resistance are also urgently needed. Genome-wide studies should facilitate these efforts. In order to facilitate genomic studies of \textit{P. vivax}, a protocol was developed to assist \textit{P. vivax} sample preparation for whole genome sequencing. The APMEN Vivax Working Group has facilitated the sharing of this protocol with the Country Partners - an instructional video is available for online viewing (http://vimeo.com/52196472). Once effective markers have been identified, the existing network of APMEN researchers will be well placed to investigate the utility of the markers in helping to identify areas of with reduced drug susceptibility.
What were the outcomes from the workshop?
During the workshop, the delegates discussed how a more practical and safe primaquine treatment regimen could be developed and deployed. It was agreed that until alternative drugs are available, the focus would be on optimising the use of primaquine; this was likely to require evaluation of shorter high dose primaquine dosing, and complementary strategies to improve adherence such as directly observed therapies. There was enthusiastic discussion on the nature of a study design that would be acceptable across all APMEN countries. A hotly debated subject was the need for a control arm, so that primaquine efficacy could be established after accounting for the wide diversity in background relapse rates. The ethics of withholding primaquine therapy in the control group was found to be a pivotal issue, with divided opinion.

Is it ethical to have a control arm in an antirelapse clinical trial?
The ethics of including a control arm in the trials were reviewed. It was acknowledged that a major challenge in estimating the efficacy of primaquine arises from our inability to distinguish relapsing from new *P. vivax* infections, and variation in the timing and rate of relapse across regions. Inclusion of a control arm in the study design is essential if the absolute antirelapse efficacy is to be defined. A control arm is also important for the assessment of the tolerability and safety of primaquine, which can result in haemolysis, sometimes with severe even fatal consequences, as well as gastrointestinal upset and other side effects. Malaria itself may be associated with similar symptoms, so a control group is required to determine whether the adverse events observed in a trial are attributable to the administration of primaquine or to malaria.

In *P. vivax* endemic countries where primaquine use is not advocated, participants agreed that there are no ethical issues regarding withholding primaquine therapy in the control arm. In *P. vivax* endemic countries where primaquine is included in treatment policy, the issue of a control arm is more challenging. This situation requires a careful ethical review with special consideration of the following factors:

- Globally, *vivax* malaria causes a considerable disease burden. Treatment for recurrent *P. vivax* episodes in the form of primaquine is available, but the optimal use of the drug is unknown and treatment is underutilized, resulting in the continued transmission of *P. vivax* malaria.
- National and WHO treatment guidelines focus on the need for prompt and effective treatment of erythrocytic stage malaria parasites but diverge markedly on recommendations for radical treatment. The WHO recognises that current recommendations for primaquine are based on insufficient data. The current WHO guidelines state that in areas of sustained transmission, the benefits of primaquine are not considered to outweigh the risks.
- Safe administration of primaquine is generally considered to require the exclusion of G6PD deficiency. Few health care providers in *P. vivax* endemic settings have routine access to the facilities to diagnose G6PD deficiency. In the absence of G6PD deficiency screening, most health care providers are reluctant to prescribe primaquine.
- Even when recommended, primaquine is not routinely prescribed. Reasons for this omission include safety concerns, uncertainty over efficacy and a widely held belief that relapses are unavoidable, relatively benign, and can be treated simply by repeated blood schizontocidal treatments.
- When primaquine is routinely prescribed, intake is generally unsupervised, poorly adhered to, and hence, lacks effectiveness. In fact, most *P. vivax* patients globally do not receive effective antirelapse treatment.
- Reassuringly, the use of control arms in previous and ongoing primaquine trials is well established, having been a key component of at least 18 published studies.

What were the main recommendations of the workshop?
The workshop participants agreed that patient safety is of primary concern and cannot be compromised. It was agreed that all participants of the planned trials should be treated immediately with highly effective blood schizontocidal therapy to ensure cure of the acute infection. However the hypnozoites of *P. vivax* lie dormant in the liver and are not associated with any symptoms, until a relapse is triggered. Patients should be monitored closely for early indications of recurrent parasitaemia and febrile illness. This is usually done at weekly, then at monthly, intervals. Since relapses can occur after many months, delegates agreed on the need for following up patients for a prolonged period (at least 6 months).

Key aspects of the multi-centre study design included the following:

- Inclusion of a control arm to understand the impact, tolerability and safety of primaquine
- G6PD screening of all study participants
- Active and close monitoring of study participants at weekly, then at monthly, intervals for early indications of parasitaemia recurrence, and onset of illness
- Immediate treatment of study participants for blood stage parasitaemia during their initial presentation, and at each subsequent recurrence
- Follow up of study participants for 12 months.
There were differences of opinion as to which primaquine regimens should be evaluated. Some workshop participants endorsed the immediate assessment of high dose primaquine regimens, while others preferred to evaluate low dose primaquine regimens first. Some sites were reluctant to evaluate a high dose primaquine regimen as long as there was insufficient evidence that low dose regimens were failing. In such sites, a comparison of low dose primaquine with a control arm may be appropriate.

It was agreed that pilot studies could provide important information about the challenges that will be faced in the conduct of clinical trials. The experience from these pilot studies would help inform the design and implementation of larger trials. The pilot studies that were subsequently funded, and are currently ongoing, are discussed below in Section 5.3.

**What needs to be done next?**

There were fundamental differences in the optimal trial design that would be most informative both within and between study sites. In view of current antimalarial policy and degrees of chloroquine resistance, it was apparent that different schizontocidal therapies will need to be used depending on local drug efficacy of both *P. falciparum* and *P. vivax*.

Following the workshop APMEN has funded three trials taking place in four countries. These trials have all commenced and are conducted either by or in close collaboration with National Malaria Control Programs.
In addition to the APMEN funded research projects, a number of APMEN Partner Institutions are participating in primaquine studies. During the APMEN Annual Meetings, Country Partners are also provided with updates on research progress and outcomes from these trials.

### 4.2.3 GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY DETECTION WORKSHOP IN INCHEON, KOREA – MAY, 2012

#### What was the rationale of the workshop?

If the elimination of *P. vivax* is to be achieved in a reasonable time frame, it is imperative that radical cure of hypnozoites can be applied safely, effectively and to as wide a population as possible. Primaquine treatment has the potential to induce severe haemolysis in G6PD deficient individuals, and this is a key obstacle to deploying antirelapse therapy widely across all APMEN countries. Improving diagnosis and management of G6PD deficiency is essential to increase confidence in a treatment with a possible major adverse outcome.

#### What were the objectives of the workshop?

The workshop aimed to review the key knowledge gaps in G6PD deficiency, with regard to the safe clinical deployment of 8-aminoquinoline treatment regimens, and to assess the tests available for evaluating an individual’s G6PD activity status.

#### What were the outcomes of the workshop?

Following presentations from Country Partners, Partner Institutions and pharmaceutical industry representatives the following were identified as the key knowledge gaps:

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<tr>
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<th>Questions this project addresses</th>
<th>Implications and Applications</th>
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<tbody>
<tr>
<td>Bhutan</td>
<td>Dr Kinley Penjor, Mr Tobgyel Tobgyel and Dr Yeshey Dorjey National Vector Borne Disease Control Program</td>
<td>What is the clinical and parasitological efficacy of chloroquine for uncomplicated vivax malaria in Bhutan? What is the relapse rate in patient receiving standard dose primaquine in Bhutan?</td>
<td>This is the first antimalarial treatment efficacy study to be conducted in Bhutan. Since the intense drug pressure of parasite in low endemic areas can encourage the emergence of drug resistance, it is important to monitor the therapeutic response to look for early sign of reduced susceptibility. So far this study has highlighted that the current policy of chloroquine and low dose primaquine is working. Patient recruitment and follow up is on-going.</td>
</tr>
<tr>
<td>Vanuatu &amp; Solomon Islands</td>
<td>Professor Ivo Mueller Walter and Eliza Hall Institute</td>
<td>What is the safety and efficacy of standard primaquine treatment regime compared to a high-dose regimen to prevent relapse?</td>
<td>Major advances have been made in overall malaria control in Vanuatu and to a lesser extent Solomon Islands. This has been attributed to the success of bed nets, vector control measures and case management of clinical diseases. Relapses are likely to represent the final and most difficult hurdle to overcome on the road to elimination in Vanuatu and the Solomon Islands. Patient recruitment and follow up is on-going.</td>
</tr>
<tr>
<td>Malaysia</td>
<td>Dr Timothy William – Queen Elizabeth Hospital Artesunate-mefloquine vs. chloroquine in patients with acute uncomplicated <em>P. vivax</em>: a randomised open label trial in Sabah, Malaysia</td>
<td>Is the fixed combination of artesunate-mefloquine superior to chloroquine in uncomplicated <em>P. vivax</em> infection in Sabah Malaysia?</td>
<td>Ministry of Health guidelines currently recommend chloroquine and primaquine as first line treatment for the radical cure of uncomplicated <em>P. vivax</em> malaria. Case reports indicated the potential for chloroquine resistance in <em>P. vivax</em> in Sabah and Peninsular Malaysia, which has been confirmed by this study. Data from this study will inform treatment policies regarding the optimal choice of first line treatment for uncomplicated malaria in this region.</td>
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</table>
Treatment

- What is the safe yet efficacious dose of 8-aminoquinolines for the radical cure of *P. vivax* malaria?

- How much haemolysis is expected in patients with uncomplicated *P. vivax* malaria who are not treated with primaquine?

- What is the threshold of G6PD activity that stakeholders, including regulatory agencies, consider sufficient to safely administer standard 8-aminoquinoline regimens?

- What is the relationship between 8-aminoquinoline dose and risk of haemolysis in G6PD normal and deficient individuals?

- What is the degree of haemolysis (i.e. proportion of red cell lysis) that constitutes an unacceptable clinical risk to the patient?

Diagnosis/testing

- What are acceptable test characteristics of rapid diagnostic G6PD tests in various populations and field conditions?

Biology

- How do G6PD genotype, enzyme activity and co-factors interact?

- How do G6PD enzyme activity and severity of haemolysis correlate?

Other

- Prevalence mapping of G6PD deficiency (G6PDd) and variant types, as well as haemolysis risks across major malaria endemic settings.

Cost-analysis of G6PD deficiency tests, and the risks versus benefits of deploying or withholding primaquine regimens for *P. vivax* infections.

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**Box 1: G6PD deficiency and the difficulties of treating malaria:**

Human red blood cells (RBCs) do not have a cell nucleus and hence, cannot produce large amounts of enzymes essential for cell function and maintenance. After production in the human bone marrow, RBCs rapidly lose their ability to produce enzymes and any other cell maintaining substances. Hence, RBCs have a limited life span (approximately 120 days). Glucose-6-Phosphate Dehydrogenase (G6DP) is an enzyme essential to the cells energy system, and ability to cope with environmental stress. Approximately 400 million people worldwide suffer from mutations in the G6PD gene, resulting in a G6PD enzyme with compromised stability (or shorter half-life) and RBCs deficient in G6PD enzymatic activity. G6PD deficiency varies from very mild to severe; however, most forms of G6PD deficiency do not compromise health and quality of life of affected individuals, under normal circumstances. Some pathogens (e.g. malaria parasites), environmental agents (e.g. fava beans) and classes of drugs (e.g. 8-aminoquinolone antimalarials), may induce a process that causes the disintegration of G6PD deficient RBCs; a process called haemolysis. The degree of haemolysis depends on two key factors:

1. The severity of the G6PD enzyme deficiency
2. The degree to which RBCs are exposed to haemolysis-causing agent

Treatment of malaria with an 8-aminoquinolone is the only current treatment for killing the dormant stages of the malaria parasite (hypnozoites), and is also used to reduce gametocyte carriage of *P. falciparum* as part of malaria elimination programs. Due to the risk of haemolysis, many health authorities and medical staff are hesitant to use these drugs. It is crucial to understand the degree to which certain drugs can provoke haemolysis across a spectrum of G6PD activities. Establishing this relationship will help to develop clear guidelines to ensure that the majority of malaria patients can be treated safely with 8-aminoquinolones, and thus achieve radical cure. The development of rapid diagnostic tests will help medical staff to promptly assess whether a malaria patient is eligible for radical cure with an 8-aminoquinolone.

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Box 2: Measuring G6PD activity

Enzyme activity is expressed in terms of how active an enzyme is, for a defined amount of red blood cells (RBCs). The gold standard measure of G6PD activity is a sophisticated method known as spectrophotometry that requires considerable laboratory infrastructure.

In a clinical setting, the ideal would be cheap, reliable handheld devices which provide a quantitative result for G6PD activity, comparable to spectrophotometry. There are a number of less expensive G6PD tests that return either a positive or negative (dichotomous) result; however, no information beyond that (qualitative test).

One of the difficulties associated with assays for G6PD deficiency is the likelihood of false diagnosis in women. The gene coding for the G6PD enzyme is located on the X chromosome which is present as a single copy in men, but as two copies in women. All RBCs are derived from this one/two copies of the gene. Since all RBCs of men show the same enzyme activity, the average enzyme activity derived from quantitative or qualitative tests reflect enzyme activity at the cellular level. Since women have two copies of the same gene, it is possible that a woman can carry one gene for normal enzyme activity and one for reduced enzyme activity (heterozygous status), resulting in two distinct populations of RBCs: G6PD normal and G6PD deficient cells. The G6PD normal RBC sub-population may mask the G6PD deficient subpopulation, in quantitative and qualitative tests, resulting in a false G6PD status. Methods such as flow cytometry address this problem, by measuring enzyme activity at the cellular level, however this technique is complicated, expensive and labour intensive.

In order to determine the exact identity of a G6PD variant, a number of DNA-based genotyping procedures exist. While technically demanding, the outcome provides an exact picture of the underlying G6PD variant. In future, genotyping may have the potential to identify known gene polymorphisms that are associated with different haemolytic risks. Many genotyping techniques depend on knowing the regional prevalent mutations, and risk misclassifying an unknown deficiency trait. For clinical purposes, measuring the enzyme activity either through qualitative assays or through quantitative assays remains the best and most feasible practice.

What were the main conclusions of the workshop?

- The participants emphasised that the exploration of G6PD activity and the risk of 8-aminoquinolone induced haemolysis is of high priority in malaria research:
  - Data were presented demonstrating that all patients receiving high dose 8-aminoquinolines experience a drop in haemoglobin concentration; haematological status is an important consideration in case management
  - Data on haemolytic events in the course of standard 8-aminoquinolone treatment need to be collected and correlated to G6PD activity of the respective individual
  - Data on haemolysis attributable to malaria infection alone need to be collected
  - As G6PD deficiency is X-linked, a special focus needs to be put on G6PD patterns of females who are at significantly higher risk than men of not being recognized as G6PD deficient by standard tests, and who may eventually suffer from haemolysis in the course of 8-aminoquinolone treatment.
  - A clear, clinical definition of significant haemolysis needs to be developed.

The workshop concluded on the importance of deriving a quantifiable and universally applicable cut off for G6PD activity, and the haemoglobin level above which primaquine could be safely given. This would ensure that anti-relapse medication can be targeted safely to those who need it most;

- Data is needed on phenotypic and genotypic distribution of G6PD deficiencies in areas where populations are at risk of malaria infections
- An in-depth field evaluation of currently available diagnostic assays (quantitative and qualitative) is needed to determine the most robust tools suitable for field deployment
- There is emerging evidence that single dose primaquine (0.25 mg base/kg) can be safely administered even in G6PD deficient individuals, with potential to block transmission of *P. falciparum*
- Studies are needed to assess the risk of haemolysis versus the haematological consequences of recurrent bouts of *P. vivax* malaria if radical cure is not provided.

The proceedings of the workshop were published in the Malaria Journal in 2013 (Section 4.1.3)22.
What needs to be done next?

APMEN has funded several projects either focused on G6PDd exclusively or as a component, through the Country Partner Technical Development Program. These projects are providing important information on testing novel diagnostics, assessing the prevalence of G6PDd, and identifying new genetic G6PD variants associated with reduced enzyme activity. This work is nearing completion and results will be shared with Country Partners.

APMEN G6PD RESEARCH PROJECTS – PROGRESS IN 2014

<table>
<thead>
<tr>
<th>Location</th>
<th>Project</th>
<th>Questions this project addresses</th>
<th>Implications and Applications</th>
</tr>
</thead>
</table>
| Cambodia | Dr Saorin Kim, Pasteur Institut Pasteur du Cambodge Glucose 6 phosphate enzyme activity dynamics and G6PD qualitative test performance in G6PD deficient Cambodian patients undergoing weekly primaquine for acute uncomplicated vivax malaria | What is the degree of haemolysis that constitutes an unacceptable clinical risk to the patient? What is the degree of haemolysis that constitutes an unacceptable clinical risk to the patient? What are acceptable test characteristics of rapid G6PD tests in various populations and field conditions? | Set within a primaquine clinical trial, this open label case control study is looking to:  
- Evaluate Fluorescent Blood Spot (FST) against spectrophotometry  
- Investigate if G6PD activity varies during P. vivax malaria treatment  
- Assesses is FST is sufficient to predict haemolysis in G6PD deficient individuals treated with primaquine  
Quantifying the presumed risk of haemolysis and assessing whether the FST may be an accurate predictor of haemolysis will inform health departments and officials on the applicability of FST for facilitates with limited diagnostic facilities. |
| China | Dr Zhou, Jiangsu Institute for Parasitic Diseases Population Screening for G-6-PD deficiency in China using an enzyme assay on filter paper dried bloodspots | What are acceptable test characteristics of rapid G6PD tests in various populations and field conditions? | This research is looking at the prevalence of G6PD in both central and southern China to guide the widespread use of primaquine as a radical cure in these regions.  
This study has confirmed that the high and scattered prevalence of G6PD does not support the use of primaquine in either province without prior G6PD testing.  
Efforts are now focused on identifying a reliable, easy to use and cost effective G6PD test. The results will be of significant benefit for any future screening programs working with large numbers of people. |
| Indonesia | Dr Ari W. Satyagraha, Eijkman Institute for Molecular Biology Prevalence of G6PD Deficient Individuals in Bangka | Prevalence mapping of G6PD deficiency and variant types as well as haemolysis risks across major malaria endemic settings. | The Ministry of Health has targeted Bangka Island as malaria free by 2015. Providing detailed data on the local distribution of G6PD deficiency is essential for any malaria elimination program as is the case for Bangka Island.  
Having a detailed pictured of G6PD deficiency among the local population will assist the local and national health officials in planning and implementing malaria intervention programs, especially in relation to the widespread deployment of primaquine. |
| | Dr Jontari Hutagalung, University of Gadjah Mada Spatial analysis of the incidence of G6PD mutations in Plasmodium vivax malaria infection in South Central Timor (SCT), East Nusa Tenggara (ENT) Province, Indonesia | Prevalence mapping of G6PD deficiency and variant types as well as haemolysis risks across major malaria endemic settings. | The local prevalence on G6PD deficiency was understood to be high in SCT and ENT however reliable data is not readily available.  
This project is collecting and analysing data on the distribution of G6PD deficiency and malaria cases in a region where the malaria and infant mortality rates are twice the national average. Processing of samples and the analysis of the results is on-going.  
Ultimately the prevalence of G6PD and the associated risk of haemolysis will determine the most appropriate approach to delivering safe and effective radical cure in these two regions. |
is becoming an increasing public health threat. Over recent
years, it has become apparent that there are similarities in
the challenges faced in detecting *P. falciparum* Artemisinin Resistance (ArtRes) and *P. vivax* Chloroquine Resistance (CQR), particularly the difficulty in defining the patients harboring drug resistant parasites, and the lack of molecular markers of resistance.

What were the objectives of the workshop?

The Resistance Workshop had the following objectives:

- Review the clinical and public health consequences of drug resistance
- Promote the importance of monitoring antimalarial drug resistance, using standardised methods, and sharing results from different locations
- Gather regional perspectives on surveillance of artemisinin resistant *P. falciparum*, and review alternative strategies for its treatment, containment and elimination. Discussion on the ways to improve the monitoring and coordination of chloroquine-resistant *P. vivax* surveillance.

<table>
<thead>
<tr>
<th>China*</th>
<th>Prof Gao Qi, Jiangsu Institute for Parasitic Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Improve the accuracy of <em>P. vivax</em> case reporting using molecular methods</td>
<td>What are acceptable test characteristics of rapid <em>P. vivax</em> tests in various populations and field conditions?</td>
</tr>
<tr>
<td>This project developed LAMP (loop isolated mediated isothermal amplification) a molecular method that is able to <em>P. vivax</em> infections in low resource settings. The visualized LAMP method offers a sensitive, rapid and economic method for detecting low density <em>P. vivax</em> infections in resource-limited field settings. This is an important tool for detecting infections which if left untreated might continue to sustain infection.</td>
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<table>
<thead>
<tr>
<th>Indonesia</th>
<th>Dr Ari W. Satyagraha, Eijkman Institute for Molecular Biology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survey of G6PD variants of Sumba Island and development of PCR primers for each</td>
<td>Prevalence mapping of G6PD deficiency and variant types as well as haemolysis risks across major malaria endemic settings. What are acceptable test characteristics of rapid G6PD tests in various populations and field conditions?</td>
</tr>
<tr>
<td>The results of this survey have direct implications for the application of 8-aminooquinolones for Sumba Island. Precise knowledge on the distribution and degree of G6PD deficiency within the local population aid in designing strategies and treatment guidelines. In addition the first generation of the Carestart G6PD RDT (Accessbio) was evaluated. Compared to spectrophotometry, the Carestart G6PD RDT was found to have sensitivity and specificity of 73% and 99% respectively.</td>
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<tr>
<th>Republic of Korea</th>
<th>Dr Jung-Yeon Kim, Center for Disease Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evaluation of sensitivity and specificity of RDTs using microscopy and PCR in vivax malaria detection</td>
<td>What are acceptable test characteristics of rapid <em>P. vivax</em> tests in various populations and field conditions?</td>
</tr>
<tr>
<td>This project had both a diagnostics and surveillance focus. This project had both a diagnostics and surveillance focus. The diagnostics components of this project have provided important information that will aid in the selection of effective diagnostics for use in health centres in South Korea. Results published.</td>
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<table>
<thead>
<tr>
<th>Philippines</th>
<th>Dr Fe Espino Esparmza, Research Institute for Tropical Medicine</th>
</tr>
</thead>
<tbody>
<tr>
<td>A prevalence study of Glucose-6-Phosphatase Dehydrogenase deficiency and operational issues in applying the test in resource poor areas in the Philippines</td>
<td>What are acceptable test characteristics of rapid G6PD tests in various populations and field conditions?</td>
</tr>
<tr>
<td>This project determined the distribution of G6PD enzyme activity among randomly selected high school students and compared four RDTs to determine the test most suitable for widespread deployment. G6PD deficiency prevalence was almost 8x higher (15.2% vs. 1.9%) as had been reported earlier, possibly due to different ethnic origin of the target population compared to former studies. This finding highlights the importance to consider the ethnic background of individuals with regards to G6PD. Considering the operational characteristics of the assays evaluated none of the test met the requirements of local health centres. This result emphasizes the need for an easy to use, cost effective and reliable diagnostic G6PD test.</td>
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4.2.4 THREAT OF ANTIMALARIAL RESISTANCE TO THE ELIMINATION OF MALARIA WORKSHOP IN BALI, INDONESIA – MARCH 2013

What was the rationale of the workshop?

The emergence and spread of drug resistance is a major threat to the elimination of malaria. Patients treated with antimalarials to which the Plasmodium strain has reduced susceptibility have a prolonged course of illness, the severity of disease increases, and may eventually result in death if effective antimalarials are not given in a timely fashion. The global strategy for the treatment of malaria is currently dependent upon Artemisinin Combination Therapy (ACT), thus the rise of Artemisinin Resistance in the Greater Mekong region poses a huge threat to the elimination of malaria. Drug resistance in *P. vivax* is not restricted to artemisinin alone. Although less severe than in *P. falciparum*, drug resistance is becoming an increasing public health threat. Over recent years, it has become apparent that there are similarities in...
What is antimalarial drug resistance and what are the consequences?

The consequences of emerging drug resistance include progressive and prolonged morbidity, anaemia and increased mortality. In pregnancy, the multiple febrile episodes of malaria associated with treatment failure result in lower birth weight, poor birth outcomes and increased perinatal mortality. There are known associated socioeconomic costs resulting in reduced school performance, lost productivity and resulting substantial macro-economic costs. The rise of multidrug resistance and recurrent parasitaemias undermine malaria control programmes if not monitored and addressed adequately.

One of the early symptoms of drug resistance is delay in the initial parasite clearance in patients, following treatment. This is associated with an increase in gametocyte carriage in the blood, resulting in an increased transmission potential of the parasite to new hosts. As a result, the overall number of cases within a population rises, and there is an increased risk of epidemics. As resistance increases, malaria parasites are no longer cleared from the body in the presence of antimalarial drugs. Relapses occur after treatment has been completed. Initially, these occur late (6-9 weeks after treatment) and are often overlooked, or regarded as new infections. As resistance takes hold, the median time to recrudescence gets shorter as the parasite is able to survive in higher blood concentrations of the drug. In the late stages, the parasites continue to multiply even after high doses of the drug are given; patients can deteriorate, develop severe disease and die.

Where are the main hotspots for drug resistance?

Most research work on antimalarial drug resistance has focused on P. falciparum, with chloroquine resistance first reported in 1957 in the eastern regions of Cambodia. Fifty years later, the Thai-Cambodian border and Thai-Myanmar border remain the main hotspots for the emergence of multidrug resistant (MDR) P. falciparum. This species is now resistant to chloroquine, sulfadoxine-pyrimethamine, mefloquine, and mostly recently, to artemisinin.

The epicentre of P. vivax chloroquine resistance appears to be the island of New Guinea, where chloroquine resistance was first reported in 1989; clinical trials have demonstrated unequivocal evidence of high-grade resistance with early clinical deterioration requiring hospitalization, delayed parasite clearance, and early recurrent parasitaemia. Partially effective drug treatment has been proposed as an important contributing factor to associated reports of severe P. vivax malaria. More recently, reports reveal declining chloroquine efficacy against P. vivax from Ethiopia, Madagascar, India, western Indonesia, South Korea, Myanmar, Thailand, Brazil and Columbia.

How can antimalarial drug resistance be tracked?

Recognising and defining the scale of the problem is critical in combating the public health threat of drug resistance. Based on resistance data from representative sets of samples collected within an area, treatment guidelines can be revised and intensive control and containment strategies implemented. To achieve this requires the application of
sensitive and robust tests and clinical trials. The use of insensitive methods has long been a major confounding factor in defining antimalarial drug resistance. This is highlighted by a study by Stepniewska et al. who found that the popular 14 day assessment of antimalarial drugs had a very low sensitivity (between 0 and 37%) to detect treatment failures of *P. falciparum*. Extending the follow up to 28 days or longer increased the sensitivity of the test considerably\(^\text{24}\). For much of the 1990s, clinical trials failed to detect the emergence of chloroquine resistance. When appropriate tests were conducted, it became apparent that chloroquine and antifolate resistance had already spread throughout much of Asia and Africa.

The interpretation of clinical efficacy trials is more complex for *P. vivax* than *P. falciparum*, since *P. vivax* recrudescence (treatment failure from the initial infection) is confounded not only by new infections (reinfections), but also the occurrence of relapses, arising from activation of the dormant liver stages. Although genotyping can determine patients with heterologous infections (co-infection with different genotypes), and distinguish these patients from those considered treatment failures, it cannot distinguish between homologous infections (similar genotypes) arising from either a true recrudescence, or a relapse from hypnozoites generated from the prior blood stage infection. The confounding effect of such relapses varies considerably between geographical locations, both for the absolute risk of relapse and the timing at which these occur.

In the workshop discussions, similarities were noted between both *P. falciparum* and *P. vivax*, with regard to the difficulty in defining clinical resistance. Whereas conventional clinical efficacy trials focus on documenting the recurrence of parasitaemia, closer attention is required towards the early parasite clearance. In patients with *P. falciparum* treated with artemesunate, the slope of clearance is an informative measure of resistance (more information can be found on the WWARN website, www.wwarn.org). For *P. vivax*, early parasite clearance is a good marker of drug efficacy, which is not confounded by relapses from the liver stage.

Better clinical protocols are needed to define early parasite clearance in *P. falciparum* and *P. vivax*, as well as better ways of distinguishing between relapses and recrudescent infections. Although *P. vivax* cannot be sustained in continuous ex vivo culture, short term culture can provide measures of ex vivo drug susceptibility. The identification of molecular markers of drug resistance for artemisinin resistance in *P. falciparum* and chloroquine resistance in *P. vivax* would be an enormous step forward to monitoring
resistance on a molecular basis. If respective markers can be identified, collecting and providing evidence of developing resistance within given areas and populations would be hugely facilitated.

**What did the workshop achieve?**

The workshop emphasised the huge threat posed to the elimination agenda from the emergence and spread of antimalarial drug resistance.

Several priority areas were identified:

- Standardised methodologies to facilitate collection and sharing of drug efficacy data across the Network. These include deriving a common protocol for *P. vivax* drug efficacy monitoring, and identification of molecular markers of resistance for ArtRes in *P. falciparum* and CQR in *P. vivax*.

- The roles of active and passive surveillance for identifying locations of ongoing transmission (hot spots) and populations at greatest risk (“hot pops”) were reviewed. There was agreement that active case detection would need to be enhanced as malaria is eliminated from endemic areas.

- The potential role of mass drug administration (MDA) was raised. Results of studies showing high levels of subpatent parasitaemia which are missed by routine microscopy suggest that testing and treating would not detect the major reservoirs that are sustaining transmission. The proposed use of MDA was received with mixed enthusiasm, with several issues identified that would need to be addressed if countries were to deploy such a strategy. The first was whether MDA, using a combination of newer antimalarial drugs, is feasible, safe, acceptable and efficacious as a tool for eliminating falciparum malaria. This question is currently being addressed by ongoing, multi-centre studies. If countries decide in future to proceed with MDA, the main challenge will be community engagement and involvement. MDA can only be successful and effective if the majority of residents in the targeted population understand the purpose of the MDA, and participate.

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5. BUILDING THE EVIDENCE

After identifying the main challenges for *P. vivax* elimination, the next phase of the strategy is to **Generate the Evidence** needed to address these. Generating Evidence has been a key component of the Vivax Working Group agenda since 2009. To assist with this APMEN allocated a budget to the Vivax Working Group to support country-led research activities. The Vivax Working Group funds and provides technical assistance to country-led research activities as part of the APMEN Country Partner Technical Development Program.

This program supports research projects that address the research priorities that are set and reviewed by Working Group members. Aligning the APMEN Country Partner Technical Development Program to these priorities provides Country Partners with the means to direct a regional operational research agenda that may provide some of the evidence needed to change policy and practice. The program is also helping develop regional research capacity.

5.1 APMEN COUNTRY PARTNER TECHNICAL DEVELOPMENT PROGRAM

Since 2009, the Working Group has been coordinating and overseeing the APMEN Country Partner Technical Development Program (previously APMEN Small Grants Program). This program funds research projects that focus on the priorities defined and reviewed at each APMEN annual meeting by Country Partners and Partner Institutions. The activities within this program provided an opportunity for National Malaria Control Programmes and Network Partners to build national research and technical skills, and undertake projects that may contribute the evidence needed to influence policy and practice.

Research projects are assessed against the following criteria:

**Feasibility**
- Precise objectives
- Sound methodology and study design
- Logistical challenges of the proposed work (e.g. time frame, number of isolates, number of centres)
- Track record of researchers and collaborators in conducting research and delivering outputs
- Relevant expertise of associated partners

**Significance**
- Proposal addresses APMEN priority themes
- Has regional implications across member countries
- Has relevance for National Malaria Control Programme Treatment Policy
- Presents innovative ideas in unique settings

**Budget**
- Provides value for money (e.g. add-on studies to existing projects)
- Provides pilot data for subsequent application to other funding sources

APMEN has a well constituted panel of peer reviewers, all of whom have experience in reviewing research applications, including ethical assessment, in at least one of the following: their own institution, in international panels, in the Australian National Health and Medical Research Council (NHMRC), or equivalent funding groups. Each proposal was reviewed by at least two peer reviewers and then sent to an executive Selection Panel, made up from the APMEN Secretariat and Vivax Working Group Coordinating Team, for final assessment.

The Vivax Working Group Coordinating Team provides technical assistance on proposal development, ethics review, field and laboratory implementation, data collection and analysis, reporting and publication.

This section provides a description of the projects and activities undertaken as part of the APMEN Country Partner Technical Development program, according to the priority themes defined by the Group.
5.2 ACHIEVEMENTS OF THE VIVAX WORKING GROUP FOUR PHASE STRATEGY

The main achievement from the Vivax Working Group four phase strategy has been the facilitation of knowledge transfer across borders and between country and research partners. In doing so, the Working Group has been able to build research capacity across the Network Research partners have been able to generate the tools required to facilitate elimination activities. Country Partners continue to assist researchers to focus on the translational aspects of their work, and empower individual stakeholders to plan and implement new projects and elimination activities.

Identifying Knowledge Gaps

- Four literature reviews have been undertaken to address the three research priorities:
  - The status of malaria research in the member countries
  - The evidence for the clinical efficacy of primaquine antirelapse treatment
  - The key knowledge gaps in G6PD diagnostics

The geographic spread and extent of drug resistant vivax malaria

Building Consensus

- Since 2010, the Vivax Working group have convened at APMEN annual meetings and technical workshops to share knowledge and best practice:
  - Vivax Research Priorities – Feb 2010 (Colombo, Sri Lanka)
  - *Plasmodium vivax* Genotyping – May 2011 (Sabah, Malaysia) and May 2012 (Incheon, Korea)
  - Planning primaquine clinical trials - September 2011 (Jiangsu, China)
  - G6PD deficiency diagnostics - May 2012 (Incheon, Korea)
  - Threat of antimalarial resistance to the elimination of malaria - March 2013 (Bali, Indonesia)
  - Site visits to discuss local issues and capacity building
  - Regular liaison with Working Group representatives to assess progress, challenges, and emerging regional priorities
  - Opportunistic meetings at major international conferences, promoting the advocacy of the goals of *P. vivax* elimination.

Generating the Evidence

- The Vivax Working Group has a budget to support country lead research activities as part of the “APMEN Country Partner Technical Development Program”.
- These grants focus on country-specific components with greatest relevance to the research priorities.
- Applications selected following scientific peer review and ethical approval
- In total 21 projects have been funded to generate knowledge on surveillance (16), diagnostics (4) and clinical trials (3).
- Support in the design, implementation, analysis and reporting provided by the Working Group coordinating team and research partners.

Influencing Policy and Activities

- The final part of the Working Group strategy is to translate new information and evidence into practice.
- Each species of malaria has intrinsic biological differences that determine the optimal strategies for combating it; new tools and approaches will need to be deployed to overcome these. However, many of the malaria control issues are common to all species, emphasising the importance of health systems strengthening and an integrated program.
- After four years the Working Group work plan and priorities are being re-assessed and adapted. Future APMEN meetings will continue to review the current challenges of malaria elimination and build upon our successes to ensure a dynamic environment from which to eliminate malaria from the region.
BUILDING THE EVIDENCE // PG 37

5.3 RESEARCH PRIORITIES OF THE VIVAX WORKING GROUP

In 2011, the Working Group convened in Kota Kinabalu, Malaysia. During this meeting, priority areas of the Working Group were defined by Country Partners and Partner Institutions. Based on criteria of critical importance to regional elimination, available resources and viability, three themes were selected: Surveillance, Diagnostics, and Treatment. These themes have since guided the activities of the Working Group, including research projects funded and undertaken, workshops, and trainings.

- SURVEILLANCE

What are the main challenges?

In regions with high malaria endemicity, surveillance is aimed at detecting and treating patients presenting to healthcare facilities with symptomatic disease; this is known as “passive” surveillance. However, in low transmission settings, a large proportion of people are asymptomatic with sustained low density or submicroscopic infections; if individuals do not suffer from discomfort from their infection, they are unlikely to seek medical attention even though they can still transmit malaria. The ability of P. vivax to lie dormant in the liver for weeks or months needs special consideration. Hypnozoites are inactive, sparse and cannot be detected with the currently available diagnostic tools. There is a need to understand the evolving epidemiology of P. vivax malaria and to develop surveillance methods to detect asymptomatic cases, so that individuals carrying parasites can be identified and treated to prevent them from transmitting the disease. Once detected, parasite populations need to be assessed for their origin (locally transmitted, or imported from another location) and whether they are likely to respond well to treatment (i.e., to assess potential drug resistance).

Host characteristics play an important role in the treatment of P. vivax malaria. The only available drug for radical cure, primaquine, can cause severe side effects in patients with Glucose – 6 – Phosphate Dehydrogenase (G6PD) deficiency. G6PD deficient is one of the most common enzyme defects in humans. Whilst G6PD deficiency offers some protection against P. vivax infection, it increases the risk of haemolysis following administration of some drugs, including primaquine. The potential risk of hemolysis following primaquine, results in dosing strategies that are often guided more by safety concerns than by efficacy data. These concerns are particularly important in settings where G6PD testing is not done due to limited resources. Better understanding of the extent of G6PD deficiency in given populations can help to adapt treatment strategies for radical cure of P. vivax.

Why are these challenges important?

As the number of P. vivax malaria cases decreases and malaria elimination becomes feasible, surveillance needs...
to shift from passive to active detection of malaria. In order to identify all infections, surveillance needs to be extended from health care facilities toward communities, to diagnose and treat asymptomatic carriers, known as “reservoirs of infection”. The goal of active surveillance is to detect all individuals infected with *P. vivax* in the community (including those who are asymptomatic), treat them and prevent transmission. Detection of low density parasitaemia is difficult since many of the current diagnostic tests lack adequate sensitivity. Active surveillance requires considerable resources; hence, a cost effective option is to focus on the most likely populations (“hot pops”) in the most likely areas (“hot spots”). However, there is no consensus on how these high-risk populations and areas should be identified.

Depending on the prevalence of G6PDd the risks for side effects from radical cure can vary. In addition the risk for haemolyses varies with the G6PDd. Understanding the prevalence of G6PD deficiency in populations at risk of malaria, characterizing the variants that occur and getting more knowledge about the spatial distribution and diversity of those variants is important to design better treatment strategies for 8 aminoquinolones and for malaria elimination.

**DIAGNOSTICS**

**What are the main challenges?**

Diagnostic tests for malaria are now available which can reliably detect clinical cases of *P. vivax* infection. Microscopy and rapid diagnostic tests may be sensitive and specific enough to confirm malaria among ill patients presenting for care, but this does not extend to subclinical infections with very low parasite densities. The latter play a major role in sustaining the transmission potential of malaria in the final stages of malaria elimination. For conducting malaria epidemiological surveys, reactive infection detection and control activities in low transmission areas, diagnostics with higher sensitivity are required. The detection of subclinical *P. vivax* infections is particularly difficult as hypnozoites cannot be detected with the tools currently available.

Once *P. vivax* malaria is diagnosed, the initial priority is to treat the blood stages of infection with a schizontocidal drug. To eliminate dormant liver stages, any treatment needs to include primaquine. Ensuring that primaquine can be administered safely requires identifying patients with G6PD deficiency, who are at greatest risk of haemolysis. The currently available tests to assess G6PD activity are expensive and complicated to use in routine patient care. Furthermore, G6PD activity is a continuous variable, yet the available point-of-care diagnostic tests are qualitative (i.e. results are given as positive or negative). A better understanding is needed of the optimal outputs for these qualitative tests, and the relevance that these have with regard to patient safety. Alternately, point-of-care quantitative tests would provide a continuous measure of G6PD activity and the level of enzyme activity that constitutes an unacceptable risk of haemolysis.

**Why are these challenges important?**

Early diagnosis is critical to ensure early treatment of *P. vivax* infection, to prevent progression of disease, and to reduce ongoing transmission of malaria via the mosquito vectors. Affordable diagnostic tests that can quickly and reliably detect *P. vivax*, even at very low density parasitaemia, are needed to identify infected individuals. This process is essential for the implementation of strategies based on screening followed by treatment. Quantitative G6PD tests that can be used widely and reliably in the field are needed for the safe administration of 8-aminoquinolines, such as primaquine. In the absence of assured safety, many health care providers are reluctant to prescribe 8-aminoquinolines.

**TREATMENT**

**What are the main challenges?**

The radical cure of *P. vivax* infection requires killing of both the asexual peripheral parasitaemia (schizontes) and the latent dormant liver stages (hypnozoites). The treatment of the peripheral parasitaemia is being confounded by the spread of resistance to chloroquine, the first line treatment in the majority of APMEN countries. The treatment of the latent dormant liver stage still relies on primaquine, despite safety concerns about the use of this drug in G6PDd individuals.

**Why are these challenges important?**

An inability to achieve radical cure of all stages of the parasite results in recurrent infection, increased transmission, and the spread of resistant parasites.

Radical cure of *P. vivax* infection requires administration of highly effective schizontocidal drugs in combination with primaquine; however, in practice, treatment is often suboptimal. Primaquine is often not prescribed for fear of patient safety, patients may adhere incompletely to a full course of treatment, or the infecting parasite is resistant to the drugs. To facilitate elimination, the radical cure of *P. vivax* needs to be optimised to ensure that treatment can be given safely and effectively to all patients harbouring parasites.
6. SURVEILLANCE

APMEN recognises the importance of both host and parasite surveillance tools to facilitate malaria elimination and prevent resurgence. Within the surveillance theme, the Working Group has facilitated the funding of 13 projects in five countries to address the specific challenges of detecting subpatent infections, identifying imported malaria cases, seeking important reservoirs of infection, ensuring early detection of outbreaks, detection of transmission hotspots, and identifying G6PD deficient patients at risk of primaquine-induced haemolysis.

Activities in parasite surveillance included developing simple laboratory methods for detecting low level *P. vivax* infections in limited resource sites\(^25\). Such methods will be particularly important in pre-elimination regions, where subpatent infections may continue to sustain transmission, even as the number of symptomatic patients declines. In addition, standardized assays were established for genotyping *P. vivax* parasites, and these were applied by APMEN partners in Indonesia, Sri Lanka, China, Bhutan, Malaysia and the Republic of Korea. These studies demonstrated the utility of parasite genotyping to assess the impact of interventions on parasite transmission in different endemic settings, and to identify outbreaks\(^26\). The data is currently being pooled across the countries to identify shared reservoirs of infection between countries, and to assess the distinction of imported from local cases.

Activities in host surveillance included identifying populations at greatest risk of harbouring parasites, as well as patients with G6PD deficiency (G6PDD). In Bhutan, a novel method used mobile technology to assure more complete and timely reporting of clinical cases. In China, a project investigated how sero-epidemiologic and spatio-temporal trends of malaria related to declining malaria endemicity. Population screening of G6PDD was undertaken in China and Indonesia. APMEN has supported the mapping of G6PD deficiency in Cambodia, Philippines, China, and Indonesia, and the information from these studies has been shared with the Malaria Atlas Project (MAP), an APMEN Partner Institution that is collating a global map of G6PD distribution\(^27\).
TABLE 1: PROJECTS ADDRESSING IMPROVED SURVEILLANCE OF *P. VIVAX*

<table>
<thead>
<tr>
<th>Projects</th>
<th>Parasite Surveillance</th>
<th>Host Surveillance</th>
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<tbody>
<tr>
<td>China</td>
<td>Prevalence surveys</td>
<td>Genetic diversity</td>
</tr>
<tr>
<td>Improving the accuracy of <em>P. vivax</em> case reporting using molecular methods*</td>
<td>✔</td>
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<tr>
<td>Population screening for G-6-PD deficiency in China using an enzyme assay on filter paper dried bloodspots</td>
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<td>✔</td>
</tr>
<tr>
<td>Sero-epidemiological analysis for monitoring malaria elimination in China</td>
<td></td>
<td>✔</td>
</tr>
<tr>
<td>Target malaria elimination intervention in China using spatial-temporal distribution analysis</td>
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*see also ‘Diagnostics’ section

Brief summaries of Projects addressing improved surveillance of *P. vivax*

Prof Gao Qi (Jiangsu Institute of Parasitic Diseases, Wuxi, Jiangsu, People’s Republic of China) conducted a study entitled *Improving the accuracy of *P. vivax* case reporting using molecular methods*. One component of this study, detailed in section 6.2, assessed diagnostic tools for *P. vivax*. A second component involved genotyping 161 *P. vivax* isolates sourced from central China and imported cases. The genotyping data demonstrated unstable transmission dynamics in central China, with frequent outbreaks presenting a risk to resurgence. Parasite gene flow was moderate between Anhui and the recently malaria-free Jiangsu Province, demanding dedicated surveillance in Jiangsu to prevent the re-introduction of malaria. The 7 markers used provided little resolution between the central Chinese and imported cases demonstrating the need for extra markers to identify imported cases in this region. **Status:** Study complete. A manuscript is in preparation.

Dr Zhou (Jiangsu Institute of Parasitic Disease, Wuxi, Jiangsu, People’s Republic of China) conducted a study entitled *Population screening for G6PD deficiency in China using an enzyme assay on filter paper dried blood spots*. In this project, 10,000 individuals in Central and Southern China were screened for G6PD deficiency. The study set out to identify the G6PD status in defined ethnic populations in central and...
southern China and assess hospital based malaria incidence rates. Respective data are essential for successful radical cure campaigns supporting malaria elimination. Status: Study ongoing.

Dr Cao (Jiangsu Institute of Parasitic Diseases, Wuxi, Jiangsu, People’s Republic of China) conducted a study entitled Sero-epidemiological analysis for monitoring malaria elimination in China. The specific objectives of this project were to establish new laboratory based methods to identify populations with prior malaria exposure in Jiangsu and Hainan. The resulting data were mapped, and compared to results from standard detection methods. This new approach may support standard surveillance methods to identify potential reservoirs of infection that can be specifically targeted in the course of malaria elimination projects. Status: Study complete, a manuscript is being prepared for publication.

Dr Shui-Sen Zhou (National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention, Shanghai, People’s Republic of China) conducted a study on the Target malaria elimination intervention in China using spatial-temporal distribution analysis. A national database was assembled based on the Annual Parasite Index (API) records between 2001 and 2010. Annual incidence rates are being mapped and predictive incidence inferred. Although malaria incidence has been declining since 2006, closer scrutiny of API in selected high transmission provinces is being undertaken as part of vigilance for resurgence and sporadic outbreaks. The findings will be used to monitor and evaluate the impact of elimination activities. Status: Study ongoing.

Dr Jung Yeon Kim (Korea National Institute of Health, Korea CDC, Republic of Korea) conducted a project on the Evaluation of sensitivity and specificity of RDTs using microscopy and PCR in vivax malaria detection. In the course of this project, the team undertook diagnostic and parasite host surveillance studies. The diagnostic study is detailed in section 6.2. The parasite surveillance involved genotyping 50 P. vivax isolates, including 47 local Korean isolates and 3 suspected imported cases at 9 markers. High diversity was observed in the Korean population, possibly fuelled by imported cases. Moderate resolution was observed between the local and imported cases, demonstrating the potential utility of genotyping to confirm imported cases in South Korea. For host surveillance, the team documented the prevalence of G6PD deficiency among all patients recruited. The results will contribute to improving field based surveillance diagnostics. Status: Analysis is underway; an extra 50 P. vivax isolates are being genotyped.

Dr Noviyanti (Eijkman Institute for Molecular Biology, Jakarta, Indonesia) is studying the Genetic diversity of P. vivax in Indonesia on Bangka and Sumba Islands. In a cross sectional survey of 1500 patients were tested and 75 (5%) patients found to be positive for P. vivax. Molecular analysis of the positive cases is being used to inform the transmission dynamics of malaria within the affected population. This study aims to assess whether infection in these two islands originate from a common reservoir. The findings will help inform suitable strategies to reduce transmission. Status: A manuscript is in preparation for publication. Preliminary results were presented in May, 2013, at the Advances in Plasmodium vivax malaria research meeting in Barcelona in May 2013.

Dr Satyagraha (Eijkman Institute for Molecular Biology, Jakarta, Indonesia) has conducted a study entitled A survey of G6PDd variants on Sumba Island and development of PCR primers for each. This study aims to document the prevalence of G6PD deficiency in Sumba, and the presence of any novel G6PD variants. In this study, 5.9% of individuals were G6PD deficient; those with G6PDd were further analysed for enzymatic activity, and a novel G6PD variant was discovered. The study has implications for defining at risk populations who may be exposed to high dose primaquine regimens. Status: Oral presentations have been given at the following meetings:

- 13th FAOBMB Congress, 25-29 November 2012, Bangkok, Thailand
- DxG Advisory Workshop, 4-5 October, 2012, Bangkok, Thailand
- Advances in Plasmodium vivax malaria research, 29-30 May, 2013, Barcelona, Spain

Mr Hutagalung (University of Gadjah Mada, Yogyakarta, Indonesia) conducted a study Spatial analysis of the incidence of G6PD mutations in Plasmodium vivax malaria infection in South Central Timor (SCT), East Nusa Tenggara (ENT) Province, Indonesia. A total of 555 patients from 5 districts in ENT province were assessed for G6PD deficiency, anaemia and malaria. Malaria prevalence was low (<2%), but G6PD deficiency was moderately high (17.3%). Patients with malaria and G6PD deficiency are currently being mapped, to inform decisions on the benefit of radical cure, versus the risks of primaquine induced haemolysis. Status: Analysis is ongoing.

Dr Satyagraha (Eijkman Institute for Molecular Biology, Jakarta, Indonesia) is conducting a study on the Prevalence of G6PD Deficient Individuals in Bangka Island, in order to assess G6PD enzyme activity and the prevalence of malaria in 1500 individuals. Participants with low G6PD activity are being assessed for new G6PD variants. The study is documenting populations at risk of malaria, and the likely risks of deploying radical cure. Status: Study ongoing.

Dr Ekawati (Eijkman Institute for Molecular Biology, Jakarta, Indonesia), conducted a Community survey on knowledge, attitude and practice of malaria intervention (diagnosis &
conducted a project entitled Assessing the prevalence of malaria parasites in displaced populations who have returned or resettled in the post conflict districts of Kilinochichi, Mullaitivu and Mannar in Sri Lanka. Between 2011 and 2012, a total of 7000 participants were enrolled and tested for malaria with microscopy and rapid diagnostic tests. The G6PD activity of all participants was evaluated. No cases of malaria were documented by any of the test methods applied, the overall prevalence of G6PD deficiency within the study population was 2.9%. The study emphasises the degree to which Sri Lanka is nearing elimination. Status: Study complete.

Dr Abdullah (Institute for Medical Research, Jalan Pahang, Kuala Lumpur, Malaysia) conducted a Study of Drug sensitivity profile and Molecular Genotyping of Plasmodium vivax isolates in Sabah, Malaysia. The drug sensitivity study was proposed to use ex vivo methods to assess the efficacy of chloroquine in P. vivax isolates in Sabah. The aim of the genotyping study was to assess the impact of ongoing interventions on the stability of P. vivax transmission in Sabah. Using genotyping data from 97 samples, the study demonstrated unstable transmission with frequent outbreaks, possibly resulting from imported cases. Status: Study complete and results published29.

Dr Wangchuk (Department of Public Health, Public Health Laboratory (PHL), Ministry of Health, Bhutan), is conducting a Molecular Assessment of P. vivax transmission dynamics in Bhutan. The study aims to genotype the pre-treatment and treatment) for vivax malaria in Indonesia. In ten, randomly selected villages, 743 respondents were interviewed, of whom 87% had reasonable knowledge about malaria’s cause and symptoms, with the majority of respondents aware that untreated malaria could be dangerous and life-threatening. Staff at five primary health centres (PHCs) were also questioned. Two PHCs relied solely on clinical diagnosis, and in those with a microscopy service only half had received formal training. Artemisinin in combination therapy (ACT) was available for all malaria patients. The study presented key knowledge gaps of the local population and caretakers, and highlights that knowledge about malaria and its diagnosis and management could be improved. Status: Study ongoing.

Dr Randeniya (Department of Zoology, Faculty of Science, University of Colombo, Sri Lanka) assessed the Dynamics of Plasmodium vivax parasite populations during malaria elimination efforts in Sri Lanka, with low transmission and unstable malaria to assess the impact of ongoing malaria interventions on the stability of local transmission and risks of imported malaria. In addition, the diversity of the P. vivax circumsporozoite surface protein gene was assessed to examine its suitability as a vaccine candidate. A total of 60 clinical P. vivax isolates were genotyped at the Pvcsps gene as well as the APMEN markers. The Pvcsps study demonstrated high diversity and several sequences unique to Sri Lanka, with implications for vaccine development. Status: The Pvcsps results have been published28. Genotyping is underway.

Dr Galappathy (National Anti-Malaria Campaign Headquarters)
any recurrent, \textit{P. vivax} infections detected during a clinical trial (see below). The aim is to distinguish new infections from relapses, and define the local relapse patterns in Bhutan. The data will also inform the degree to which new malaria cases are imported or locally acquired. \textbf{Status:} Study ongoing.

**Dr Togbay (Vector Borne Disease Control Programme)** is conducting a study on \textit{Malaria elimination in Bhutan using mobile technology for disease mapping and early diagnosis}. The aim of this study is to develop web-based and mobile technology surveillance tools for case detection and monitoring, for the national malaria elimination program. The study is complemented by a cost-effectiveness evaluation and feasibility study that may facilitate the translation of these approaches to other sites. \textbf{Status:} Study ongoing.

**Dr Espino** conducted a prevalence study of Glucose-6-Phosphate Dehydrogenase deficiency and operational issues in applying the test in resource poor areas in the Philippines (see also ‘Diagnostics’ section). The team enrolled students at local high schools and found 15.1\% of 621 participants to be G6PD deficient. This high rate of G6PD deficient individuals may have implications for the deployment of radical cure of vivax malaria in the area. \textbf{Status:} Study complete; a manuscript is being prepared for publication.

**Collaboration with other partners**

Population screening of G6PDd was performed in many of the above described projects. APMEN is supporting the mapping of G6PD deficiency in Cambodia, Philippines, China, and Indonesia and the information from these studies will be included into initiatives such as the Malaria Atlas Project (MAP), an APMEN Partner Institute, who is collating global maps of G6PD distribution. \textbf{Status:} Study ongoing.

### What still needs to be done?

Remaining challenges to surveillance include:

- The need for further information on identifying the major reservoirs sustaining continued transmission in different endemic settings.
- Updating maps, defining areas and populations presenting reservoirs of infection. This will help to focus malaria control efforts to where they are needed most.
- Identifying areas with low level parasitaemia in the absence of clinical disease is particularly important.
- Devising suitable site-specific strategies for population based sampling will help to ensure that cost effective novel assays and diagnostic tools can be applied to detect the remaining reservoirs of infection.

With ever-increasing international travel, imported cases and identify their geographic origin. The pooled multi-country parasite genotyping data will provide important insights for the development of a molecular tool for determining the geographic origin of an infection.

The increasing prevalence of chloroquine resistance in \textit{P. vivax} is a major threat to containment and elimination efforts. Simple and cost-effective methods are required for high-throughput surveillance of chloroquine resistance. Molecular markers of resistance would greatly facilitate these surveillance efforts.

A better understanding of the variants of G6PD deficiency and relative risk for hemolyses will help to adapt treatment strategies for radical cure.

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7. DIAGNOSTICS

The Working Group is collaborating with international experts and organisations such as the Foundation for Innovative New Diagnostics (FIND) to evaluate and develop novel diagnostics. APMEN-supported projects have evaluate rapid diagnostic tests, distinguishing P. vivax from other species of infections (Republic of Korea), and have also found that loop-mediated isothermal amplification (LAMP) has similar test characteristics to PCR, but requires fewer resources (China).

To improve the diagnosis of G6PDd, the Working Group convened a meeting in Republic of Korea in 2012, which allowed country and international experts to discuss the current knowledge of G6PD tests (section 5.2.4). Subsequent projects in Cambodia and the Philippines were funded to assess the performance and operational issues of novel tests coming on to the market. Brief summaries of Projects addressing improved diagnostics of P. vivax

Dr Espino (Research Institute for Tropical Medicine, Philippines) has conducted study entitled A prevalence study of Glucose-6-Phosphate Dehydrogenase deficiency and operational issues in applying the test in resource poor areas in the Philippines. In this study, 621 high school students were assessed, using four G6PD rapid diagnostic tests. All patients were tested with a field test and a laboratory quality control method, and the results were compared. Between 50 - 79% of all deficient samples were correctly identified by one of the field tests; however, specificity was low. The study highlighted the technical challenges of deploying current G6PD screening tests in resource poor areas in Palawan. Status: Study complete. A manuscript is under preparation.

Dr Gao Qi (Jiangsu Institute of Parasitic Diseases, Wuxi, Jiangsu, People’s Republic of China) conducted a study entitled Improving the accuracy of P. vivax case reporting using molecular methods. A novel DNA test (LAMP) was assessed for its ability to diagnose malaria. Compared to microscopy, the sensitivity and specificity of the LAMP method were 98.3% (95% CI: 91.1-99.7%) and 100% (95% CI: 88.3-100%), and were in close agreement with a gold standard method (nested PCR). The new method ensured low risk of contamination. The study is helping to develop robust, sensitive P. vivax diagnostic tools for field settings, and detection of asymptomatic parasitaemias. Status: Study complete. Results published.

Dr Jung Yeon Kim (Division of Malaria and Parasitic Diseases, National Institute of Health, South Korea) conducted an ‘Evaluation of P. vivax Diagnostic Methods and Assessment of G6PD Prevalence and P. vivax Population Structure in Korea’.

<table>
<thead>
<tr>
<th>TABLE 2. PROJECTS ADDRESSING IMPROVED DIAGNOSTICS OF P. VIVAX</th>
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<tr>
<td><strong>Parasite Diagnostics</strong></td>
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<tr>
<td><strong>Funded Projects</strong></td>
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<tr>
<td>Philippines</td>
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<tr>
<td>China</td>
</tr>
<tr>
<td>Republic of Korea</td>
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<tr>
<td>Cambodia</td>
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*see also ‘Surveillance’ section
The development and improvement of G6PD point of care tests is urgently needed to facilitate field based rapid diagnosis of vulnerable populations. Tests which can produce quantitative readout of enzyme activity (Biosensors) will help to improve our understanding of the relationship between G6PD enzyme activity and the risk of primaquine-induced haemolysis.

Dr Kim (Institute Pasteur du Cambodge, Phnom Penh, Cambodia) looked at Glucose 6 phosphate enzyme activity dynamics and G6PD qualitative test performance in G6PD deficient Cambodian patients undergoing weekly primaquine for acute uncomplicated vivax malaria. The study is assessing the performance of the G6PDNAPD spot test over time, in patients presenting to clinics with P. vivax malaria, and during the subsequent clinical recovery. Its objective is to determine whether the performance of the fluorescent test changes in febrile patients. The study has implications for the predictive value of testing G6PD activity, and identifying patients at risk of haemolysis. Status: Study ongoing.

What still needs to be done?

Detection of low level parasitaemia infections remains a top priority. If these infections sustain transmission, then it is crucial that they are identified and destroyed. This calls for active case detection and the investigation of the feasibility, safety and effectiveness of suitable treatment strategies. An example of the latter is mass drug administrations in populations with a high prevalence of asymptomatic carriers.

This study assessed four RDTs in a study of 253 patients with malaria, and compared them to the gold standards of nested-PCR and microscopy. The investigators found that two of the evaluated tests detected >99% of all positive cases, whereas two inferior tests detected 93.0% and 94.7%, respectively. The results have helped to inform the choice of suitable rapid diagnostics, and also whether infections can be classified as locally acquired or imported. Status: Study published.33

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8. TREATMENT

The emergence of drug resistant *P. vivax* has been underestimated, and is likely to be undermining the elimination of *P. vivax* malaria.

In 2013, the Working Group convened a workshop on antimalarial resistance in Bali, which brought together Country Partners, Partner Institutions and international experts. The Working Group has supported three treatment trials in Bhutan, Malaysia, and Vanuatu & the Solomon Islands. These trials have been undertaken in regions with little or no prior experience in the conduct of clinical trials, and with limited resources. The Working Group also supported a community survey in Indonesia on knowledge, attitudes and perceptions of *P. vivax* malaria diagnosis and treatment in Indonesia.

**Brief summaries of Projects to improve treatments for *P. vivax* infection**

The Vector Borne Disease Control Program of the Ministry of Health in Bhutan is conducting a trial to Assess Parasitic clearance and recurrence rates among patients with vivax malaria on chloroquine and primaquine therapy. This trial is assessing the efficacy of chloroquine among children and adults with uncomplicated *P. vivax* malaria, as gauged by the risk of recurrent malaria at day 28. Primaquine is given at day 28 and patients followed for a year to look for late recurrences. **Status:** Study ongoing.

The Evaluation of safety and efficacy of two primaquine dosing regimens for the radical treatment of *Plasmodium vivax* malaria in Vanuatu and Solomon Islands is supported the Walter and Eliza Hall Institute and Ministries of Health in both the Solomon Islands and Vanuatu. The aim of the study is to compare the efficacy of different treatment regimens for primaquine in preventing early relapses from *P. vivax* in the Solomon Islands and Vanuatu. The secondary aim is to measure the safety and toxicity of primaquine when administered as a standard or high-dose regimen. **Status:** Study ongoing.

Dr William (Queen Elizabeth Hospital, Sabah, Malaysia) is conducting a clinical trial of Artesunate-mefloquine vs. chloroquine in patients with acute uncomplicated *P. vivax*: a randomised open label trial in Sabah, Malaysia in Kudat and Kota Marudu District Hospitals. The aim of this study is to determine whether the fixed combination of artesunate-mefloquine is superior to chloroquine in uncomplicated *P. vivax* infections in adults and children. **Status:** Study ongoing.

**What still needs to be done?**

Clinical trials require considerable resources and time but are critical to evaluate the efficacy of routine drugs as well as new drugs and regimens. Standardized methods for defining schizontocidal and antirelapse efficacy are needed in *P. vivax* clinical trials, in order to generate results that allow a direct comparison.

In the course of successful elimination programs, the fraction of symptomatic *P. vivax* cases among all treatment recipients will gradually decrease, whereas the proportion of asymptomatic individuals will increase. Exposing asymptomatic patients to potentially harmful drugs will require additional cost-benefit studies to gain a good understanding of the impact respective programs have on an individual as well as on a community level.

### TABLE 3: PROJECTS TO IMPROVE TREATMENTS FOR *P. VIVAX* INFECTION

<table>
<thead>
<tr>
<th>Funded Projects</th>
<th>Schizontocidal Treatment</th>
<th>Antirelapse Studies</th>
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<tbody>
<tr>
<td>Bhutan</td>
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<tr>
<td>Artesunate-mefloquine vs. chloroquine in patients with acute uncomplicated <em>P. vivax</em>: a randomised open label trial in Sabah, Malaysia</td>
<td>✓</td>
<td>✓</td>
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9. INFLUENCING POLICY AND PRACTICE

The last decade has brought significant gains in the global fight against malaria, with the malaria endemic countries of the Asia Pacific region making a substantial commitment to malaria control and elimination. Although there has been a reduction in the incidence of disease and associated morbidity and mortality, huge challenges remain if these gains are to be sustained, and ensure the complete elimination of the parasite. Strategies to achieve this will vary, according to the local endemic setting and resources available. Whilst strengthening of current malaria control activities is vital, this alone may not be enough to achieve elimination, since it is clear that the greater the pressure put on the parasite to survive, the greater its propensity to evolve new strategies for its own survival. APMEN brings together a unique partnership of stakeholders from National Malaria Control Programmes, policy makers, researchers, funding bodies and the World Health Organization, providing a forum to encourage the collaboration and innovation that will be crucial to achieving the ultimate goal for regional elimination of malaria.

The occurrence of different species of infection is particularly important in the Asia Pacific. Strategies based on early diagnosis, highly effective treatment with ACTs and vector control, have been key in the fight against *P. falciparum*, but are less efficient for the other human pathogenic malaria species such as *P. vivax*, *P. malariae* and *P. knowlesi*. This is apparent in the rising proportion of non-*falciparum* infections in many co-endemic regions. The Working Group was formed to bring greater attention to the elimination of *P. vivax*. Working together with Network Partners, the Working Group has focused on translational themes likely to have the greatest impact for National Malaria Control Programmes.

**Surveillance** can be broadly separated into characterising parasite, and host, populations. Since a high proportion of *P. vivax* infection can be asymptomatic, the former requires both active and passive case detection, and even then it is not yet possible to detect the liver stage parasites, which can lie dormant for many months. The risk of *P. vivax* relapse varies hugely across different endemic settings. Understanding the relapse patterns, and the relative contributions that locally acquired infection and imported cases make towards outbreaks is critical if limited resources are to be mobilised in the most efficient manner. For instance, the response to high rates of new infections might be very different to that due to high relapse rates; or where the predominant source originates from returning travellers or migrants, rather than from local reservoirs of infection. Novel genotyping tools have been developed that will allow discrimination of locally acquired and imported infections, and the elucidation of the underlying relapse patterns. Furthermore, multiplicity of infection (multiple strains infecting a single person) may provide a useful surrogate marker of background transmission dynamics that can also inform suitable control strategies, and monitoring of policy impact.

Host surveillance can help identify populations at greatest risk of infection, or most vulnerable to adverse effects of interventions. With regard to the former, the Vivax Working Group has been working closely with the APMEN Surveillance Group to develop strategies for case detection of “hot spots” and “hot pops”, so that control efforts can be prioritised. The Working Group has also been investigating human populations with reduced G6PD activity, who may be at risk of haemolysis due to administration of primaquine radical cure. The risk of haemolysis varies with the dominant local G6PD enzyme variants. Community surveys, coupled with investigation of known and novel variants, will help to gauge the risks and benefits of increasing coverage with primaquine-based regimens for radical cure. In collaboration with the
Malaria Atlas Project (MAP), a global map of prevalence and variants is being generated that will underpin these policy decisions.

The diagnosis of patients with *P. vivax* is also challenging and, as for surveillance, can be divided into the challenges associated with the parasite or the host. *P. vivax* survives at lower density infections than *P. falciparum*, making the diagnosis of parasites by microscopy and RDTs more difficult. New RDTs with improved sensitivity for blood stages have become available, as well as novel techniques such as LAMP and PCR, capable of detecting subpatent infections. The application of these tests needs to be balanced against the extra cost and logistics required to deliver them reliably. As the incidence of malaria reduces, such techniques need to become more cost effective, since they can be targeted to outbreak situations or to high risk populations. The most important diagnostic for the host is a reliable point of care test for G6PD deficiency. In areas where the risk of haemolysis with an 8-aminoquinoline is high, prior testing is essential to ensure patient safety. In the absence of reliable tests at an affordable price, many health carers refrain from prescribing radical cure for *P. vivax*, considering the health risk of haemolysis greater than the health risk of a future relapse. This is one of the most important impediments for the broad application of 8-aminoquinolones to eliminate malaria. After years of neglect, resources are now being directed toward overcoming this challenge. The Working Group has been working with partners at FIND and PATH to develop and test suitable diagnostics to achieve this.

Treatment of all stages of the parasite is then needed to kill all remaining parasites. This requires administration of both schizontocidal (blood stage) and hypnozonticidal (liver stage) antimalarial agents. Schizontocidal treatments are being undermined by the emergence and spread of drug resistant *P. vivax*, often hard to detect in the early stages, since recurrences are assumed to be relapses rather than recrudescence infections. Better methods are needed to detect early signs of CQR *P. vivax*, including identifying molecular markers that can be applied easily at large scale, to highlight emerging parasite populations. The comparative benefits of chloroquine versus ACTs for all species of infection need to be addressed. The ultimate challenge is the reliable and safe delivery of the radical cure of *P. vivax*. Current methods require long courses of primaquine which are rarely adhered to. Tafenoquine, a novel 8 aminoquinoline, can be given as a single dose which will improve adherence significantly; however, it will likely require reliable diagnosis of G6PD status prior to administration. The Working Group has been building clinical trials capacity and embarking on comparative drug studies to investigate novel therapeutic strategies.

Over the last four years, the Vivax Working Group has made significant progress in addressing many of these key issues. Having reviewed the existing evidence, identified priorities and implemented a process to gather relevant additional evidence, it is vital that this knowledge and experience is shared between partners, and where possible, translated into practice to impact malaria.

The themes of research need to be reviewed and regularly updated to ensure that they meet the needs of the Country Partners, to help achieve their elimination targets. Each species of malaria has intrinsic biological differences that influence the optimal strategy for combating it. However, whilst *P. vivax* is particularly challenging, many of the malaria control issues are common to all species; and thus, the importance of health systems strengthening and delivery of care cannot be overemphasised. Some countries, such as Sri Lanka and South Korea, highlight the importance of sustaining efforts, shifting towards measures to reduce reintroduction of malaria from across international borders. Finally, the evolution of parasite and vector resistance to drugs and insecticides threaten to turn back the hard earned successes of the last ten years. Now, four years into the APMEN program, there is an opportunity to once again review the current challenges of malaria elimination facing the region, to build upon existing experience to refine efforts, and to increase the capacity to ensure our ultimate goal of malaria elimination.
REFERENCES


### COMPLETE LIST OF APMEN VIVAX WORKING GROUP RESEARCH GRANTS

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<td>Artesunate-mefloquine vs. chloroquine in patients with acute uncomplicated <em>P. vivax</em>: a randomised open label trial in Sabah, Malaysia</td>
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Improving the accuracy of P. vivax case reporting using molecular methods

BACKGROUND

China initiated its malaria elimination program in July 2010, and has set the goal to eliminate malaria by 2020. Outbreaks of P. vivax in the early 2000s highlighted the major risk of resurgence with this species, and the importance of maintaining diligent surveillance. Due to the low levels of parasitaemia of P. vivax infections, malaria diagnosis is often missed by microscopy and RDTs. These cases are often asymptomatic and yet can represent a potentially important reservoir of infection. PCR-based diagnostic methods have high sensitivity and specificity but are often not widely used owing to economic and practical limitations. Improving molecular tools for diagnosis of low density infections and surveillance of outbreaks and imported cases is key to achieving China’s elimination goals.

The current study proposed to develop a novel molecular method, known as loop-mediated isothermal amplification (LAMP), for the sensitive detection of P. vivax infections in low resource settings. In addition, P. vivax genotyping of isolates from central China and imported cases was proposed to assess the local transmission dynamics in central China and to assess the utility of genotyping to confirm imported cases.

AIMS AND OBJECTIVES

i. To develop LAMP for detection of low density and sub-microscopic P. vivax infections in field settings.

ii. To determine the parasitological and clinical characteristics of P. vivax infections in pre-elimination settings.

iii. To assess the transmission dynamics of P. vivax infections in central China, and assess the utility of genotyping to confirm imported cases.

METHODS

A standard LAMP reaction using primers targeting the P. vivax 18S ribosomal RNA gene, was adapted into a visualized LAMP method by adding a microcrystalline wax-dye capsule containing the DNA fluorescence dye SYBR Green I prior to the initiation of the reaction. SYBR Green I dye fluoresces when bound to double-stranded DNA, enabling detection of amplified parasite-positive samples. Blood samples were collected on filter paper from febrile patients with suspected malaria. DNA was extracted using a simple boiling method and tested by the visualized LAMP method for P. vivax infection. The same samples were assessed for infection by microscopy and using a standard nested PCR assay.
For *P. vivax* genotyping, samples from central China and imported cases were collected from patients attending local health centers in Anhui and Jiangsu Province between 2008 and 2010. Dried blood spots were extracted using the Qiagen kits and genotyped at 7 of the APMEN consensus markers at the Australian Army Malaria Institute, Brisbane. Standard population genetic analyses were undertaken using the vivaxgen data analysis platform (vivaxgen.menzies.edu.au).

**RESULTS AND INTERPRETATION**

The results of the LAMP study have been published. Of the 89 samples tested, the sensitivity and specificity of the LAMP method were 98.3% (95% CI: 91.1–99.7%) and 100% (95% CI: 88.3–100%) compared to microscopy, and were in close agreement with the nested PCR method. The closed system method ensured low risk of contamination.

The genotyping study has been published. A total of 161 *P. vivax* samples were collected for genotyping, of which 128 could be genotyped successfully. Polyclonal infections were infrequent in 94 isolates from Anhui (4%) and 25 from Jiangsu (12%), with a trend for increasing frequency from 2008 to 2010 (2 to 19%), correlating with the rise of imported cases. Population diversity, as measured by the expected heterozygosity ($H_e$) was high in both provinces and across the years tested ($H_e=0.8–0.85$). Differentiation between Anhui and Jiangsu was modest ($F_{ST} = 0.1$). Several clusters of isolates with identical genotype profiles were observed across both Anhui and Jiangsu, possibly reflecting occasional epidemic transmission dynamics. None of 5 imported isolates shared identical haplotypes to any of the central Chinese isolates.

**CONCLUSIONS AND IMPLICATIONS**

The visualized LAMP method offers a sensitive, rapid and economic method for detecting low density *P. vivax* infections in resource-limited field settings. This is an important tool for detecting infections which if left untreated might continue to sustain infection.

Population genetic analyses of *P. vivax* in central China highlighted unstable transmission, with limited barriers to gene flow between the central provinces. The challenge of imported cases and risks of resurgence emphasize the need for continued surveillance to detect outbreaks early. Although parasite genotyping has potential to inform the management of outbreaks, further studies are required to identify suitable marker panels for resolving local from imported infections.

1. Report APMEN VxWG Genotyping Workshop May 2011
2. Tao et al. Parasites and Vectors 2011, 4:115
Target malaria elimination intervention in China using spatial-temporal distribution analysis (stage 1&2)

**BACKGROUND**

The People’s Republic of China launched its Malaria Elimination Program in July 2010, and significant gains are being made to achieve its goals. The country has a highly effective malaria case reporting system to monitor progress, with a long history of using Annual Parasite Index data to monitor elimination activities. The Malaria Atlas Project (MAP) is working with the country partners who developed this system to adapt this approach to the global scale for the surveillance of both *P. falciparum* and *P. vivax*.

**AIMS AND OBJECTIVES**

The overall aim of the study is to map the annual changes in China’s malaria situation during the last decade.

Specific aims included:

i) to produce accurate malaria prevalence map at the county level in China

ii) to establish mathematical models for assessing interventions and predicting optimal elimination strategies

**METHODS**

A database was assembled based on the Annual Parasite Index records from 2002–2010. All the data came from National Malaria Annual Report, including the number of malaria cases and the incidence of malaria both at the county and provincial levels. The investigators assessed the trends in the malaria including different time periods and the malaria annual incidence at both national level and in special areas to explain the trends between 2002 and 2010. The investigators also mapped the malaria probability distribution based on the GIS database by the spatial local interpolation method in the extension function. The predictive incidence probability map and semi-variance function was produced by unbiased criterion. A cross-validation technique was used to evaluate the fitness of the distribution maps by plotting the error distribution map.

**STATUS**

The study is ongoing and currently in the second phase.
RESULTS

After years of decline, malaria incidence in China increased in 2002, the recorded incidence was in 2002 was 3.48 and in 2006 4.7 per 100,000yrs (Figure 1). After 2006, malaria incidence decreased yearly to 0.94 per 100,000yrs in 2010. Malaria cases reported from Anhui, Henan, Hubei, Jiangsu and Shandong province, which are located in the Central and Southern part of China (Yunnan and Hainan province) contributed to most of prevalence changes (Figure 2). The distribution of reported cases of malaria in 2006 is shown in Figure 3.

ANALYSIS AND IMPLICATIONS

Spatio-temporal mapping offers great potential to guide and evaluate malaria elimination interventions, by presenting accurate and visible information. GIS technology is being used to present historical maps of malaria prevalence and establish the spatiotemporal models that will provide the baseline information and gauge progress of malaria control programs and evaluation of suitable malaria elimination interventions.
Sero-epidemiological analysis for monitoring malaria elimination in China

BACKGROUND

Malaria elimination requires robust surveillance for ongoing transmission at the community level. An ideal measure for this purpose would reflect malaria inoculation over time. The current key transmission measures include the prevalence of the parasite within human blood or within the vector (entomologic infection rate, EIR); however these do not provide information on long term trends and may be imprecise. Due to the short life span of the vector and the short-lived nature of individual infections, estimates based on EIR or parasite prevalence require frequent and large scale re-sampling.

By contrast the serological response to malaria infection (i.e. presence of anti-malarial antibodies) is relatively long-lived. Although the exact duration of anti-malarial immunity is debated, even in the worst-case scenario, antibodies persist markedly longer than human infections or mosquito lifespan.

Most recently the serological assessment of malaria transmission has been revolutionised by the availability of large quantities of standardized antigens through recombinant technology and the microplate ELISA technology. These technical advances have been mirrored by statistical and mathematical modelling advances which allow in depth analysis to provide detailed information on changes in population based exposure to malaria. Both the technical and analytical elements are being adapted specifically to address the issue of malaria elimination

AIMS AND OBJECTIVES

The overall goals of this project are to describe the prevalence of anti-malarial antibodies as indicators of malaria transmission in the context of malaria elimination in the central China (Jiangsu Province) and to explore the utility of serology as a surveillance tool to support intensive malaria control, foci identification, and elimination evaluation.

METHODS

In the course of a cross sectional survey 4,800 participants are enrolled in 24 villages in the province of Jiangsu. Demographic data and information on recent travel as well as two drops of blood (100µl) are collected from all participants. Malaria microscopy, PCR, IFAT and ELISA are performed on all collected blood samples.
PRELIMINARY RESULTS

To date a total of 472 samples were collected and analyzed. Preliminary results show that sero-prevalence increases with age and *P. vivax* sero-prevalence is higher than *P. falciparum*. 

*P. vivax* marker screening has been carried out and six *P. vivax* recombinant antigens with high immunogenicity were identified. Respective markers were validated on sera of individuals who had had *P. vivax* infections 5, 12 and 30 years ago were screened. Concluding results are pending.

ANALYSIS AND IMPLICATIONS

China’s malaria elimination program was launched in July, 2010. This project explores the utility of ELISA as a surveillance tool to support intensive malaria control, identification of foci of parasite reservoirs sustaining transmission, and the evaluation of elimination strategies. This study investigates the role of ELISA in achieving this and if successful this will be integrated into China’s standard surveillance program.

The preliminary results reflect the higher prevalence of *P. vivax* compared to *P. falciparum* within the study area. Increasing rates of sero-positivity with age reflect the prolonged time of exposure. The identified markers will refine the established method and show the potential of the assay to assess long term trends.

Roll out has the potential to accelerate the malaria elimination progress not only in China, but also across other APMEN countries.
Population screening for G-6-PD deficiency in China using an enzyme assay on filter paper dried bloodspots

BACKGROUND

China launched its malaria elimination program in July 2010. In the course of which, there has been increasing use of primaquine for the radical cure of patients with vivax malaria patients. Primaquine can induce haemolysis in G6PD deficient individuals, and this is of high interest to the implementing authorities.

Hainan province, in the south of the country, is home to a number of ethnic minorities such as Miao and Li. This area remains endemic for both P. vivax and P. falciparum. In contrast Jiangsu province in Central China is inhabited by Han Chinese, with the last case of P. falciparum reported in 1990 and P. vivax incidence dropping from 1 million cases in 1972 to 13 in 2011.

The WST8/1-methoxy PMS (Dojindo, Japan) method is a convenient tool for mass screening of G6PD activity. The colorimetric readout of the assay can be interpreted qualitatively by the naked eye as well as quantitatively using a micro-plate reader. Samples can be collected on dried blood spots, and thus the assay is well suited for large scale community based surveys.

AIMS AND OBJECTIVES

This study aims to assess the prevalence of G6PD deficiency in both central and southern China to guide the widespread use of primaquine radical cure in these regions.

METHODS

More than 10,000 individuals in Jiangsu and Hainan province are being enrolled at local health care facilities. Half of all participants are recruited among patients attending the health care facility due to fever (≥37.5°C) and the other half are healthy individuals attending health care facility for routine medical examination. Ethnic and demographic data is collected from all participants. A drop of blood is collected on filter paper from all participants and stored at 4°C. All blood spots are evaluated for G6PD activity at a reference center using the WST8/1-methoxy PMS method (Dojindo, Japan). G6PD enzyme activity is read quantitatively with a microplate reader.
PRELIMINARY RESULTS

3210 individuals of Han ethnic background from Jiangsu province (1520 males/1690 females) were enrolled and assessed and no case of G6PD deficiency was detected. In contrast, 12.10% (n=670) of 5538 individuals from Hainan province were classified as G6PD deficient, with 4.21% (n=233) suffered from severe deficiency. Also in Hainan province a significantly higher (p=0.0016) prevalence of G6PD deficient individuals was observed among the ethnic group of Li (n=318, 13.70%) compared to members of the ethnic group of Han (n=277, 10.74%). The study is on-going, results will be published at study end.

ANALYSIS AND IMPLICATIONS

The significant differences in G6PD deficiency prevalence in between different areas of the Peoples Republic of China and in between different ethnic groups residing within the same area highlight the need for studies as the above and the challenge malaria elimination provides. The risk of haemolysis in G6PD deficient individuals following primaquine treatment is considerable however the drug is the only compound effective in the treatment of dormant \textit{P. vivax} liver stages. While mass drug administration of primaquine could possibly provide an effective way to eliminate vivax malaria form the entire area, the high and scattered prevalence of G6PD reported here does not support this application in either province.

The observed high variation in prevalence of G6PD deficiency instead calls for testing of all vivax patients prior to primaquine treatment. However this can only be facilitated if reliable, easy to use and cost effective G6PD test devices for broad application become available and are evaluated. In order to address this need the WST 8 / 1 – methoxy PMS method (Dojindo, Japan) is being evaluated. The results will be of significant benefit for any future screening program that considers including large numbers of participants. To the authors knowledge this is the largest ever evaluation study to be carried out on this methodology and will provide data on the applicability of the method as a mass screening tool for G6PD.
Evaluation of P. vivax diagnostic methods, assessment of G6PD prevalence and P. vivax population diversity in South Korea

BACKGROUND

Indigenous vivax malaria was successfully eliminated from South Korea in the late 1970s, but has re-emerged since 1993. Initially, the re-introduced cases were only found in soldiers serving in the Demilitarized Zone (DMZ) bordering North Korea. Over time, an increasing number of cases have been observed in the civilian population and in regions distal to the DMZ.

Early diagnosis, immediate and effective treatment, and diligent surveillance are essential to contain the re-emerging parasite population and prevent further resurgence. However, limited accuracy of microscopy at peripheral health centers, uncertainty regarding the prevalence of G6PD deficiency and safe deployment of primaquine, and imported malaria challenge these efforts.

AIMS AND OBJECTIVES

i. To compare the sensitivity and specificity of four RDTs available in South Korea against microscopy and two molecular assays used by the national CDC.

ii. To define the prevalence of G6PD deficiency in South Korean patients with P. vivax infection.

iii. To determine the genetic diversity of the South Korean P. vivax population and assess the utility of genotyping to identify imported cases.

METHODS

The diagnostic performance of four RDTs (CareStart™, SD Bioline, NanoSign, and Asan Easy) was compared to PCR and microscopy using venous blood samples from malaria patients presenting at public health centers (sampling sites in Figure 3). All tests were performed at the national CDC laboratory, and were undertaken within 6 hours of venipuncture. Microscopy was undertaken on Giemsa-stained thick blood smears. For the molecular assays, DNA was extracted using the QIAamp blood kits (Qiagen), and PCR was undertaken using nested and real-time assays targeting the 18S rRNA gene.

G6PD testing and parasite genotyping were undertaken on fresh blood samples collected within the framework of the diagnostics study. G6PD testing was done using the CareStart G6PD and Trinity Biotech G-6-PDH tests. Genotyping was undertaken using the 9 APMEN markers and methods1. Population genetic analysis was undertaken using the vivaxgen data analysis tool (vivaxgen.menzies.edu.au).

1. Korea National Institute of Health, Korea CDC, Seoul, Republic of Korea
2. Gyeongsang National University, Jinju, Republic of Korea
3. Center for Genome Science, National Institute of Health, Korea CDC, Osong, Republic of Korea
4. Menzies School of Health Research, Darwin, Australia
5. Australian Army Malaria Institute, Brisbane, Australia
RESULTS AND INTERPRETATION

The results of the diagnostics study have been published\(^2\). A total of 253 fresh blood samples were examined. The sensitivities and specificities of the four RDTs were assessed against the nested-PCR and microscopy results. The CareStart\(^{\text{TM}}\) and SD Bioline RDTs demonstrated higher sensitivities (99.4 and 98.8%, respectively) than NanoSign and Asan Easy (93 and 94.7%, respectively).

The results of the G6PD study have been published\(^3\). Of 1,044 blood samples tested using the CareStart G6PD test, none were G6PD deficient. However, slightly elevated level of G6PD activity was observed in 14 of 1,031 samples tested with the Trinity G-6-PDH test.

Forty six indigenous and 3 imported \(P.\) \(vivax\) cases have been genotyped. A further 50 isolates are currently being processed. The preliminary data demonstrates lower population diversity than observed in tropical endemic regions (expected heterozygosity = 0.56). The 3 imported cases had distinct genotype profiles from the indigenous cases (Figure 4).

CONCLUSIONS AND IMPLICATIONS

The RDT study provided important information that will aid in the selection of effective diagnostics for use in peripheral health centers in South Korea. The preliminary genotyping data demonstrates the potential for molecular tools to inform on imported \(P.\) \(vivax\) cases in South Korea. Further analyses are ongoing.

1. Report APMEN WkG Genotyping Workshop May 2011
**Genetic diversity of *P. vivax* in Indonesia**

**BACKGROUND**

Approximately 60% of Indonesia’s 239 million people live at risk of infection from malaria. Close to 50% of all confirmed cases are due to *P. vivax*. High levels of multi-drug resistant *P. vivax* and associated life-threatening disease in the eastern province of Papua and threaten to undermine elimination goals.

In April 2009, the Ministry of Health announced a renewed malaria control and elimination strategy employing an island by island approach in the effort to achieve nation-wide elimination by 2030. The extensive heterogeneity in malaria epidemiology across Indonesia suggests that interventions which have been successful in one setting may not necessarily have the same impact in another (Figure 1). Without the restrictions of political boundaries, and with increasing travel for social and economic purposes, a further challenge is the risk of resurgence in pre-elimination areas fuelled by local reservoirs of infection in high endemic areas.

**AIMS AND OBJECTIVES**

To set the foundations for comprehensive parasite transmission surveillance in Indonesia, a study was undertaken to characterize the genetic diversity of *P. vivax* isolates in the intermediate and high transmission settings of Bangka-Belitung and Sumba (Nusa Tenggara Timur); Figure 1. Specific aims were:

i. To establish *P. vivax* genotyping in Indonesia.

ii. To compare the genetic diversity and transmission patterns of *P. vivax* isolates in an intermediate (Bangka) and high (Sumba) endemic setting to assess the prospects for elimination in both.

iii. To determine whether Bangka and Sumba share common reservoirs of *P. vivax* infection, providing insights for the island-by-island approach.

**METHODS**

Nine polymorphic markers defined by the APMEN vivax working group were genotyped in 66 *P. vivax* isolates from Bangka and 40 from Sumba using the APMEN markers and methods. All genotyping was undertaken at the Eijkman Institute.

To address the questions raised by aims ii and iii, standard population genetic measures were calculated using vivaxgen, an online data analysis tool developed in collaboration between Eijkman and Menzies (vivaxgen.menzies.edu.au).
RESULTS

Expected heterozygosity ($H_e$) was high in Bangka ($H_e = 0.84$) and in Sumba ($H_e = 0.86$), indicating high population diversity in both regions despite differences in endemicity. However, the diversity of individual infections, as determined by the Multiplicity of Infection (MOI), was higher in Sumba (MOI = 1.85) than Bangka (MOI = 1.47), consistent with higher transmission in Sumba.

The unadjusted genetic differentiation between the two islands was very low ($F_{ST} = 0.04$), indicating that the two populations share the same reservoir of infection. However, after adjusting for the extensive marker diversity using the standardized genetic differentiation, moderate differentiation was observed ($F_{ST}’ = 0.27$).

Moderate clustering of the isolates by island was observed with neighbour-joining analysis (Figure 2). The neighbour-joining analysis further illustrated the extensive diversity in both populations, with very few infections exhibiting the same genotype profile.

CONCLUSIONS

The analysis of multiplicity of infection suggested that it may have utility as a measure of local transmission intensity. However, it remains unclear what mechanism or reservoir is maintaining high levels of diversity in both the intermediate and high transmission settings. Relapsing infections and/or parasite exchange between islands might have important roles. Comparative assessments of *P. falciparum* diversity and transmission dynamics in Bangka and Sumba are underway with support from the Malaria Transmission Consortium. These results may provide insights into the impact of relapse on the population diversity.

Further parasite genotyping surveillance is planned in other regions of Indonesia, and may provide further insights on important reservoirs of infection.


FIGURE 1

Figure 1: Prevalence map of *P. vivax* malaria in Indonesia (Malaria Atlas Project).

Figure 2: Unrooted neighbor-joining tree illustrating the relatedness between *P. vivax* isolates from Bangka (red) and Sumba (green).
Survey of G6PDd variants on Sumba Island and development of PCR primers for each

BACKGROUND

G6PD enzyme deficiency plays a crucial role in the radical cure of *P. vivax* infections with 8-aminoquinolones. Unrecognized G6PD deficient individuals treated with 8-aminoquinolones are at high risk for severe and potentially life threatening side effects. Knowledge on type and distribution of G6PD deficiency among affected populations is accordingly crucial to determine the risk of side effects.

Little is known on the prevalence of G6PD variants in Indonesia. Most surveys conducted to date are either based on qualitative or genetic assays. While qualitative assays discriminate between normal and deficient individuals they do not provide information on exact enzyme activity. Most genetic surveys on the other hand are based on sequencing and are restricted to known variants. In consequence any unknown variant is erroneously classified as a G6PD normal variant.

Accordingly there is a high need for additional primers that will allow identifying a broader scope of G6PD variants in an effective and precise way.

AIMS AND OBJECTIVES

This study aims to detect known and new G6PD variants in West Sumba, Indonesia and design respective primers for use in Polymerase Chain Reaction (PCR).

METHODS

The study is being conducted in five villages in West Sumba, South West Sumba, and Central Sumba, Indonesia and 2500 individuals are randomly selected and enrolled. All participants are tested for G6PD deficiency using the fluorescent blood spot test. A total of 8ml of blood is collected from all participants found to be G6PD deficient as well as a randomly selected subsample of 50 G6PD. All samples are subsequently transported to the Protein Laboratory of the Eijkman Institute for Molecular Biology in Jakarta for enzyme purification and assessment of physical properties. DNA is extracted from all samples and scanned for known and new G6PD variants. In addition the first generation of the Carestart G6PD RDT (AccessBio) was evaluated on the first 1168 samples collected and the results were compared to spectrophotometry (Trinity Biotech, Ireland).
RESULTS

The prevalence of G6PD deficiency within all tested samples from Sumba was 5.2%. The three most common G6PD variants in Sumba are Vanua Lava, Viangchan and Chatham. All of these belong to WHO Class II G6PD Enzyme Classification (severe). One new variant was discovered at position 17089 T>G (Cys > Gly) as a heterozygous condition. Enzyme kinetics of this new variant showed a preference for its cofactor NADP rather than its own substrate, G6P. The investigators will be collaborating with PATH and EOCRU to continue their G6PD work in Sumba and develop primers for the new variant discovered.

Compared to spectrophotometry, the Carestart G6PD RDT was found to have sensitivity and specificity of 73% and 99% respectively.

ANALYSIS AND IMPLICATIONS

The results of this survey have direct implications for the application of 8-aminoquinolones for Sumba island. Precise knowledge on the distribution and degree of G6PD deficiency within the local population will allow designing strategies and treatment guidelines for vivax malaria within the region that consider G6PD deficient individuals.
Spatial Analysis of The Incidence G-6-PD Mutations in *Plasmodium vivax* Malaria Infection in South Central Timor (SCT), East Nusa Tenggara (ENT) Province, Indonesia, 2013

**BACKGROUND**

The province of East Nusa Tenggara (ENT), Indonesia, has one of the highest annual parasite indices (API > 15‰) in Indonesia with the malaria and infant mortality rates (MMR and IMR) twice that of the national average. Within ENT the South Central Timor district (SCT) contributes the greatest number of malaria, with 18,722 reported cases in 2011 alone. Among all reported cases *P. vivax* and *P. falciparum* are the two dominant species. The local prevalence of G6PD deficiency is said to be high, however reliable data from the area have not yet been collected.

**AIMS AND OBJECTIVES**

The primary aim of this project was to define the spatial distribution of G6PD deficiency and malaria cases in SCT. Secondary aims were to assess risk factors associated with G6PD deficiency, and compare differences in signs and symptoms among deficient and non-deficient malaria positive participants. Novel G6PD variants were screened from G6PD deficient samples.

**METHODS**

A cross sectional survey enrolled 556 randomly selected and healthy participants over 14 years of age from 5 districts at SCT district, between July and August 2013. All participants completed a short questionnaire and underwent a physical examination. Venous blood was collected to measure haemoglobin levels, and to prepare thick and thin malaria blood smears that were later confirmed by Nested PCR. G6PD enzyme activity was measured on all collected samples using spectrophotometry (*Randox* kit quantitative test, cat: 410, UK). G6PD deficiency was defined as an enzyme activity <6.97 IU/gHb following the manufacturers recommendations. A subset of G6PD deficient samples were sequenced for new G6PD variants. Cases of malaria and G6PD deficiency were mapped and spatial analysis was done using geostatistical SatScan and clustering methods (30').

This study was approved by Ethical committee Faculty of Medicine University Gadjah Mada (Ref: KE/FK/85/EC).
RESULTS

A total of 556 individuals (male : female = 229:326) from 5 health centres were enrolled, all except one were included in the subsequent analysis. Age at time of enrolment ranged from 14 to 95 year (median 43 years). A total of 96 (17.3%) participants were G6PD deficient and 21 (3.7%) had borderline deficiency (≥6,97-7,2 U/g Hb). The observed incidence of malaria was 16.2 per 1000 population. In total 9 patients were found to be positive for malaria (6 \textit{P. vivax} and 3 \textit{P. falciparum}), the overall prevalence was 8 (1,7%) in G6PD normal patients, 0 % in G6PD intermediate and only 1 (0,2%) in G6PD deficient individuals.

Of the parameters assessed (age, sex, duration of stay in the area, malaria slide result, ethnicity) only haemoglobin varied significantly between G6PD normal (mean Hb=11.7 g/dl) and deficient individuals (mean Hb=13.3 g/dl); p<0.001. The only risk factor associated with malaria was individuals not using insect repellents regularly.

Processing of samples and the analysis of the results is ongoing. The results will be published once all molecular work is completed and a detailed map on G6PD deficiency is made for the study site.

<table>
<thead>
<tr>
<th>Study site</th>
<th>Total enrolled</th>
<th>G6PD deficient (%)</th>
<th>Malaria positive (Microscopies) N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oinlasi</td>
<td>100</td>
<td>39 (7.1)</td>
<td>0 (0)</td>
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<td>Oe’ekam</td>
<td>100</td>
<td>15 (2.7)</td>
<td>0 (0)</td>
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<tr>
<td>Panite</td>
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<td>19 (3.4)</td>
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<td>5 (0.9)</td>
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<td>135</td>
<td>15 (2.7)</td>
<td>4 (0.7)</td>
</tr>
<tr>
<td>Total</td>
<td>555</td>
<td>96 (17.3)</td>
<td>9 (1.6)</td>
</tr>
</tbody>
</table>

ANALYSIS AND IMPLICATIONS

G6PD deficiency was high within the study population. However the cut–off, recommended by the manufacturer of the spectrophotometry assay (Randox, UK), is set conservatively and thus the true prevalence of individuals at risk of haemolysis could be lower. This highlights the urgent need for in depth studies on the G6PD enzyme activity to gauge the risk of haemolysis following different primaquine treatment regimens. The risk of severe haemolysis within an individual will depend upon the type of variant present. Ultimately the prevalence of G6PD and the associated risk of haemolysis will determine the most appropriate approach to delivering safe and effective radical cure.
Prevalence of G6PD Deficient Individuals in Bangka Island

BACKGROUND

Data on the prevalence of G6PD deficiency are scattered, however essential for the countries national elimination program. The primary diagnostic tool for previous data collection was a qualitative colorimetric assay. While more cost effective than quantitative formats, qualitative diagnostic tools for G6PD deficiency only distinguish between normal and deficient individuals and there is a considerable variation among manufacturers on what level of enzyme activity to use as the cut-off for defining deficiency. Hence surveys based exclusively on qualitative methods may not provide a clear picture on the distribution of enzyme activity among local populations. Use of primaquine treatment regimens for *P. vivax* radical cure can induce severe haemolysis in G6PD deficient individuals, it is therefore important to have a detailed understanding of the prevalence of local G6PD deficiencies in the populations that maybe exposed to the drug.

AIMS AND OBJECTIVES

The Indonesian Ministry of Health has targeted Bangka Island as malaria free by 2015. In order to support this goal the study aims to screen for G6PD deficiency in Bangka using a quantitative approach. The study results will provide a detailed picture of the distribution of enzyme activity among the local population of Bangka.

METHODS

A total of 500 to 600 randomly selected, healthy participants fitting pre-defined inclusion criteria (age > 5 year, Hb ≥ 10g / dL, no severe illness, informed consent) and living in two districts of Bangka island were enrolled. Blood samples were collected for the quantitative assessment of G6PD activity and for DNA isolation. All DNA isolates are currently being genotyped or sequenced. Participants with unknown G6PD variants are re-visited and an additional blood sample is taken for detailed biochemical characterization of the G6PD enzyme.

This study was approved by Ethical committee Faculty of Medicine University Gadjah Mada (Ref: KE/FK/85/EC).
RESULTS

The study is ongoing. During sample collection between November and December 2013 a total 615 subjects (330 and 285 subjects from Bangka and Middle Bangka Districts respectively) were screened. While no malaria cases were detected, 3.25% (20/615) of all participants were G6PD deficient. Currently DNA extraction is ongoing and PCR/RFLP for G6PD common variants have just started.

ANALYSIS AND IMPLICATIONS

Providing detailed data on the local distribution of G6PD deficiency is essential for any malaria elimination program as is the case for Bangka island.

In the late stages of malaria elimination the proportion of asymptomatic malaria patients increases in relation to symptomatic patients. To eliminate malaria all infected patients need to receive radical cure to reduce transmission potential. However treating asymptomatic patients with primaquine treatment requires careful and strong consideration of the potential risks of haemolysis in an otherwise healthy individual.

This study provides important information on the risks of widespread deployment of primaquine that will assist the local and national Indonesian malaria elimination program for the planning and implementation of malaria intervention programs. It is also building a strong national capacity and expertise in G6PD diagnostics and analysis at the Eijkman Institute for Molecular Biology in Jakarta.
Community survey on knowledge, attitude and practice of malaria intervention (diagnosis & treatment) for vivax malaria in Indonesia

BACKGROUND

In 2009 approximately 175 million Indonesians were at risk of infection with vivax malaria. South Bengkulu district is located on the south-west coast of Sumatera in the Indonesian archipelago. The district has a population 150,000 and land area of 1,186.10 km² and suffers from stable transmission of *P. vivax*: PvAPI > 0.1 per 1,000 populations per year and prevalence (PvPR<sub>1-99</sub>) estimated at 5%. The national malaria control programs aims to eliminate malaria from the district by 2020.

AIMS AND OBJECTIVES

This study assesses the knowledge, perceptions and treatment-seeking behaviour in vivax like illnesses and documents household prevention practices among the local population. The study is also evaluating malaria treatment facilities serving the study population.

METHODS

A cross sectional survey was conducted in April 2013 in the districts of South Bengkulu, Indonesia. Ten villages in five sub-districts were randomly selected by using probability proportional to size (PPS) sampling strategy. Information on demographic and socioeconomic background, knowledge and perceptions of malaria, treatment seeking behavior and prevention practices were collected in questionnaire based community interviews. Information on diagnostic practices, treatment provided for malaria-like illness and resources for malaria diagnostics at local laboratories were collected via semi structured questionnaires in five primary health care centers (PHC).

RESULTS

A total of 281 interviews were conducted, with 20% (n=58) of all interviewees reporting an episode of febrile illness, which could be laboratory confirmed in 29% (n=17) cases. In total, 87% respondents had heard about malaria and 71% correctly identified mosquito bites as main cause of disease. Other factors reported to cause malaria were unsanitary houses (11%) and bad weather (8%). The most common malaria symptoms named were fever and/or chills (80%). The majority of respondents were aware that untreated malaria was extremely dangerous and life-threatening. Approximately 63%
of respondents reported owning a bed net, but 96% of these nets were non-insecticide treated. On average, three people (range 0-7) per household slept under bed nets. Only 20% of all participants’ households had been treated with indoor residual spraying (IRS) at least once. Approximately 20% (58/281) of all respondents had suffered from malaria within the past year and 29% (n=17) of all respondents sought self-treatment, before seeking professional help.

A total of five Primary Health Care Units (PHCs), serving the sampled communities were assessed. Two PHCs relied solely on clinical diagnosis. Half of all microscopists (n=4) had not received formal training. Artemisinin combination therapy (ACT) was available for all patients with confirmed malaria diagnosis, primaquine as a radical cure drug for vivax malaria and relapse prevention was inconsistently given in the primary health centers.

STATUS
The analysis of this study is ongoing.

PRELIMINARY ANALYSIS AND IMPLICATIONS
During this community based survey, most people stated a basic understanding of malaria, its main causes and transmission, symptoms and consequence of not being treated. However the study highlights that community awareness on malaria needs to be strengthened and this is likely to enhance patient avoidance of malaria and early presentation to clinic.

The current diagnostic infrastructure is insufficient with almost half of all PHCs assessed relying exclusively on a clinical diagnosis for malaria. At facilities were malaria microscopy was available half of the local staff had not received formal training. If malaria elimination is to be achieved by 2020 for the entire Bengkulu district in Sumatera, these knowledge gaps and lack of diagnostic infrastructure need to be addressed.

patients need to receive radical cure to reduce transmission potential. However treating asymptomatic patients with primaquine treatment requires careful and strong consideration of the potential risks of haemolysis in an otherwise healthy individual.

This study provides important information on the risks of widespread deployment of primaquine that will assist the local and national Indonesian malaria elimination program for the planning and implementation of malaria intervention programs. It is also building a strong national capacity and expertise in G6PD diagnostics and analysis at the Eijkman Institute for Molecular Biology in Jakarta.
Dynamics of *Plasmodium vivax* parasite populations during malaria elimination efforts in Sri Lanka, with low transmission and unstable malaria

**BACKGROUND**

Sri Lanka has experienced a marked reduction in malaria cases over the past decade, with more rapid decline in the incidence of *P. falciparum* than *P. vivax*. As the country approaches elimination, it now faces the challenges of declining immunity in the host community, unstable transmission, imported malaria, and the associated risks of outbreaks and resurgence. Diligent surveillance of the parasite is critical to prevent resurgence in this setting. By addressing genetic regions under selection as well as those in neutral (non-selected) regions of the genomes, it may be possible to gain insights on the impact of declining incidence on both transmission dynamics and host immune pressure. The *P. vivax* circumsporozoite gene (*Pvcsp*) encodes a vaccine target candidate expressed on the surface of the sporozoite stage parasite, and is therefore a suitable gene to assess host selective pressures.

**AIMS AND OBJECTIVES**

The study proposed to investigate the genetic diversity and evidence for selection in *Pvcsp* in Sri Lankan isolates, with comparison to global isolates. In addition, genotyping at neutral markers in Sri Lankan *P. vivax* isolates collected 10 years apart, in 2000 and post-2010, was proposed to support the National Malaria Control Program’s surveillance efforts by informing on the impact of the declining incidence on the local parasite population diversity and transmission dynamics.

Specific aims were:

i. To determine whether the *P. vivax* populations in two endemic regions of Sri Lanka had been subject to a “bottleneck” or “founder” effect as a result of aggressive intervention.

ii. To assess the impact of the intervention efforts on the prevalence of polyclonal infections by comparing frequencies in isolates collected in 2010 and post-2010.

iii. To compare the impact of the intervention efforts on the diversity and population structure of *P. vivax* isolates collected in 2000 and post-2010 at a) putatively neutral loci, and b) the *Pvcsp* locus.
METHODS

Blood samples were collected from *P. vivax* patients attending hospitals in two malaria-endemic regions, Anuradhapura and Katagarama, between 2010 and 2013. Samples collected within the framework of other studies conducted in the same regions in the early 2000s were also processed for temporal evaluations. DNA was extracted using the QIAamp blood kits (Qiagen), and *P. vivax* infection confirmed by speciation PCR. The central repeat domain of the *Pvcsp* gene was examined by capillary sequencing. Genotyping of putatively neutral loci will be undertaken using the APMEN consensus markers and methods.

RESULTS

The central repeat domain of *Pvcsp* was successfully sequenced in 60 *P. vivax* infections from Anuradhapura and Katagarama. All amino acid sequences corresponded to the VK210 variant. Nineteen of the amino acid haplotypes defined were exclusive to Sri Lanka, adding to 57 other haplotypes of the VK210 variant identified in a global set of *P. vivax* isolates (Figure 1). In the global sample set, the central repeat domain of both VK210 and VK247 variants was under purifying selection not diversifying selection. Genotyping at the neutral markers is ongoing.

CONCLUSIONS AND IMPLICATIONS

The *Pvcsp* study results have been published. The extensive diversity observed in this antigen presents a challenge to vaccine development. The unique geographic clustering of the *Pvcsp* VK210 variant also has implications for vaccine development.

1. Report APMEN VxWG Genotyping Workshop May 2011
2. Dias et al. *Gene* 2013, 518(2)
Assessing the prevalence of malaria parasites in displaced populations who have returned or resettled in the post conflict districts of Kilinochichi, Mullaithivu and Mannar in Sri Lanka

BACKGROUND

After 30 years of conflict in the north of Sri Lanka an end to the war was finally announced in May 2009. During the conflict over 60% of all malaria cases in the country were being reported to the north where the prevailing situation had impeded the implementation of the malaria control activities that had been so successful in the rest of the country. The lack of proper diagnostic facilities in these areas and difficulties in reporting undermined the accuracy of reported estimates of the number of malaria cases.

In 2009 the National Malaria Control Program announced the aim for malaria elimination, setting a goal for the elimination of Plasmodium falciparum malaria by 2012 and P. vivax malaria by 2014.

AIMS AND OBJECTIVES

The study was set up to understand the epidemiology of malaria in the post-conflict areas better, strengthen the surveillance activities and warn of potential future malaria epidemics.

Specific aims included:

i) documenting the parasite prevalence in the post-conflicts districts of Kilinochichi, Mullaithivu and Mannar

ii) estimating the prevalence of G6PD deficiency among a sub sample of the study population

iii) describing the genetic diversity of the parasite population among the malaria positive patients in the study population
METHODS

The study was carried out between 2011 and 2012 and was designed as a cross sectional survey using 70 clusters (each comprising 100 people) in the 3 districts. After informed consent was obtained, a Public Health Field Assistant or Health Assistant from the Regional Malaria Office collected blood from a finger prick. Thick and thin blood smears were prepared for microscopic evaluation. At the same time blood was collected on a filter paper for molecular analysis. Nested PCR was used to determine and confirm species diagnosis and detect submicroscopic infections. PCR positive cases were planned to be genotyped using the APMEN consensus markers. An additional drop of blood was collected in a subgroup for G6PD testing using fluorescence blood spot tests. Basic demographic data including age, sex, occupation, history of fever within 24 hours and past history of malaria infections were recorded.

RESULTS

A total of 7,000 samples collected from the three districts, all were negative for malaria. Out of these samples a total of 1000 were tested for the presence of malaria parasites by PCR and were also all found to be negative. Therefore no genotyping was performed. G6PD testing was conducted in one province only with deficiency detected in 2.9% (11/375) of patients.

STATUS

The study is now completed and report submitted.

ANALYSIS AND IMPLICATIONS

This is the first large scale epidemiological study carried out in the post-conflict areas in Sri Lanka. The results indicate that Sri Lanka continues towards its goal of and even in this troubled area malaria remains low.

Subsequent surveillance has revealed that the number of indigenous cases in the country in 2011 was 124, a marked reduction from 2010 when more than 680 cases were reported. The biggest threat for malaria elimination in Sri Lanka, similar to other countries close to elimination, is a resurgence of disease due to imported malaria cases.
Molecular genotyping of *P. vivax* in isolates in Sabah, Malaysia

**BACKGROUND**

Malaysia has committed to eliminating malaria by 2015 in the Peninsular region and 2020 on the island of Borneo. Although there has been a rapid decline in malaria incidence, *P. vivax* presents a major challenge, due to its dormant hypnozoite stage, greater asymptomatic asexual carriage and early gametocyte production enhancing the parasite’s transmission potential.

The region most widely affected by malaria is Sabah (Borneo), with an estimated 24.5% of the population living at risk of infection. Imported malaria presents a further challenge in this region. Sabah shares borders with the Malaysian state Sarawak in the south-west, Kalimantan (Indonesia) in the south-east, and the islands of the Philippines to the north - *P. vivax* transmission persists in all of these border regions.

**AIMS AND OBJECTIVES**

The study proposed to use *P. vivax* genotyping to assess the impact of ongoing intervention efforts on *P. vivax* transmission dynamics in different endemic settings in Sabah.

Specific aims were:

i. To learn the methods for *P. vivax* genotyping for use in future surveillance efforts.

ii. To compare the genetic diversity and transmission patterns of *P. vivax* isolates in a low (Kota Marudu) and very low (Kota Kinabalu) endemic setting (Image 1) to assess the prospects for elimination in both.

iii. To generate representative *P. vivax* genotyping data to contribute to the broader APMEN objective to assess the utility of genotyping to identify imported cases.

**METHODS**

The study focused on Kota Kinabalu and Kota Marudu, where established collaborations with local health researchers at major health centers enabled representative sampling from patients. Additional samples were collected from active case detection surveys undertaken in Tawau district. DNA was extracted using Qiagen kits, and *P. vivax* infection confirmed by speciation PCR. Genotyping was undertaken by Dr Abdullah at Menzies (Image 2) using the APMEN consensus markers and methods. Population genetic analysis was undertaken using the online vivaxgen data analysis tool developed by Eijkman and Menzies (vivaxgen.menzies.edu.au).
RESULTS AND INTERPRETATION

A total of 97 *P. vivax* samples collected between 2010 and 2013 were included in the study, including 25 from Kota Kinabalu and 24 from Kota Marudu. Consistent with low endemic transmission, as measured by the multiplicity of infection (MOI), polyclonal infections were less common in Kota Marudu (MOI = 1.38) and Kota Kinabalu (MOI = 1.19) than in other tropical endemic regions.

However, population diversity, as measured by the expected heterozygosity (Hₑ) remained moderate in both populations, with slightly higher levels observed in Kota Kinabalu (Hₑ = 0.67) than Kota Marudu (Hₑ = 0.58) despite the lower endemicity in Kota Kinabalu. This difference might reflect imported cases, with the large economic center of Kota Kinabalu expected to experience greater migration than Kota Marudu.

Several clusters of isolates had identical genotype profiles (Figure 1) and presented at similar time points. These clusters are indicative of outbreaks, and highlight the instability of transmission.

ANALYSIS AND IMPLICATIONS

The study has been completed and published. Sabah’s shrinking *P. vivax* population appears to have rendered this low endemic setting vulnerable to epidemic expansions. The APMEN markers could be useful in detecting early outbreaks before they expand further. Migration may play an important role in sustaining *P. vivax* infection in Sabah, with important implications for malaria elimination. The pooled, multi-center APMEN genotyping study will be important to assess the utility of genotyping to identify imported cases.

2. Abdullah et al. PLoS ONE 2013, 8(12) e82553 +++MISSING IN TEXT+++
Molecular assessment of *P. vivax* transmission dynamics in Bhutan

**BACKGROUND**

Bhutan has made substantial progress in achieving malaria elimination by 2016, although the greatest gains have been made for *P. falciparum* rather than *P. vivax*. The sustained transmission of *P. vivax* may reflect either importation of cases across the long southern border with India or the failure to achieve parasitological cure. Imported cases may increase the risk of drug resistance spread and outbreaks in communities with limited immunity.

The Bhutanese National Malaria Control Guidelines recommend chloroquine (5-10mg/kg) plus primaquine (0.25mg/kg daily for 14 days) for the treatment of *P. vivax* infection. An APMEN funded pilot trial (Study 108-08) is underway to investigate the clinical-parasitological efficacy of this regimen. This complementary study proposes to genotype *P. vivax* infections in the trial pre-treatment and at recurrence. The baseline (pre-treatment) data will inform on the local transmission patterns including identification of outbreaks. In addition, *P. vivax* cases from non-nationals will be genotyped to assess the importance of imported malaria as a reservoir sustaining local transmission.

**AIMS AND OBJECTIVES**

The broad objectives of the study are to:

i. Facilitate monitoring of the *P. vivax* cure rate and relapse dynamics in Bhutan with the current National drug policy.

ii. Characterise the local *P. vivax* transmission dynamics in Bhutan.

Specific aims are to:

i. Confirm *P. vivax* mono-infection in the pilot trial using PCR-based assays.

ii. Characterise recurrent infections using the genetic and temporal information.

iii. Characterise the baseline *P. vivax* diversity and structure in Bhutan.

iv. Determine whether the Bhutanese parasites are genetically different from the non-national parasites.
METHODS

Fifty patients with microscopy-determined *P. vivax* mono-infection will be enrolled at health centres in sentinel sites in the southern districts of Samtse, Dagana, Sarpang and Samdrupjongkhar. Clinical and epidemiological patient details including recent travel history will be recorded on a clinical record form. A 5 ml venous blood sample will be collected from the pre-treatment and recurrent samples arising during the 12 month follow up in the pilot trial, and from consenting non-national patients presenting at the health centres. DNA will be extracted using Qiagen kits, and *P. vivax* mono-infection will be confirmed by PCR using species-specific primers. Genotyping will be undertaken on the *P. vivax*-confirmed infections at the APMEN consensus markers using the consensus methods\(^1\).

RESULTS AND INTERPRETATION

Patient recruitment began in May 2012 and is ongoing. To date, 7 samples have been collected including 5 day 0 samples from Bhutanese patients enrolled into the pilot trial, and 2 from non-national patients.

DNA extraction and *Plasmodium spp.* confirmation PCR has been established at the Public Health Laboratory in Thimphu confirming *P. vivax* mono-infection in the 5 Bhutanese samples. Molecular processing is underway on the non-national samples.

Preliminary analysis demonstrated different genetic profiles in each of the 5 samples, all of which exhibited multiple clone infections, with one infection appearing to have at least 4 different clones.

ANALYSIS AND IMPLICATIONS

Having established molecular methods to confirm *Plasmodium spp.* at the Public Health Laboratory, the technologists/technicians are now sufficiently trained to implement these methods on samples collected at various sentinel sites in southern Bhutan. In this area where malaria cases have fallen to pre-elimination levels, these molecular techniques will facilitate more accurate and sensitive diagnosis of malaria.

The study is ongoing to address aims ii to iv. The preliminary data suggests that the Bhutanese *P. vivax* population is diverse with a high rate of polyclonal infections. These features are normally observed in high transmission settings, suggesting that the Bhutanese *P. vivax* population may be largely sustained by an external reservoir of infection, such as that due to imported cases. Further analysis of non-national samples from bordering India will enable assessment of whether this region is the source of importation.

1. Report APMEN VxWG Genotyping Workshop May 2011
Malaria elimination in Bhutan using mobile technology for disease mapping and early diagnosis

BACKGROUND

In Bhutan the number of confirmed malaria decreased from 39,852 reported cases in 1994 to only 82 cases in 2012, with the associated malaria-related deaths falling in the same period from 62 deaths to one. By 2012 most of the remaining indigenous cases were due to P. vivax (57%). Bhutan is planning to achieve “zero local malaria transmission” by 2018 and WHO malaria-free certification by 2020.

AIMS AND OBJECTIVES

The overall aim of this study is to use mobile technology for disease mapping and early diagnosis case in Bhutan.

The specific objectives are as follows:

i) to develop the online febrile and malaria information system using web-base and mobile technology for implementation in Bhutan.

ii) to train health care personnel at the selected health facilities both at operational and management levels regarding application of mobile technology and geographical information system for decision making.

iii) to assess epidemiology of febrile and malaria in implementation areas regarding case detection of febrile and malaria cases, case management and treatment outcomes of malaria patients among resident and migrant populations in the project implementation areas.

iv) to assess the cost effectiveness and expansion feasibility of disease mapping using mobile technology for malaria elimination in Bhutan.

v) to assess for possibility of future expansion of the system to other disease surveillance in the country.
METHODS

This study is being carried out in five health facilities of Sarpang district (Figure 1), which contributes more than 60% of the country’s malaria cases. An online information system for malaria and febrile episodes using web-based and mobile technology was developed. Health care personnel at selected health facilities are trained both at operational and management levels regarding application of mobile technology and geographical information system for decision-making. The outcomes measured are: time taken to receive case reports, cost of instituting and sustaining the system, epidemic situations averted through efficient reporting systems, malaria hot spots mapped for targeted impacts, and assessing the pattern of parasite clearance.

RESULTS AND STATUS

A mixed methodology of evaluation was employed to evaluate the project including goal-based and goal-free evaluations. Technology acceptance model (TAM) was used in assessing the users’ perceptions and attitudes towards the project as well as assessing the potential users’ opinion on project expansion. The data captured on mobile phone, as presented on the GIS mapping, revealed that there were follow-ups in 14 of 19 malaria-infected cases and 8 breeding sites were investigated.

Data from a formal survey and interviewing staff during the project conclusion phase suggested that users accepted the implemented system. Publications are currently in preparation.

CONCLUSION

The quantitative and qualitative findings based on information captured during and at the conclusion of the evaluation suggested that the project was a success. The system can generate an evidence-based and traceable epidemiology reports to assess the situation at different levels and has the potential to inform decision making for effective case management and vector control and moving forward towards the target of elimination.
A prevalence study of Glucose-6-Phosphate Dehydrogenase deficiency and operational issues in applying the test in resource poor areas in the Philippines

BACKGROUND

In 2009 approximately 40% of all malaria cases in the Philippines were reported from the province of Palawan, of which 22% were diagnosed as P. vivax. In the preceding 2 years approximately 12,000 malaria cases were reported per year, resulting in an Annual Parasite Index (API) of 14.31 per 1,000 population. In order to prevent relapses from vivax malaria the Research Institute for Tropical Medicine has recently embarked on a clinical trial to assess the efficacy of primaquine as hypnozoitocidal treatment. To minimize the risk of severe adverse events for patients receiving primaquine treatment, it is important to ensure good knowledge on the prevalence of G6PD deficiency and this requires reliable and accurate point of care tests for the diagnosis G6PD deficiency.

AIMS AND OBJECTIVES

The aim of the study is to determine the prevalence of G6PD enzyme deficiency among randomly selected high school students attending local schools and assess a battery of assays for G6PD deficiency to determine the test most suitable for widespread deployment.

METHODS

The study was undertaken in Puerto Princesa City, Palawan between December 2011 and November 2012. Blood samples were collected from randomly selected high school students. Informed written consent was collected from parents or legal guardians. G6PD enzyme deficiency was assessed using a fluorescent spot method (Procedure 203, Trinity Biotech), a colorimetric test (G6PD WST-8 Assay, Dojindo Co), a rapid diagnostic test (Carestart, Access Bio) and spectrophotometry (Procedure 345, Trinity Biotech). The rapid diagnostic test was tested repeatedly, on venous and capillary blood, deficiency was defined as enzyme activity < 146 IU / 1012 RBCs.

All tests kits were further assessed for their operational characteristics, such as required storage conditions, additional supply required, ease of result interpretation and cost / test.
TABLE 1: SENSITIVITY AND SPECIFICITY OF G6PD SCREENING KITS

<table>
<thead>
<tr>
<th>Test Type</th>
<th>Sensitivity (%) (95% CI)</th>
<th>Specificity (%) (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluorescent Spot Test</td>
<td>59.0 (45.7 - 71.2)</td>
<td>98.0 (96.3 - 99.0)</td>
</tr>
<tr>
<td>Colorimetric Test</td>
<td>52.5 (39.4 - 65.2)</td>
<td>98.8 (97.4 - 99.5)</td>
</tr>
<tr>
<td>Rapid Diagnostic Test - capillary</td>
<td>50.0 (31.1 - 68.9)</td>
<td>93.4 (89.6 - 95.9)</td>
</tr>
<tr>
<td>Rapid Diagnostic Test - venous</td>
<td>78.6 (58.5 - 90.9)</td>
<td>97.5 (94.6 - 98.9)</td>
</tr>
</tbody>
</table>

*Fluorescent Spot Test (Procedure No. 203, Trinity Biotech); Colorimetric Test (G6PD WST-8 Assay, Dojindo Co.); RDT - Rapid Diagnostic Test (Carestart™, Access Bio); CI – Confidence Interval

RESULTS

Among 621 tested students, 15.1% were G6PD deficient as determined by the fluorescent blood spot test. The performance of three screening tests was evaluated on a subset of samples from 578 blood samples collected (table).

Assessment of the operational utility of the test kits revealed that techniques were often laborious. Stability of the kits, equipment, the requirement of high level of technical skills, and cost were not suitable for implementation at local health care facilities.

Status: The study is complete, and a manuscript is being prepared for publication.

ANALYSIS AND IMPLICATIONS

The Philippine Newborn Screening Program (PNSP) for 2009 reports the G6PD deficiency rate to be 1.9%, significantly lower than the 15.2% reported the current study. Possible explanations for this include that the populations in the PNSP report and the current were different, highlighting the diversity of G6PD variants among different ethnic populations. This emphasizes the importance of defining G6PD status and variants in the population to be exposed to G6PD.

The sensitivities of the point of care tests evaluated were between 50 and 59% when tested on capillary blood, too low to support the use of any of the tests as a reliable diagnostic test. Interestingly sensitivity of the RDT improved to 78.6 when performed on venous blood, however still too low for field application. A large fraction of G6PD deficient individuals would erroneously be treated with primaquine and consequently be exposed to the risk of severe haemolysis if decision to treat was based on any of the test outcomes.

Considering the operational characteristics of the assays evaluated none of the test was found to meet the requirements to be used in a local setting. The result emphasizes the need for an easy to use, cost effective and reliable diagnostic G6PD test. Further research into the operational characteristics of currently available and future test formats is urgently needed to identify a product that will facilitate the safe use of primaquine in malaria elimination projects.
Glucose 6 phosphate enzyme activity dynamics and G6PD qualitative test performance in G6PD deficient Cambodian patients undergoing weekly primaquine for acute uncomplicated vivax malaria

BACKGROUND

The Glucose 6 Phosphate Dehydrogenase enzyme (G6PD) is essential in maintaining the integrity of Red Blood Cells (RBCs). Individuals with a reduced G6PD activity are at an increased risk of severe side effects (haemolysis) if exposed to external triggers such as the antimalarial primaquine.

The Fluorescent blood spot (FST) assesses the activity of the G6PD enzyme in RBCs. For this purpose a drop of blood is added to a filter paper and dried. If the substrate NADP+ is added to the dried blood spot test fluorescent NADPH is produced in the presence of a functional enzyme. The FST will fluoresce if exposed to UV light. If the enzyme activity is very low no such reaction will take place, the FST will remain dark under UV light.

AIMS AND OBJECTIVES

This project aims to:

i) evaluate the test performance of FST against a widely accepted and precise method, spectrophotometry

ii) investigate if G6PD activity varies within the course of a vivax malaria treatment

iii) assess whether the test is sufficient to predict haemolysis in G6PD deficient individuals that are treated with primaquine.
METHODS

This study is an open label case control study within a primaquine clinical trial currently being conducted in vivax malaria infected Cambodians. A total of 100 G6PD deficient and 50 G6PD normal participants of the clinical trial are enrolled. The diagnosis of normal or deficient G6PD activity is based on the FST performed on site.

The FST and spectrophotometric testing is done at the Institute Pasteur, Pnomh Penh. The result of the FST and spectrophotometry are linked to the clinical profile from the data in clinical trial. Testing on site and at the reference centre is repeated four times over the course of the clinical trial on days 0, 7, 28 and 56.

STUDY STATUS

The study is ongoing.

DISCUSSION

The study will provide an important contribution to the knowledge on pre-test probabilities for the FST. The investigators hypothesise that the risk of a false normal result in malaria patients may be high as vivax malaria induces the production of red blood cells, known to exhibit higher G6PD activities than older cells.

Specifically in the light of radical cure with primaquine a false positive result can have a significant clinical impact. A vivax patient with very low G6PD activity that remains unrecognized is at risk of receiving primaquine treatment, rendering the patient at high risk of severe haemolysis. Quantifying the presumed risk of haemolysis and assessing whether the FST may be an accurate predictor of haemolysis will inform decision makers on the applicability of the FST for a number of different settings with poor diagnostic facilities.
Parasitic clearance and recurrence rates among patients with vivax malaria on chloroquine and primaquine therapy

BACKGROUND

The low-lying southern regions of Bhutan bordering with the Indian states of Assam and West Bengal are at high-risk for malaria transmission. The confirmed malaria cases in Bhutan declined by 98.7% from 1994 to 2010. The majority of indigenous cases (59.9%) are due to *Plasmodium vivax*, and only 32% and 8% due to *Plasmodium falciparum* and mixed infections respectively. Despite significant progress malaria caused by *Plasmodium vivax*, remains an important public health problem in Bhutan. Bhutan aims to achieve zero local malaria transmission in Bhutan by 2016 and World Health Organization malaria-free certification by 2020. Progress towards the elimination of vivax malaria will require optimization of treatment regimens, including knowledge of chloroquine (CQ) and primaquine (PQ) efficacy and awareness of potential resistance to the currently used drugs.

AIMS AND OBJECTIVES

The aim of this study is to assess the therapeutic response of CQ plus PQ treatment for uncomplicated vivax malaria. Specific objectives are:

i) To measure the clinical and parasitological efficacy of CQ among adults and children older than 12 months of age with uncomplicated vivax malaria treated with 10mg/kg on day 1, 2 and 5mg/kg on day 3.

ii) To measure risk of *P. vivax* recurrence in patients within 1 year of receiving a 14 day regimen of low dose PQ (0.25mg/kg x 14 days).

METHODS

The study started in 12 sentinel sites in the most affected districts in the south of the country, bordering India and was extended to 23 additional sites in May 2014 due to limited enrolment of cases.

Patients recruited are admitted and treated with CQ for the first 3 days (day 0, 1 and 2) and with supervised PQ for 14 days (day 28 to 41). All drug administration is directly observed. Parasite clearance and clinical evaluations are monitored daily whilst an inpatient. Patients are asked to return for follow up visits on day 7, 14, and day 28.
The proportion of patients parasitaemic on day 1, 2, 3 will be calculated and the cumulative risk of recurrence on day 28, 6 month and 12 month calculated using survival analysis. The relapse rates following the administration of PQ will be estimated with 95% confidence interval.

RESULTS

The study was started in April 2013. Currently a total of 24 patients have been enrolled into the study. Out of these 24 patients, 4 patients were lost to follow up. No recurrent infections have been observed by day 28. PCR sample were collected for all 24 patients and are currently being analysed.

STUDY STATUS

The study is ongoing and is due to end in 2015.

STUDY IMPLICATIONS

This is the first antimalarial treatment efficacy study to be conducted in Bhutan. The slow recruitment reflects the difficulty of monitoring treatment efficacy in an area with successful control approaching the end stages of elimination. Since the intense drug pressure of parasites in such areas can encourage the emergence of drug resistance, it is important to monitor the therapeutic response to look for early signs of reduced susceptibility. Although the study is ongoing, it is reassuring in highlighting that the current policy of chloroquine and low dose primaquine appears to be working.
Evaluation of safety and efficacy of two primaquine dosing regimens for the radical treatment of *Plasmodium vivax* malaria in Vanuatu and Solomon Islands

**BACKGROUND**

Hypnozoite relapse is a key consideration in efforts to achieve elimination in settings where *Plasmodium vivax* and ovale are endemic. The Melanesian states of the Western Pacific (Papua New Guinea, Solomon Islands, and Vanuatu) represent a unique and especially prescient challenge in this respect. Major advances have been made in overall malaria control, especially in Vanuatu and Solomon Islands with >90% reductions in malaria morbidity achieved over the last 2 decades. However, as *P. vivax* and *P. ovale* remain endemic and as overall mosquito-borne transmission of all plasmodial species falls, hypnozoite-relapses from *P. vivax* will account for an ever-increasing proportion of the overall burden of malarial disease. They therefore constitute the final and most difficult hurdle to overcome on the road to eventual malaria elimination.

There is no data available that describe primaquine efficacy in Melanesian populations in Vanuatu, Solomon Islands, or Papua New Guinea. The currently recommended antirelapse treatment is a standard dose of primaquine (0.25mg/kg/day for 14 days). However, data from returned travellers suggest this may be ineffective and a higher dose (0.5mg/kg/day for 14 days) may be required.

In addition, the risks associated with local G6PD variants in Melanesia are uncertain, with data limited to a handful of case reports of haemolytic anaemia following primaquine. Therefore, a higher dose regimen could have unacceptably high risks in a setting where pre-treatment G6PD testing is likely to be impractical.

**AIMS AND OBJECTIVES**

Primary: to define and compare the efficacy of standard (0.25mg/kg/day for 14 days) and high-dose (0.5mg/kg/day for 14 days) primaquine in preventing early relapses from *P. vivax* in Solomon Islands and Vanuatu.

Secondary: to measure safety and toxicity of standard and high-dose primaquine in Melanesian adults and children in Solomon Islands and Vanuatu.
METHODS

A dual-country multi-centre three-arm randomised controlled trial comparing: (1) standard versus (2) high-dose primaquine versus (3) control (no primaquine). Eligible study patients presenting to participating health centres with symptomatic P. vivax infection are screened for G6PD deficiency and randomly assigned to their treatment arm and actively followed every 2 weeks for 3 months. All patients also receive Artemether-Lumefantrine schizontocidal treatment. The study sites include networks of participating treatment centres in Espiritu Santo, Vanuatu and Gudalcanal, Solomon Islands (Figure 1). The proposed sample size is 180 (including 90 in each country and 60 in each treatment arm).

RESULTS AND STATUS

Of 35 participants enrolled in Vanuatu, 33 have completed the 3 months of follow-up. Relapse rates were 72.7% (8/11), 41.7% (5/12) and 41.7% (5/12) in the control, standard and high-dose arms respectively (p=0.079 for difference between control vs both treatment arms combined). Genotypic analyses to distinguish reinfections from recrudescence/relapse are pending. None of the screened patients have been G6PD deficient and no significant side-effects (including intravascular haemolysis or methaemoglobinemia) have been detected. The trial is currently on hold in Vanuatu and will commence at the Solomon Islands field site in April 2014.

CONCLUSIONS AND IMPLICATIONS

The endogenous P. vivax hypnozoite relapse rate in Vanuatu is high, emphasizing the importance of hypnozoite relapse in sustaining transmission in this particular elimination setting. The preliminary data suggest a trend towards a treatment effect of primaquine for both dose regiments in preventing early P. vivax relapses. Given the impracticality of testing of patients for G6PD deficiency in routine clinical practice, the demonstration of even a modest anti-relapse efficacy of the low dose of primaquine (0.25mg-kg-day) may outweigh the risks of unsupervised high dose primaquine. If ultimately higher doses are shown to be more effective this will need to be carefully weighed against the possibly higher risks of severe hemolysis. In this instance the higher dose regimen could probably be only considered as a practical alternative if the standard regimen was shown to have no or minimal efficacy.
Artesunate-mefloquine versus chloroquine in patients with acute uncomplicated *P. vivax*: a randomised open label trial in Sabah, Malaysia

**BACKGROUND**

There are concerns that chloroquine resistant *P. vivax* is emerging in malaria endemic areas neighbouring the province of Sabah, however no prospective antimalarial efficacy studies have yet been conducted. Current Malaysian Ministry of Health guidelines specify that patients with malaria confirmed by microscopy must be admitted to hospital and only discharged once 2 negative blood smears for malarial parasites are obtained. Parasite reduction rates therefore determine duration of hospitalisation and contribute to health sector cost. Evidence for artemisinin-combination therapies as a unified treatment for all malaria species is being evaluated in this region. Unified policy with ACT has particular importance in this region where *P. knowlesi* has emerged as an important cause of severe and fatal malaria, and there is the potential for misdiagnosis and inappropriate treatment with chloroquine.

**AIMS AND OBJECTIVES**

The aim of this study is to determine whether the fixed combination of artesunate-mefloquine is superior to chloroquine for the treatment of uncomplicated *P. vivax* infection in adults and children in Sabah, Malaysia. The primary endpoint is the therapeutic efficacy of artesunate-mefloquine versus chloroquine, as defined by the assessment of microscopic parasite clearance 24 hours after initiation of treatment. Secondary endpoints include parasite clearance at day 2, 28 and 42 (both PCR adjusted and unadjusted). Prevalence of anaemia at day 28, progression to severe complications, presence of gametocytes at follow up and recurrence of *P. vivax* are also reported. Safety and tolerability are monitored on a standard questionnaire.

**METHODS**

The study sites are in Kudat, Kota Marudu and Pitas (Figure 1), where patients are being recruited at the local District Hospitals. The study is a two-arm, randomized, open label trial. Patients are being randomly allocated to treatment with either artesunate-mefloquine or chloroquine. The administration of primaquine is delayed until Day 28. All patients are followed until day 42. After day 42 patients are contacted by mobile phone every two weeks for a total follow up of 12 months to assess long-term risk of reinfection/recurrence in this region. Patients with febrile illness after
day 42, patients are asked to return to the study centers for clinical assessment and malaria microscopy, to assess for recurrent malaria.

RESULTS

The study started in January 2013 and is currently ongoing. Preliminary data as of October 2014, show that 77 uncomplicated *P. vivax* patients have been enrolled (39 in AS-MQ arm, 38 in CQ arm). In total 42% (16/38) of patients treated with chloroquine were observed to fail treatment: 3 with early treatment failure and 13 with recurrent parasitaemia before day 28. In contrast no patients who received artesunate/mefloquine and were followed up to day 28 failed treatment (Table 1).

40 patients from the same site were excluded from the randomised trial, with 28 treated with CQ and enrolled in a concurrent pathophysiology study. In total 36% (10/28) patients were observed to fail treatment: 4 with early treatment failure, 1 patient with recurrent parasitaemia each at day 19 and day 20, and 3 patients at day 28.

STUDY STATUS

The study is ongoing with recruitment of patients likely extending to June 2015. Pharmacokinetic analysis is in progress.

ANALYSIS AND IMPLICATIONS

Malaysian Ministry of Health guidelines currently recommends chloroquine and primaquine as first line treatment for the radical cure of uncomplicated *P. vivax* malaria. Case reports have highlight the potential for chloroquine resistant (*CQR*) *P. vivax* in Sabah and also Peninsular Malaysia. There is growing support for artemisinin-based combination therapy (ACT) as a unified first line treatment choice in areas co-endemic for *P. falciparum* and *P. vivax*, due to the regional increase of chloroquine-resistant *P. vivax*, and ongoing concerns over the microscopic misdiagnosis of other Plasmodium species. This study confirms the existence of *CQR* *P. vivax* in Sabah, with almost a third of patients failing chloroquine monotherapy. The data from this study will inform treatment policies regarding the optimal choice of first line treatment for uncomplicated *P. vivax* malaria in this region.

<table>
<thead>
<tr>
<th></th>
<th>AS-MQ (n=39)</th>
<th>CQ (n=38)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any recurrence during FU</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td>Still positive on day 3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Positive on day 14</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Positive after day 20</td>
<td>0</td>
<td>6</td>
</tr>
</tbody>
</table>
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Ministry of Health Bhutan  
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Ministry of Health China  
Ministry of Public Health Democratic People’s Republic of Korea  
Ministry of Health Indonesia  
Ministry of Health Lao People’s Democratic Republic  
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Ministry of Health and Medical Services, Solomon Islands  
Ministry of Health Sri Lanka  
Ministry of Public Health Thailand  
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Ministry of Health Vietnam

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United States Centers for Disease Control and Prevention  
FIND - Foundation for Innovative New Diagnostics  
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