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 pathological 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 markers1 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 published2. 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 published3. 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 (FST = 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. Tao et al., Malar J. 2011, 10:29
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 \textit{P. falciparum} and \textit{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.
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 standardised 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 analyses 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

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 People’s 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 *P. vivax* liver stages. While mass drug administration of primaquine could possibly provide an effective way to eliminate *vivax* malaria from 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.
The results of the diagnostics study have been published2. 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 CareStartTM 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 published3. 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 VxWG Genotyping Workshop May 2011
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 (FST = 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’SST = 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.

1. Report APMEN VxWG Genotyping Workshop May 2011
**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.

**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).
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 main 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 a total of 15 health centres were enrolled, all except one were included in the subsequent analysis. Age at time of enrolment ranged from 14 to 95 years (median 43 years). A total of 96 (17.3%) participants were G6PD deficient and 25 (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 P. vivax and 3 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.

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 years (median 43 years). A total of 96 (17.3%) participants were G6PD deficient and 25 (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 P. vivax and 3 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 in G6PD normal (mean Hb=11.7 g/dl) and deficient individuals (mean Hb=13.3 g/dl); p<0.001. The only sign significantly between G6PD normal (mean Hb=11.7 g/dl) and deficient patients, 0% in G6PD intermediate and only 1 (0.2%) in G6PD deficient individuals.

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.

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.

TABLE: G6PD DEFICIENT AND MALARIA POSITIVE SAMPLES (MICROSCOPIES/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>Oraiwa</td>
<td>100</td>
<td>39 (7.1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Oe vivarm</td>
<td>100</td>
<td>15 (2.7)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Panika</td>
<td>100</td>
<td>10 (3.4)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Batu Putih</td>
<td>120</td>
<td>8 (1.4)</td>
<td>5 (1.9)</td>
</tr>
<tr>
<td>Denure</td>
<td>125</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
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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/FR/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 1,186.50 km² and suffers from stable transmission of P. vivax: PvAPI > 0.1 per 1,000 populations per year and prevalence (PvPR) 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 could be 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 consequences 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. 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.

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.
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 methods1.

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 published2. 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 Kilinochchi, Mullaitivu 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 Kilinochchi, Mullaitivu 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 species-specific 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 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.

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(15,12),(991,986)

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.

**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.

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1. 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.), fluorescent spot test (procedure No. 203, Trinity Biotech), 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.

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.
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 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, and 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.

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 are prevalent in areas where pre-treatment G6PD testing is low risks in a setting where pre-treatment G6PD testing is difficult hurdle to overcome on the road to eventual malaria elimination. Therefore, a higher dose regimen could have unacceptably high incidence of severe hemolysis (0.25mg/kg/day for 14 days) and high-dose (0.5mg/kg/day for 14 days) may be required. 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 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 populations are uncertain, with data limited to a handful of case reports of haemolytic anaemia following primaquine. 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. If ultimately higher doses are shown to be more effective this will need careful weighing against the possibility 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.

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 methaemoglobinaemia) 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 antimarial 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 regions 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 up to 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.