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**Operational applications of serology for malaria surveillance  
in different transmission settings in Indonesia**

**Henry Surendra**

Thesis submitted in accordance with the requirements for the degree of  
Doctor of Philosophy of the University of London

March 2020

DEPARTMENT OF INFECTION BIOLOGY  
FACULTY OF INFECTIOUS AND TROPICAL DISEASES  
LONDON SCHOOL OF HYGIENE & TROPICAL MEDICINE

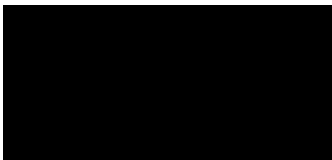
Funded by the Indonesia Endowment Fund for Education (LPDP)

Supervisors: Professor Chris Drakeley, Faculty of Infectious and Tropical Diseases  
Assistant Professor Jackie Cook, Faculty of Epidemiology and Population Health

## Declaration

I, *Henry Surendra*, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

5 March 2020,



## Abstract

Serological surveillance involves the detection of *Plasmodium* species-specific antibodies as biomarkers for monitoring recent and historical malaria transmission dynamics at population-level. These methods are particularly useful in low transmission settings where standard surveillance such as parasitological and entomological approaches are inefficient. This thesis explores the use of serological surveillance to estimate the magnitude and heterogeneity of malaria transmission using different sampling strategies, mapping techniques and serological assays in three areas of differing endemicity in Indonesia. Findings suggest that: 1) Analysis of community-based serological data can confirm the discontinuation of transmission and be used to identify high-risk areas where malaria is most likely to be reintroduced, 2) Mobile technology-based participatory mapping approaches can be used to quickly obtain spatial residential information for individuals presenting at health facilities in resource poor areas where formal addresses are typically not used and internet connectivity is limited, 3) the combination of facility-based sampling, multiplex serological assays and participatory mapping can be used as an additional surveillance method to better identify and target areas still receptive to malaria in very low transmission area conducting elimination, 4) seropositivity to Etramp5.Ag1 is sensitive and specific in predicting *Plasmodium falciparum* PCR positivity in children in a high transmission setting, suggesting its potential use as a marker of recent exposure in elimination setting. In conclusion, this thesis demonstrates the various applications of serological surveillance at different levels of malaria endemicity. Further implementation research is needed to enable the integration of these methods to the existing surveillance systems.

## Acknowledgements

The research presented in this thesis would not have been possible without helps of so many people. First, I would like to thank my supervisors Chris Drakeley and Jackie Cook for giving me the opportunity to pursue my PhD under their supervision. I am so grateful for their invaluable support and guidance in the last four years. They put so much trust on me who sometimes do not believe I can do good in my PhD.

I would like to thank Gillian Stresman and Kimberly Fornace who have been very kind to share their knowledge and skills on spatial analysis, Lindsey Wu and Nuno Sepulveda for the statistical advices, and to Tom Hall and Lotus van den Hoogen for training me on the serological assays and to Kevin Tetteh who produced the antigens used in research presented in this thesis. Thanks to Lynn Grignard who trained me the basic knowledge and skills on handling DNA samples and performing nested PCR. Thanks to all other member of Drakeley's group who have been very nice to me even though I am not a good person to chat with. Thanks also to Inke Lubis and Satria Prabowo for sharing their PhD learning experiences and for being very supportive friends during my PhD.

I am extremely grateful being part of Universitas Gadjah Mada (UGM). Without support and guidance from many people at UGM, it would not have been possible for me to do a PhD at the London School of Hygiene & Tropical Medicine. A bunch of thanks to late Prof. Supargiyono who put his trust on me and who was very supportive for my PhD and professional career. It was very sad that Prof. Supargiyono passed away three days prior to my PhD viva. Thanks to Dr. Riris Andono Ahmad, a supportive mentor who helped me find

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To my family: my wife Nurul and my lovely daughter Zizi who have been very supportive all the times; my mom and dad who taught me honesty, discipline and hard work will always be paid off; my sister Evelyn and other family members who put their trust on me and pray the best for my career.

Last, but not least, I would like to thank all field workers, district health offices and health facilities staffs as well as study participants and communities who were involved in research presented in this thesis.

## Statement of Contributions

The work described within this thesis was funded by the Indonesia Endowment Fund for Education, Ministry of Finance, Republic of Indonesia (Lembaga Pengelola Dana Pendidikan, Kementerian Keuangan, Republik Indonesia). Projects were conducted in partnership with the Centre for Tropical Medicine, Universitas Gadjah Mada (UGM), Indonesia and were part of existing collaboration between Prof. Chris Drakeley and Prof. Supargiyono at UGM. During my PhD programme, I have learnt a totally new field by performing laboratory works such as ELISA, bead-based assays and nested PCR, all of which I had never done before. I have also developed my skills in advanced statistical and spatial epidemiological analysis of serological data generated from my lab works. My role and the contributions of my collaborators to the studies included in this thesis are summarised below:

**Chapter 1** was based on literature review conducted by myself, supervised by Chris Drakeley and Jackie Cook.

**Chapter 2** describes the objectives and summary of the methods used in each research presented in this thesis.

**Chapter 3** was based on community-based cross-sectional surveys conducted in area achieving elimination, Sabang Municipality, Indonesia. All data cleaning, analysis and writing was conducted by myself. Chris Drakeley, Jackie Cook and Supargiyono (UGM) provided comments on data analysis and interpretation. Supargiyono led the field and lab works and provided the data.

***Surendra H, Wijayanti MA, Murhandarwati EH, Irnawati, Yuniarti T, Mardiaty, et al.***

*Analysis of serological data to investigate heterogeneity of malaria transmission: a*

*community-based cross-sectional study in an area conducting elimination in Indonesia. Malar J. 2019;18:227.*

**Chapter 4** was based on field evaluation and validation conducted in Kulon Progo District, Indonesia and Palawan, the Philippines. This work was in collaboration with UGM and the Research Institute of Tropical Medicine, Philippines. This chapter was co-authored by myself and Kimberly M Fornace (LSHTM), with equal contributions in designing study, final data analysis and writing. Chris Drakeley and Jackie Cook provided guidance on study design and analysis.

*Fornace KM\*, Surendra H\*, Abidin TR, Reyes R, Macalinao MLM, Stresman G, et al. Use of mobile technology-based participatory mapping approaches to geolocate health facility attendees for disease surveillance in low resource settings. Int J Health Geogr. 2018;1–10.*

\*Shared first author.

**Chapter 5** was based on quarterly health facility-based cross-sectional surveys conducted in area conducting elimination, Kulon Progo District, Indonesia. Study design, field activities, sample collection, and laboratory analysis for the OPSIN project were coordinated by myself, with support from Chris Drakeley, Jackie Cook and Supargiyono. Sample and data collection and microscopy test were the work of numerous field workers, laboratory staff, nurses and control programs at the District Health Office of Kulon Progo. Luminex assay procedures were optimised by Kevin Tetteh and Tom Hall (Drakeley's group, LSHTM). Antigens were designed/produced by Kevin Tetteh and Chetan Chitnis (Institute Pasteur, Paris). Luminex procedures were adapted/coordinated at the UGM lab by myself. Assays were mainly



conducted by myself, and supported by Alexander Ward (LSHTM). Study design for the analysis in this chapter was conceived by myself with support from Chris Drakeley and Jackie Cook. Nuno Sepulveda, Gillian Stresman, Kimberly Fornace and Lyndsey Wu (LSHTM) provided advice on statistical analyses and R code. Lynn Grignard (LSHTM) provide guidance in conducting additional work on PCR.

***Surendra H, Supargiyono, Ahmad RA, Kusumasari RA, Rahayujati TB, et al. Using health facility-based serological surveillance to predict receptive areas at risk of malaria outbreaks in elimination areas. BMC Med. 2020;18:9.***

**Chapter 6** was based on community-based cross-sectional surveys conducted in a high transmission setting, Mimika District, Indonesia. All data cleaning, analysis and writing was conducted by myself. Chris Drakeley, Jackie Cook, Supargiyono and Ric Price (Menzies Institute, Australia) provided comments on data analysis and interpretation. Ric Price led the project. Luminex assay procedures were optimised by Kevin Tetteh and Tom Hall. Antigens were designed/produced by Kevin Tetteh and Chetan Chitnis. Luminex procedures were performed by Rumbiwati at UGM laboratory and supervised by myself.

***Surendra H, Puspoprodjo JR, Supargiyono, Wijayanti MA, Murhandarwati EH, et al. Serological markers to assess malaria transmission in a high transmission setting. Malaria Journal. In preparation.***

**Chapter 7** consists of original work describing a summary of the findings presented in this thesis, general discussion and future directions.

### **Additional publications:**

I also contributed to other works that were not part of my PhD:

- Boelaert M, **The NIDIAG Consortium** (2016) *Clinical Research on Neglected Tropical Diseases: Challenges and Solutions*. *PLoS Negl Trop Dis* 10(11): e0004853.
- Ahmad RA, Ferdiana A, **Surendra H**, Sy TR, Herbianto D, et al. *A participatory approach to address within-country cross-border Malaria: the case of Menoreh Hills in Java, Indonesia*. *Global Health Action*. Submitted.
- Ahmad RA, Ferdiana A, **Surendra H**, Sy TR, Herbianto D, et al. *Joint operational action plan for malaria elimination in Menoreh Hills in Java, Indonesia*. 2018.

As part of my PhD training programme, I have also presented my work in several scientific conferences:

- **Surendra H**, Ahmad RA, Ferdiana A, et al. (2018). *A participatory approach to addressing within country cross-border malaria: the case of Menoreh Hills in Java, Indonesia*. Poster presentation at the First Malaria World Congress, Melbourne, Australia, 1–5 July 2018.
- **Surendra H**, Supargiyono, Cook J and Drakeley C (2018). *Evaluation of health facility-based surveys to monitor malaria transmission in a pre-elimination setting, Indonesia*. Poster presentation at the American Society of Tropical Medicine and Hygiene 67<sup>th</sup> Annual Meeting, New Orleans, USA, 28 October-1 November 2018.
- **Surendra H**, Fornace K, Abidin TR, Reyes R, Macalinao MLM, et al. (2018). *Use of mobile technology-based participatory mapping approaches to geolocate health facility attendees for disease surveillance in low resource settings*. Oral presentation

*at the American Society of Tropical Medicine and Hygiene 67<sup>th</sup> Annual Meeting, New Orleans, USA, 28 October-1 November 2018.*

- **Surendra H**, Supargiyono, Ahmad RA, Kusumasari RA, Rahayujati TB, et al. *Serological analysis reveals heterogeneity and predicts hotspot of P. vivax transmission in a pre-elimination setting, Indonesia. Oral and poster presentation at the 7<sup>th</sup> International Conference on Plasmodium vivax Research, Paris, France 26-28 June 2019.*
- **Surendra H**, Supargiyono, Ahmad RA, Kusumasari RA, Rahayujati TB, et al. *Use of health facility-based serological surveillance to investigate P. falciparum and P. vivax transmission dynamics in a pre-elimination setting, Indonesia. Oral and poster presentation at the 68<sup>th</sup> Annual Meeting, Maryland, USA, 20-24 November 2019.*

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## Abbreviations

AMA1	Apical membrane antigen 1
API	Annual parasite incidence
CSP	Circumsporozoite protein
DBPR2	Duffy binding protein region 2
EBA	Erythrocyte binding antigen
EBP	Erythrocyte binding protein
EIR	Entomological inoculation rate
ELISA	Enzyme-linked immunosorbent assay
Etramp	Early transcribed membrane protein
FMM	Finite mixture model
GexP18	Gametocyte exported protein 18
GLURPR2	Glutamate rich protein R2
HSP40	Heat shock protein 40
HRP2	Histidine-rich protein 2
IgG	Immunoglobulin-G
IgM	Immunoglobulin-M
IPT	Intermittent preventive treatment
IRS	Indoor residual spraying
LAMP	Loop-mediated isothermal amplification
LFA	Lateral flow assay
LLINs	Long-lasting insecticidal nets
MBCs	Memory B cells
MFI	Median fluorescence intensity
MSP-1 <sub>-19</sub>	Merozoite surface protein 1, C-terminal 19-kilodalton region
OR	Odds Ratio
PCR	Polymerase chain reaction
PR	Parasite rate
<i>Pf</i>	<i>Plasmodium falciparum</i>
<i>Pv</i>	<i>Plasmodium vivax</i>
<i>PfPR</i> <sub>2-10</sub>	<i>P. falciparum</i> parasite rate in 2-10 year olds
RBP1a	Reticulocyte binding protein 1a [amino acids 160-1170]
RBP2b	Reticulocyte binding protein 2b [amino acids 161-1454]
RDT	Rapid diagnostic test
ROC	Receiver operating characteristics
SBP	Skeleton-binding protein
SCR / SRR	Sero-conversion rate / sero-reversion rate
SD	Standard deviation
SEA	Schizont egress antigen
WHO	World Health Organization
µL	Micro-litre
95%CI	95% confidence interval

## 1. Introduction

### 1.1 Global burden and the changing epidemiology of malaria

In humans, malaria is caused by five species of *Plasmodium*. *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae* and *Plasmodium ovale* are spread from one person to another via the bite of female mosquitoes of the genus *Anopheles*, whereas *Plasmodium knowlesi* is a result of zoonotic transmission when an *Anopheles* mosquito infected by parasites circulating in a macaque then bites and infects humans (1). Of these five species, *P. falciparum* and *P. vivax* cause the greatest public health challenge because *P. falciparum* is responsible for most of the disease morbidity and mortality whilst *P. vivax* is the most geographically widely distributed and has a hypnozoite, liver stage which is extremely difficult to detect and responsible for approximately 80% of infections (1–5). In addition, the public health threat caused by *P. knowlesi* appears to be growing with the number of human *P. knowlesi* cases increasing across Southeast Asia (1,6–13) with those who visit rural, forested areas of the region most at risk (6,14,15).

Globally, there were significant reductions in the number of malaria cases and deaths between 2010 and 2017. It was estimated that the number of malaria cases had decreased from 239 million cases (95% CI: 219–285 million) in 2010 to 219 million cases (95% CI: 203–262 million) in 2017(16). In line with the cases, the estimated number of malaria deaths had also decreased from 607,000 deaths in 2010 to 435,000 in 2017. Although there were large reductions in the number of malaria cases and deaths, malaria is still a major public health problem in many countries of the world. It is reported that almost 80% of all malaria cases were in 15 African countries and in India, whilst approximately 82% of estimated *P. vivax*

malaria cases in 2017 occurred in just five countries i.e. India, Pakistan, Ethiopia, Afghanistan and Indonesia (16). The spatial distribution of age-standardized *P. falciparum* parasite rate in 2005 and 2017 and the predicted *P. vivax* parasite rate and change 2005 and 2017 can be seen in Figure 1.1 and 1.2, respectively.

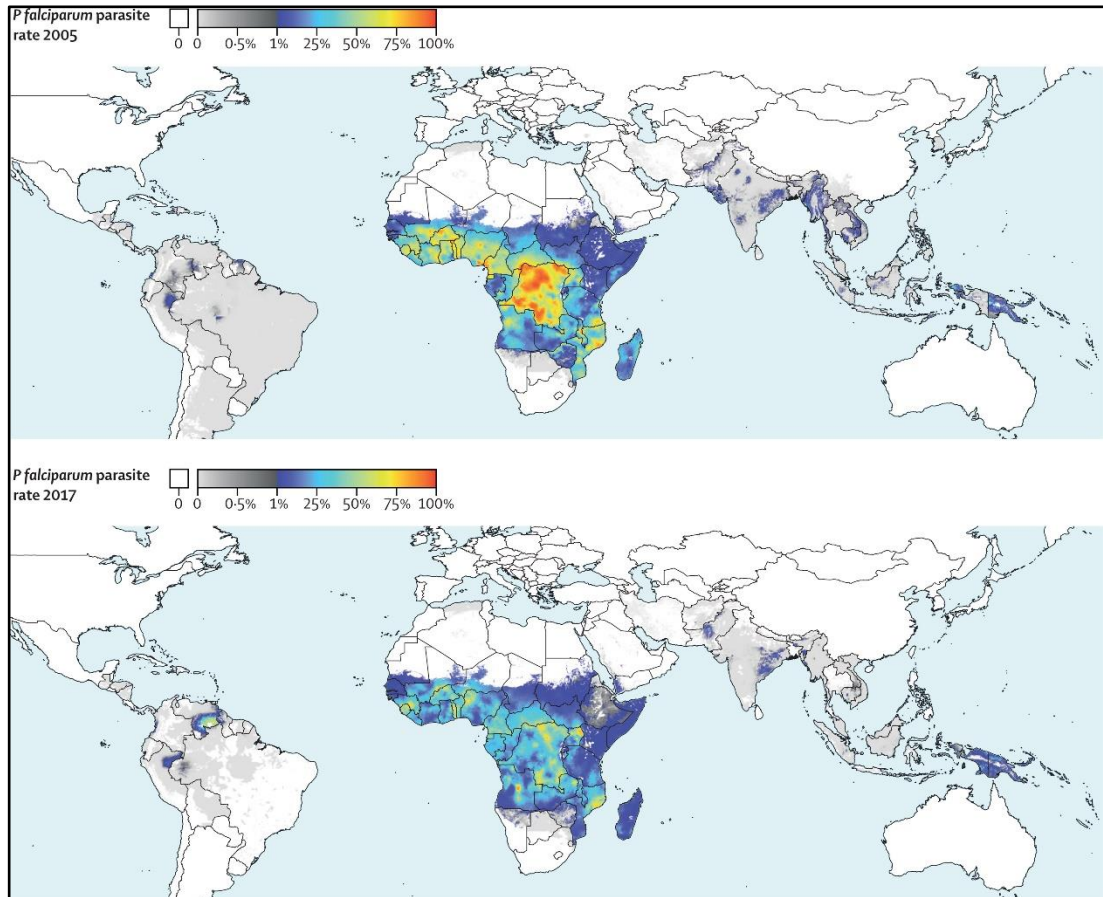


Figure 1.1 Spatial distribution of age-standardized *P. falciparum* parasite rate for children aged 2–10 years in 2005 (top) and 2017 (bottom) reproduced from Weiss et al. (17) The grey shades represent low endemic areas with a linear scale between zero and 0.01 *P. falciparum* parasite rate<sub>2-10</sub>, colors from blue to red represent areas with *P. falciparum* parasite rate<sub>2-10</sub> between 0.01 and 1. Areas without endemic *P. falciparum* are shown in white.

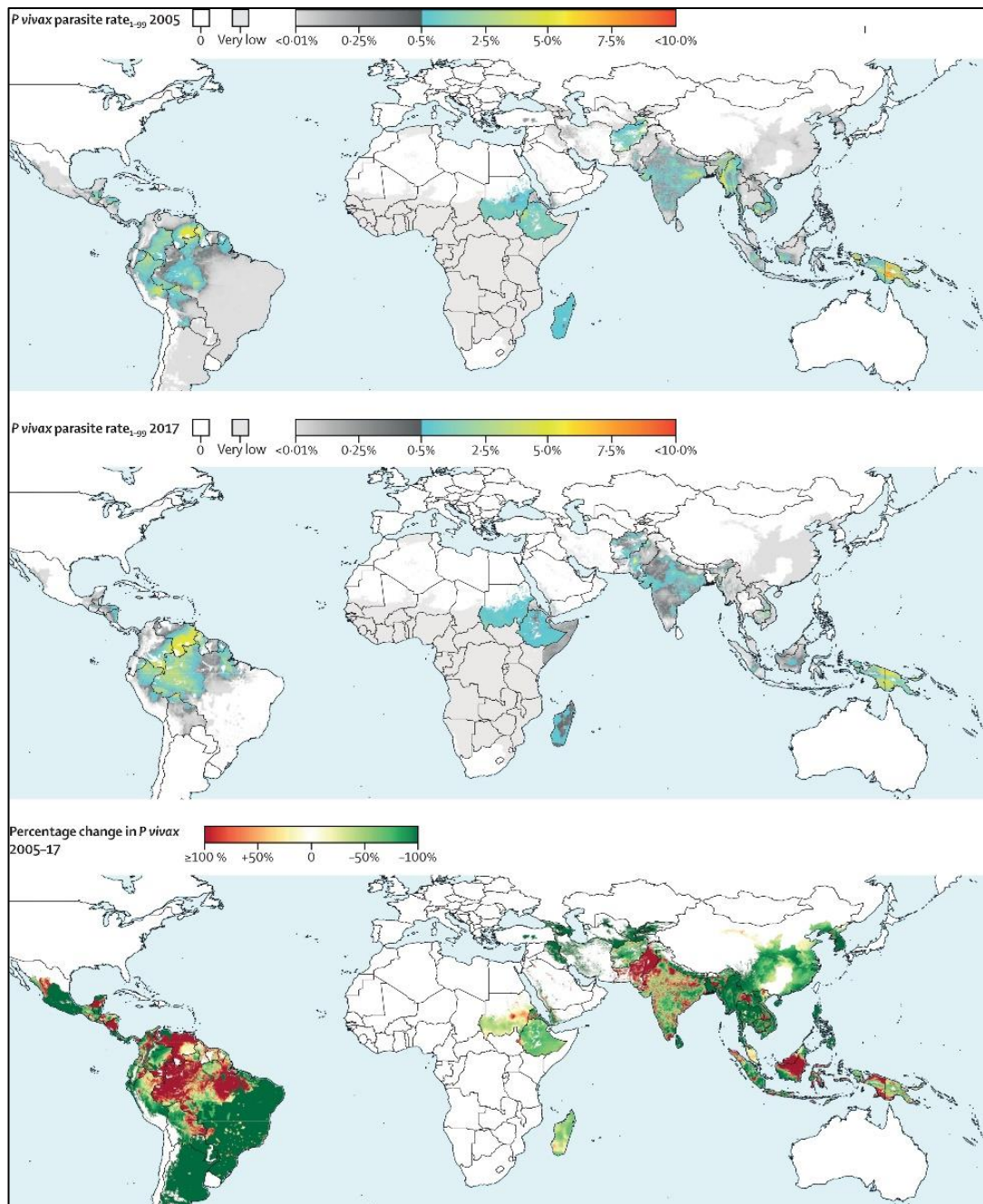


Figure 1.2 Map of *P. vivax* parasite rate and change 2005 and 2017 as shown in Battle et al.(18)

Light grey represents endemic areas with insufficient information to generate a prediction. The change in parasite rate was calculated by the value for 2005 minus 2017 divided by the 2005 value and multiplied by 100. A scale of white to green represents a decrease and from white to red represents an increase. The darkest green areas have seen a  $\geq 100\%$  decrease in prevalence, while the darkest red areas show a  $\geq 100\%$  increase in prevalence from 2005 to 2017.

The significant decline in malaria transmission in many regions has led to optimism that malaria elimination might be achieved in numerous countries (19–26). Malaria elimination is being considered as an ultimate goal and sustaining a malaria-free status will have tremendous benefits in terms of deaths and illness averted, household socioeconomics, and the growth of industrial and agricultural benefits for a country (27,28). It was reported that there are 91 countries and territories with ongoing malaria transmission (29) , 7 countries and territories certified malaria-free (29,30) and there are 21 countries with the potential to eliminate by 2020 (Figure 1.3 (31)).

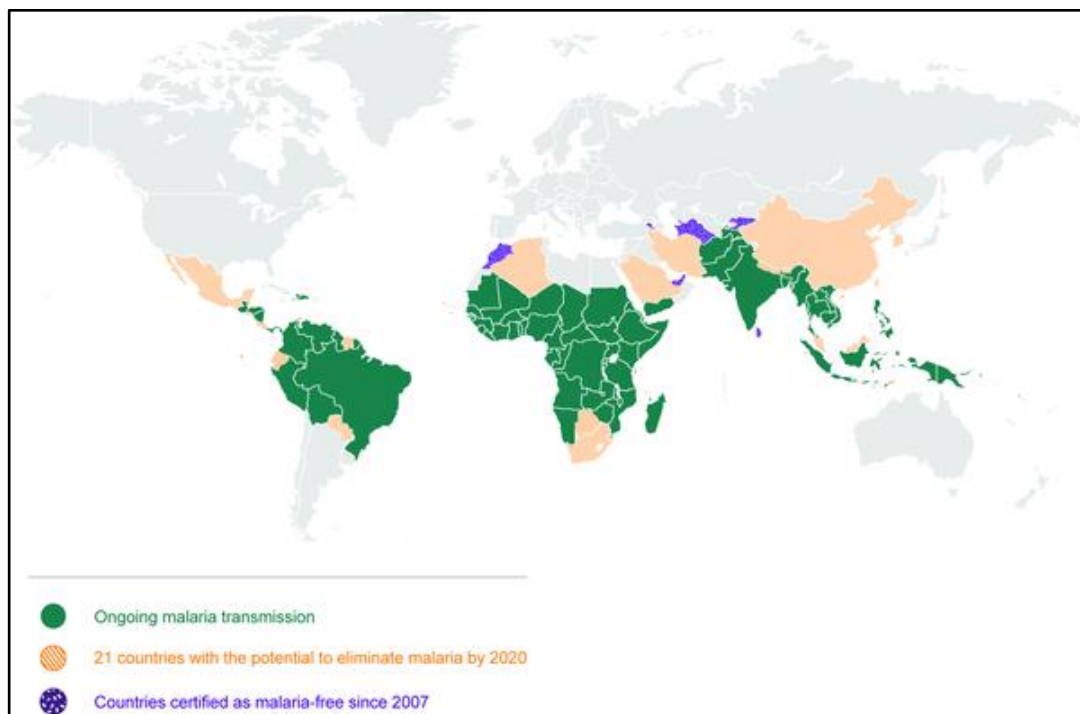


Figure 1.3 Map of 21 countries with the potential to eliminate malaria by 2020 reproduced from Rabinovich et al.(31)

A recent review by Cotter et al. (3) has provided a comprehensive picture of the changing epidemiology of malaria in areas moving from controlled low–endemic malaria to elimination. In East Asian countries, the most common epidemiological shift is the increasing

proportion of malaria in the adult male population due to occupational and behavioural factors such as plantation work and forest activities, sleeping in fields for farming purposes, and travel to endemic areas, which increases risk of exposure (32,33). Whilst in Africa, malaria risk is occupationally related to gold miners, loggers, and outdoor activities such as sleeping outdoor and social activities. Residual transmission is also typically concentrated in hard-to-reach populations whom typically have less access to malaria prevention and treatment provided by health facilities (34). Finally, imported malaria and human migration may provide a significant source of reintroduction of malaria transmission in the absence of strong public health systems (35–37).

## 1.2 Malaria surveillance in elimination

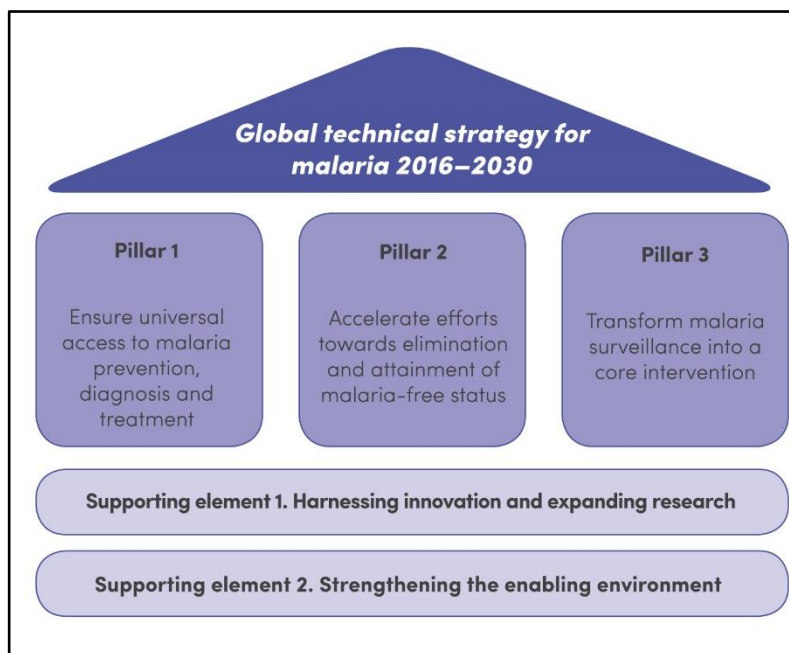


Figure 1.4 Global technical strategy for malaria 2016-2030: framework, pillars and supporting element copied from WHO (38)

Transforming malaria surveillance into a core intervention is one of three pillars of the WHO global technical strategy for malaria 2016-2030 (Figure 1.4) (38). In an elimination context, as transmission declines, monitoring changes in malaria transmission intensity and disease prevalence through surveillance becomes increasingly important to allow the evaluation of health services and control programs (19,39,40). Moreover, once elimination has been achieved, surveillance must continue in order to confirm a region’s elimination status and to ensure that outbreaks resulting from re-introduced infections are quickly identified and controlled (41). Measuring transmission in these situations is challenging as it tends to become more heterogeneous and hotspots of transmission (geographical areas where transmission intensity exceeds the average level (42,43)) are increasingly common (Figure 1.5).

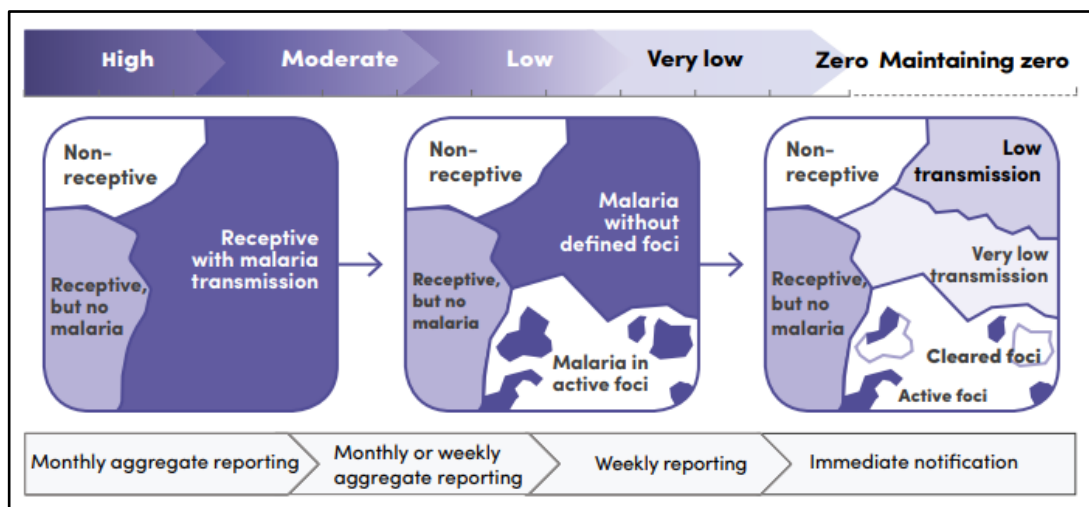


Figure 1.5 Malaria heterogeneity across the transmission continuum  
*Schematic of the increasingly focal nature of malaria as transmission decreases, requiring increased intensity and frequency of reporting from large geographical areas (e.g., district) to reporting near-real-time individual case data in small areas”. Figure from WHO (41).*

The WHO framework for malaria elimination defined the following stratification for transmission risk (Figure 1.6): 1) Receptive areas (i.e. the ecosystem is suitable for

transmission of malaria) and non-receptive areas (i.e. the ecosystem is not suitable for transmission of malaria), 2) Receptive areas with and without ongoing transmission, 3) Transmission with or without foci, 4) Degree of transmission in diffuse or focal areas.

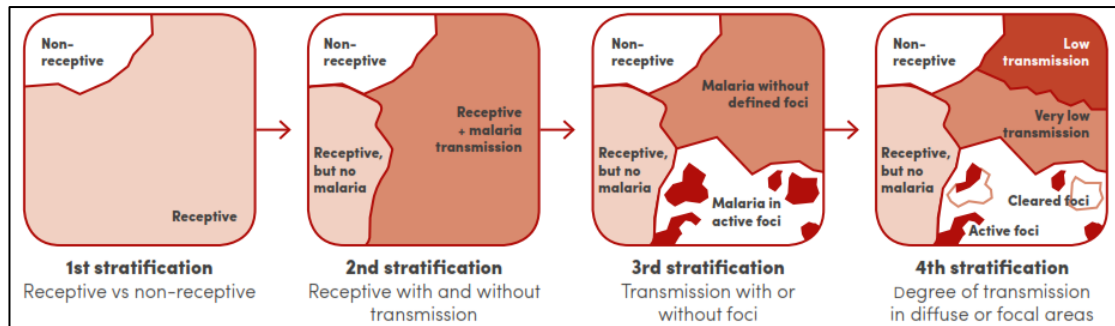


Figure 1.6 Sequential risk stratification based on receptivity and transmission intensity taken from WHO (44)

### 1.2.1 Heterogeneity of transmission

Heterogeneity of transmission occurs when a small proportion of the population is disproportionately affected and experiences the majority of the disease due to environmental, social or biological factors (45,46). To better target interventions in areas moving towards elimination, it is increasingly important to identify and target hotspots of transmission and understand the factors that may contribute to disease persistence in these locations (47,48). However, the assessment of transmission heterogeneity has been focused on national level estimates, mainly due to the availability of data (49,50). Previous studies reported that the detection of local level clusters of infection has an important role for improving understanding of the micro epidemiological patterns of disease transmission, and to ensure that control strategies are tailored to the specific epidemiological characteristics in an area as much as is feasible (42,43).



### **1.3 Approaches to quantify malaria transmission**

#### **1.3.1 Entomological**

The entomological inoculation rate (EIR) is considered the gold standard for estimating malaria transmission. EIR provides a measure of the degree of malaria exposure in the population by assessing the average number of infectious bites that a person in a given area is expected to receive per unit time (51,52). As it is difficult to directly calculate the proportion of host seeking mosquitoes that are harbouring sporozoites (53), using human landing catches is considered to be the best proxy. However, as this method involves the risk of the workers being exposed to malaria, the use of light and chemical traps have become more widely utilised as an alternative method (54,55). Despite it being considered the gold standard for estimating transmission intensity, EIR is not extensively used as it is a highly seasonal measure with extreme variability over time and space, and is difficult to estimate in areas of low transmission intensity where the density of mosquitoes is low (52,56).

#### **1.3.2 Parasitological**

##### **1.3.2.1 Microscopy**

Microscopy is considered the gold standard of malaria diagnostic tools in the field and the estimates of *P. falciparum* parasite rate (PfPR) generated from microscopy tests are the most common malaria burden metric reported worldwide (57). Microscopy is typically the recommended diagnostic in a clinical setting and involves reviewing and quantifying the presence of parasites in bloods slides that are visualised under an oil immersion microscope (58).

Although microscopy is able to detect as few as 5 parasites/ $\mu\text{l}$  of blood, its sensitivity has been reported to vary considerably with some estimates suggesting a more consistent limit of detection closer to 100-200 parasites/ $\mu\text{l}$  of blood for routine microscopy in clinical settings and likely to be more insensitive in low transmission areas where microscopists do not see malaria parasites on a regularly basis (59–61). Parasite densities tend to fluctuate in most infected individuals, with microscopically detectable malaria likely to be present at some points during each infection (62–64). It has been reported that parasite prevalence generated from microscopy tests could be negatively biased by at least 20% due to fluctuating parasite densities (65). Specifically, for *P. vivax*, it is impossible for microscopy to diagnose individuals with hypnozoites which can result in future infections.

### **1.3.2.2 Rapid Diagnostic Tests**

Malaria Rapid Diagnostic Tests (RDTs) provide an easy and less technically demanding diagnostic tool with similar sensitivity to conventional field based microscopy (66). The basis for *P. falciparum* and *P. vivax* detection by RDTs is usually detection of histidine-rich protein 2 (HRP2) and plasmodium lactate dehydrogenase (pLDH), respectively. The latest WHO malaria RDT evaluation reported that most RDTs are showing invalid rate when testing samples with parasite densities below 200 parasites/ $\mu\text{l}$  of blood (67). The RDTs are also likely to be false-positive, often caused by the detection of HRP-2 that is still circulating post clearance of infection. In addition, it was reported that the currently available RDTs were less good at detecting *P. vivax* than *P. falciparum* infection (67). For an example, a study in the high transmission area in Papua province, Indonesia reported that sensitivity of Plasmotec Malaria-3 RDT to detect *P. falciparum* and *P. vivax* was 78% and 52% when parasite densities ranged from 101-1,000/ $\mu\text{l}$  then became lower to 60% and 21% when

parasite densities were lower than 100/μl (68). Although there is a *P. falciparum* highly sensitive HRP2-based RDT with a reported detection limit 10 folds more sensitive than conventional RDTs, assessment of its field performance is still ongoing (69).

### **1.3.2.3 Molecular methods**

Molecular methods such as polymerase chain reaction (PCR) and loop mediated isothermal amplification (LAMP) are more sensitive methods for determining parasite positivity typically used in malaria research. Firstly, PCR-based methods have been found to detect as few as 1 or 2 parasites/μl of blood demonstrating the high sensitivity and specificity of these methods (70,71). It has been reported that the increased sensitivity of PCR could overall detect 50% more infections than microscopy or RDTs (59,72). However, the field application of PCR is limited by technical complexity and high cost of the assay as well as the length of time required to process samples (60). LAMP has been reported as the most advanced molecular method that may provide a field-friendly sensitive diagnostic tool that can be used in malaria endemic countries (73–75). LAMP provides an attractive alternative tool with less technical complexity, less time to obtain a result, and with similar sensitivity to PCR (73,76). These molecular methods have the potential to become useful tools to quickly detect areas of focal transmission when detecting sub patent infections becomes the priority as the malaria programmes move towards elimination (72).

### **1.3.3 Serological measures**

Malaria infections elicit the production of antibodies in humans which can be used as markers of exposure to the disease (77). Studies suggest that antibodies to parasite antigens are generated within 2 weeks after infection (78,79) with some variation depending on age

(78). Furthermore, previous studies suggested that there are differences in the production of antibodies by age, where malaria species specific antibodies may be short-lived in young children (80) but long-lived in older individuals (81).

As markers of exposure, a memory antibody response exists and can persist for many years in adults (82–85). Studies have shown that antibodies can persist for several years without re-infection in immigrants to Europe (82,83). It has also been reported that antibodies appear very rapidly in individuals re-exposed to malaria during epidemics in Madagascar (84) and in populations from which malaria had been eliminated in Vanuatu (85). However, evidence suggested that antibody responses in children are not as fixed as they are in adults, particularly in areas of seasonal malaria (80,86,87).

Serology provides an alternative approach to indirectly estimate malaria transmission by measuring human antibody responses to malaria parasite antigens. The presence of species-specific antibodies reflects historical (or current) exposure and therefore offers a more sensitive measure of transmission, particularly in low endemic settings where other approaches become less viable (88–90). This approach has been used to measure transmission intensity, primarily through seroconversion rates (SCR, i.e. the annual rate by which people seroconvert from negative to positive) derived from age-dependent measures of antibody seroprevalence. Serological estimates have been reported as alternative measures of medium and long-term transmission intensity (39,40,77,91–95) and strongly correlate with estimates of EIR, PR, and clinical incidence (39). Recently, it has been reported that SCR has a stronger correlation with EIR, compared to the correlation between PR and EIR (Figure 1.7).

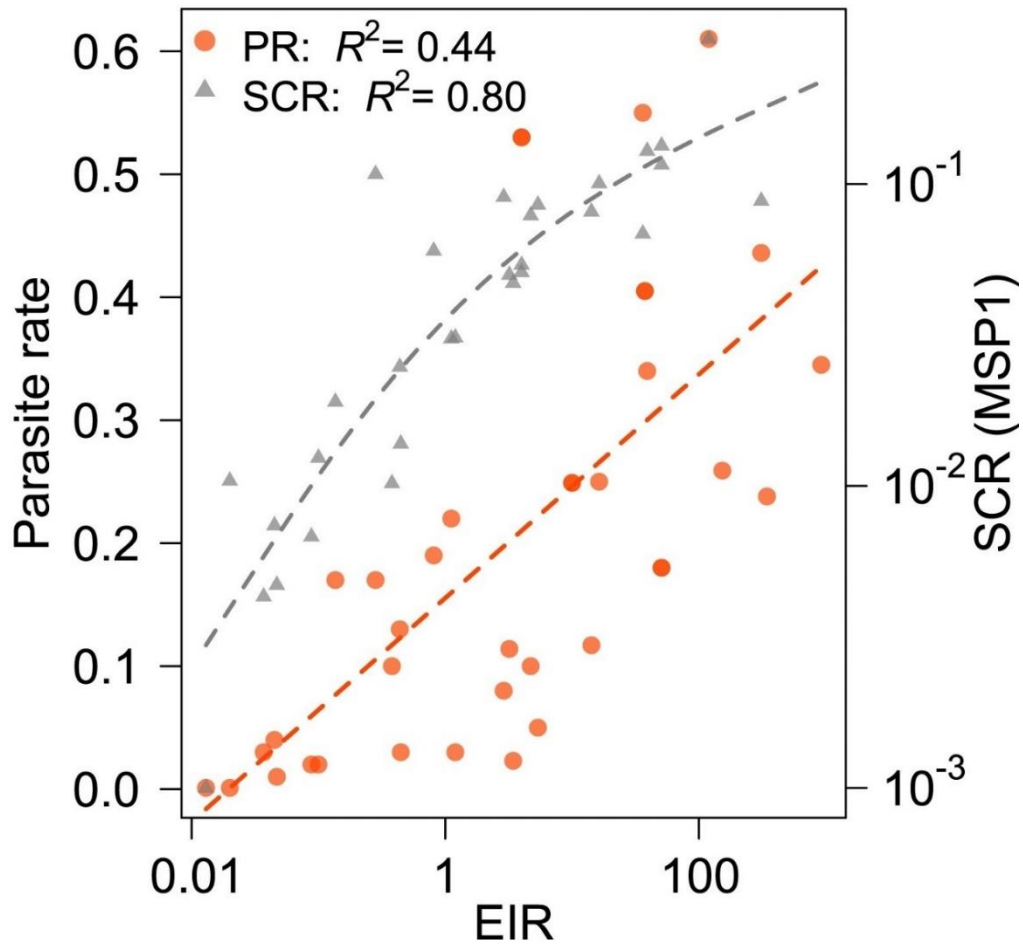


Figure 1.7 Comparison of EIR, SCR and parasite rate measurements from multiple sites taken from Greenhouse et al. (96)

#### 1.4 Potential use of serological surveillance to aid malaria elimination

##### 1.4.1 Potential application

Conventional measures such as entomological estimates and parasitaemia point prevalence become less sensitive and relatively expensive as transmission declines (59,97). The detection of *Plasmodium* species-specific antibodies as biomarkers for monitoring exposure and transmission has been utilised in several countries and is a more sensitive tool to assess population-level malaria exposure in low-transmission settings (39,98). One of the potential

applications of serological surveillance is to confirm malaria elimination and monitor for re-emergence of malaria (99). Serological surveillance could be used to verify that malaria has been eliminated since the absence of antibodies in the youngest age groups indicates that malaria transmission has been interrupted (93,98). In addition, it can also be used to identify high-risk areas (94,100) and groups suitable for active case detection (34).

Figure 1.8 describes the priority applications of serological markers of exposure in different levels of transmission intensity and different target populations. At the population level, serological surveillance can be used to generate risk stratification and measure changes in transmission due to interventions in areas where transmission is low and/or approaching zero, as well as to verify the absence of transmission once zero transmission is achieved. At the individual level, serological markers could be used to develop point of care tests to identify people who have had recent exposure to infection. This could be particularly pertinent for *P. vivax* exposure as there are currently no tests which detect the hypnozoite stage- but the presence of antibodies indicating recent exposure may help to identify individuals at risk of carrying hypnozoites in areas approaching zero transmission and also can potentially be used as early detection and response tools in areas with very low transmission setting where infections are becoming very rare.

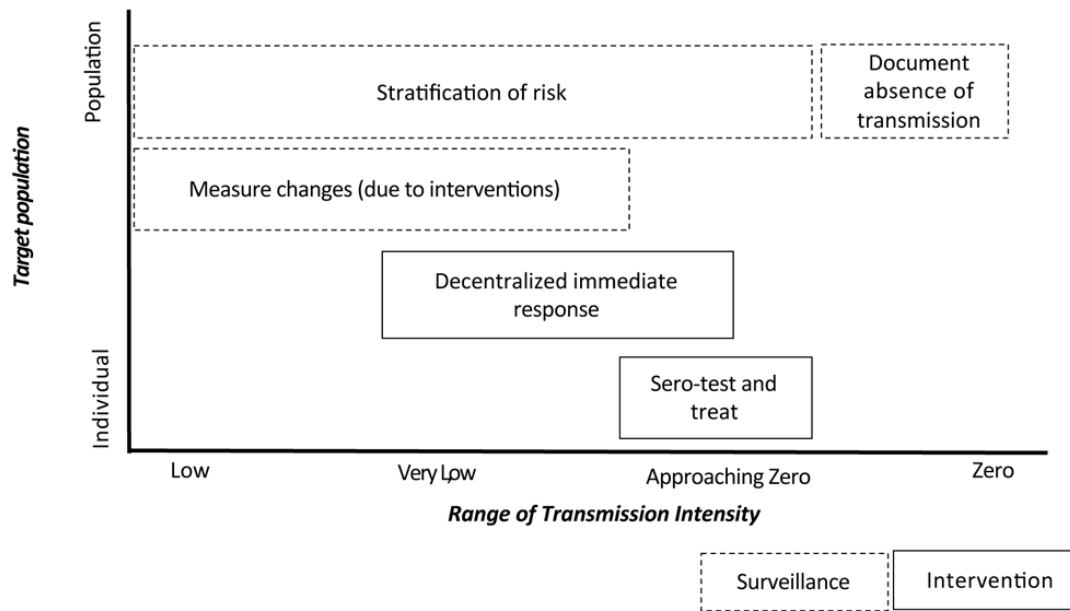


Figure 1.8 Priority applications of serological markers of exposure from Greenhouse et al. (101)

#### 1.4.2 Potential antigens for serological surveillance

Malaria parasites consist of many antigens, some of which induce strong antibody responses in humans depending on their abundance on the parasite, the size of the antigen and their availability to the human immune system. For instance, The invasion of erythrocytes involves several interactions with proteins on the merozoite surface and those associated with invasion organelles – micronemes and rhoptries (Figure 1.9) (102). These antigens are major targets for protective antibodies due to their direct exposure to the host immune system and their roles in invasion. The antibodies can act either by inhibiting parasite replication, blocking binding of merozoite ligands to their receptor or binding partners, or blocking processes required for parasite function (103–110). After initial exposure and binding to parasite antigens, naïve B cells begin to differentiate into either short-lived plasma cells that function to control initial infection or long-lived plasma cells and memory B cells (MBCs) that

contribute to the maintenance of sustained antibody-based immunity (111,112). Research suggests that short-lived plasma cells secrete primarily immunoglobulin-M (IgM) that only persists for several days to a month, while long-lived plasma cells and MBCs secrete immunoglobulin-G (IgG) that can persist for years (113).

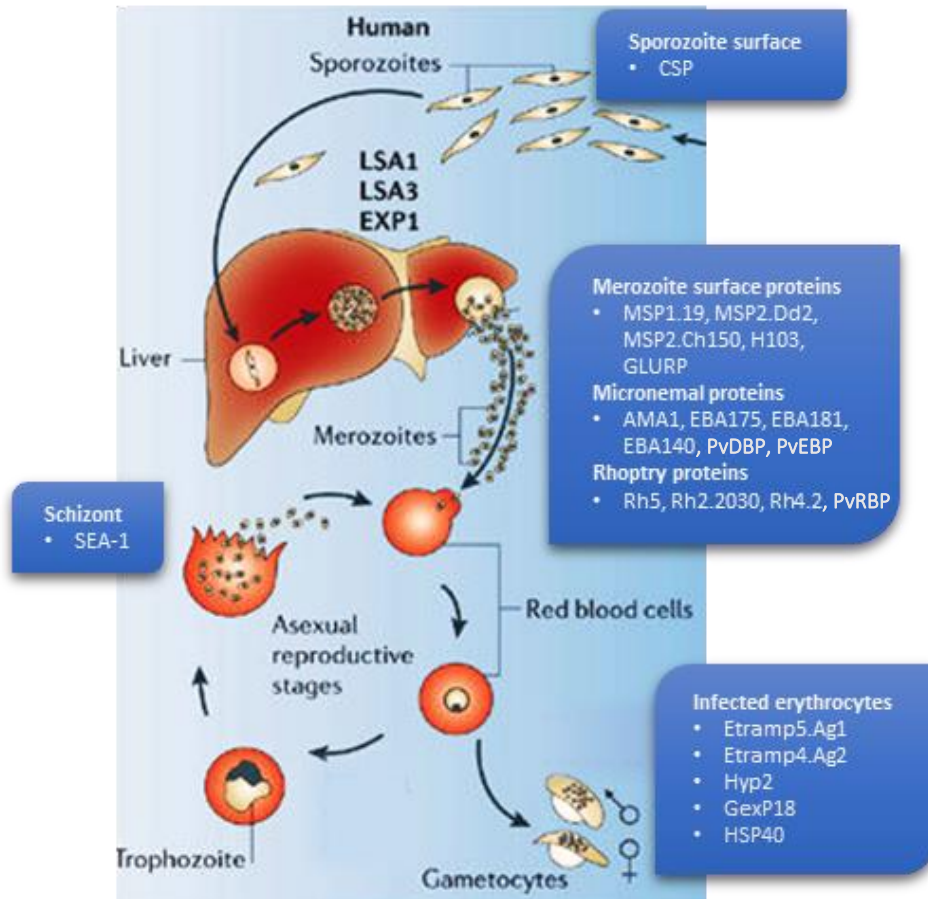


Figure 1.9 Parasite life cycle and antigens that are potential biomarkers of malaria exposure adapted from Wu 2018 (114), Winzeler et al 2006 (115) and Cowman et al 2006 (102).

A summary of antigens used in this thesis is presented in Table 1.1. Some of the well-studied *P. falciparum* antigens such as PfAMA1, PfMSP<sub>-1,19</sub>, MSP2, CSP and PfGLURP have been reported to induce long-lived antibodies that can persist years after infection (116,117) and have been used to measure transmission intensity in many endemic settings. Some of the relatively newly studied *P. falciparum* antigens such as Etramp5.Ag1, Etramp5.Ag2, Hyp2,



Gexp18, HSP40, PfSEA-1 were recently reported to be associated with short-lived antibody responses and can accurately predict days since an individual was last infected and malaria incidence in the last 12 months (118). For *P. vivax*, in addition to the antigens associated with long-lived antibodies i.e. PvAMA1 and PvMSP<sup>-1-19</sup>, there are some new antigens (PvDBP, PvEBP, PvRBP1a and PvRBP2b) which are potentially useful for detecting historical exposure and hypnozoite carriage. Of these antigens, PvRBP2b was reported as a potential antigen associated with short-lived antibodies which could be used to detect recent *P. vivax* exposure (119,120).

*Table 1.1 Summary of antigens used in this thesis*

No	Gene ID	Acronym	Description	Location	Reference
1	PF3D7_0304600	CSP	Circumsporozoite protein. Most predominant and antigenic protein on sporozoite surface. Component of RTS, S vaccine	Sporozoite	(121)
2	PF3D7_1301600	EBA140 RIII-V	erythrocyte binding antigen 140; involved in invasion	Apical organelles, micronemes	(122)
3	PF3D7_0731500	EBA175RII_F2	erythrocyte binding antigen 175; red blood cell (RBC) binding region via glycophorin A	Apical tip	(122)
4	PF3D7_0423700	Etramp 4 antigen 2	Early transcribed membrane antigen. Integral parasitophorous vacuole membrane (PVM) protein. C-terminal	Infected red blood cell (iRBC), PVM	(118)
5	PF3D7_0532100	Etramp 5 Ag 1	Early transcribed membrane antigen. Integral PVM protein.	iRBC, PVM	(123)
6	PF3D7_0402400	GEXP18	Gametocyte exported protein 18. Unknown function.	iRBC/Gametocyte	(118)
7	PF3D7_1035300	GLURP R2	Glutamate rich protein R2	Merozoite Surface	(124)
8	PF3D7_0501100.1	HSP40 Ag 1	Heat shock protein 40	iRBC	(118)
9	PF3D7_0501100.1	HSP40 Ag 3	Heat shock protein 40	iRBC	(118)
10	PF3D7_0206800	MSP2 CH150/9	CH150/9 allele of Merozoite surface protein (MSP) 2. Full-length.	Merozoite surface	(125)
11	PF3D7_0206800	MSP2 Dd2	Dd2 allele of MSP2. Full-length.	Merozoite surface	(126)
12	PF3D7_1133400	PfAMA1	Apical membrane antigen 1	micronemes	(127)
13	PF3D7_0930300	PfMSP-1 <sub>-19</sub>	19kDa fragment of MSP1 molecule.	Merozoite surface	(128)
14	PF3D7_1021800	PfSEA-1	Schizont egress antigen.	iRBC	(129)
15	PF3D7_0424100	Rh5	Receptor for human protein Basigin.	Apical tip	(130)(131)
16	PF3D7_0501300	SBP1	skeleton-binding protein; essential for translocation of PfEMP1 to iRBC surface via Maurer's cleft.	iRBC	(132)
17	PF3D7_1002000	Hyp2	Plasmodium exported protein	iRBC / PVM	(118)
18	PF3D7_1036000	H103	Merozoite surface protein 11/H101/MSP3.7	Merozoite	(133)
19	PVX_092275	PvAMA1	Apical membrane antigen 1	micronemes	(134)
20	PVX_099980	PvMSP-1 <sub>-19</sub>	19kDa fragment of MSP molecule	Merozoite	(135)
21	PVX_110810	PvDBP R2	Duffy binding protein region II	Merozoite	(136)
22	PVX_110835	PvEBP	Erythrocyte binding protein	Merozoite	(136,137)
23	PVX_098585	PvRBP1a	Reticulocyte binding protein amino acids 160–1170	Apical tip	(138)
24	PVX_094255	PvRBP2b	Reticulocyte binding protein amino acids 161–1454	Apical tip	(138)

### 1.4.3 Serological assays

The refinement of the enzyme linked immunosorbent assay (ELISA) for the detection of malaria specific antibodies has increased the applicability of sero-epidemiology (98). Briefly, the assay works by coating antigens on to high-binding micro-titre plates and all non-malarial antibodies are blocked. The bound antibodies are then detected with an enzyme linked secondary antibody. The presence of the target antigens (bound-antigen) is visualised through a colour change in the reaction, and quantified using a spectrophotometer (98). Advantages of the ELISA include high throughput capability, field applicability, and relatively low cost. Many plates can be run simultaneously. However, since antigens are tested individually and samples usually in duplicate, the time per antigen tested is relatively long. Moreover, the dynamic range (i.e. the width from the minimum to the maximum value of the response signal) is narrow in comparison with more advanced techniques, such as bead-based assays (139).

Bead-based assays (CBA: cytometric bead assays) are a relatively new technique which can measure the response to multiple antigens in a single sample simultaneously. The assay measures antibody responses as median fluorescence intensity (MFI). Current machines can perform up to 500 different tests in one sample. One of the key strengths of the assay is the ability to multiplex, which allows the potential to detect antibodies to multiple antigens at the same time therefore could lead to highly efficient testing. The time to read a single plate is relatively slow (1½–3 h), but since multiple antigens are tested simultaneously, the relative time per antigen is fast. Furthermore, the dynamic range of this assay is wide which increases granularity (level of detail) for low antibody responses (139).

The most frequently used source of antibodies in malaria serology research is serum collected on filter paper from finger-prick blood samples (140). Although still an invasive technique, it is a simple alternative to separating plasma or serum from a blood sample which may be impractical in remote field settings with limited laboratory facilities. Additionally, the same filter paper blood spots can be a source of parasite DNA for PCR-based testing (141).

### **1.5 Statistical approaches to serological data**

Seroprevalence and seroconversion rates (SCR) are two key serological metrics that can be used to measure malaria transmission in population. These metrics are sensitive to measure transmission in low transmission settings but will saturate in high transmission settings as the majority of individuals will be seropositive due to cumulative exposure to infection. In addition, the utility of these metrics depends on the choice of antibody target.

Seroprevalence is defined as the percentage of individuals in a population who are seropositive to a malaria antigen based on a defined cut-off. The seropositivity cut-off is typically determined by two methods. The first method is based on antibody responses of negative control/non-exposed individuals tested alongside the studied samples, where individuals are classified seropositive if the respective antibody levels exceed the mean plus 3 times the standard deviation of the negative population. The second method is based on the two-component finite mixture model (FMM) which relies on the basic assumption that the tested sample is composed of a mixture of latent seronegative or seropositive populations (142). Using similar criteria, individuals are then classified seropositive if the respective antibody levels exceed the mean plus 3 times the standard deviation of the seronegative population identified by the FMM.

Seroconversion rate (SCR) is defined as the annual rate by which seronegative individuals become seropositive upon malaria exposure. SCR can be used to measure population-level transmission intensity and temporal changes in transmission from a single cross-sectional sampling. Sepulveda et al. (142) have highlighted the reversible catalytic models (RCMs) as the most popular models used to estimate SCR. The models rely on assumption that individuals are born as seronegative but can convert into seropositive (seroconversion) upon malaria exposure, and then can revert to a seronegative state (seroreversion) in the absence of frequent malaria exposure. Two models used to estimate SCRs in this thesis are described below.

The first model is used to estimate population-level transmission intensity assuming that malaria transmission intensity is stable and constant over time. The seroconversion-seroreversion dynamics of each individual is described by a Markov chain with two states, seronegative ( $S^-$ ) and seropositive ( $S^+$ ). The resulting RCM is described by the following probability of an individual aged  $t$  being seropositive:

$$p_{S^+}(t) = \frac{\lambda}{\lambda + \rho_r} (1 - e^{-(\lambda + \rho_r)t}),$$

where  $\lambda$  is SCR and  $\rho$  is SRR (seroreversion rates).

The second model allows for a change in transmission intensity from  $\lambda_1$  to  $\lambda_2$  at time or age  $\tau$  assuming there was a rapid reduction of malaria transmission intensity at some time point before data collection or there are different risks of exposure due to different behaviour in different age groups. Sampled people born after the change in transmission ( $t \leq \tau$ ), will have a probability of being seropositive with constant transmission (Model 1) subject to the

current seroconversion rate  $\lambda_2$ . While people born before the change in transmission ( $t > \tau$ ) will have a probability of being seropositive that is a function of both seroconversion rates (past SCR). The  $\rho$  value included in Model 2 is a fixed value chosen based on the model with the highest log likelihood.

$$p_{S+}(t) = \begin{cases} \frac{\lambda_2}{\lambda_2 + \rho} (1 - e^{-(\lambda_2 + \rho)t}) & : t \leq \tau \\ \frac{\lambda_2}{\lambda_2 + \rho} (1 - e^{-(\lambda_2 + \rho)\tau}) + \frac{\lambda_1}{\lambda_1 + \rho} (1 - e^{-(\lambda_1 + \rho)(t - \tau)}) e^{-(\lambda_2 + \rho)\tau} & : t > \tau \end{cases}$$

Both models described above can be run using package available for Stata or R software. When running the models, the package will generate a seroconversion curve describing the fitted and observed probability for being seropositive for each age decile and estimate of the seroconversion rates. At minimum, individual data on age and seropositivity status are required to run the models.

## 1.6 Statistical approaches to assess spatial heterogeneity

Spatial analysis of malaria was historically restricted to visual comparisons of the geographical distribution of malaria burden (143). However, more robust methods are needed to identify malaria risk and disease clustering particularly in highly heterogeneous areas (47). Advances in geographical information systems (GIS) and statistical cluster detection methods has enabled the more nuanced detection of malaria hotspots (144,145). Several methods that are commonly used to detect the spatial heterogeneity of malaria are described below.

Approaches that can detect spatial clustering of the malaria burden are useful to better target surveillance and control programmes. The assumption used in identifying the clusters of disease or exposure is that the risk is assumed to be consistent across space. Methods then determine if the distribution of cases are likely to be concentrated in particular areas and can be considered as clustered (146). Several spatial analysis approaches such as kriging (i.e. a weighted moving average technique that interpolates estimates based on values at neighbouring locations and parameters from the semi-variogram) or model-based geostatistics (i.e. classical geostatistics is embedded in the framework of a generalised linear model) (147,148) have been used in predicting malaria risk and are useful to capture spatial patterns of malaria risk at different scales. However, the use of this approach is currently restricted to national and regional level mapping due to the availability of currently accessible data (17,18,149,150).

Several spatial clustering methods such as Kulldorff's spatial scan statistic, kernel density or the cumulative X test have similar assumptions and use the likelihood ratio test to determine where clustering is occurring (151,152). Of these methods, Kulldorff's spatial scan statistic which is accessible through SatScan software package (Harvard Medical School, Boston, USA) is the most popular approach used in malaria research (146,151). This approach uses a series of circles or elliptical shaped windows centred on each data point, followed by a likelihood ratio test comparing the rate inside the window to the outside. To test the null hypothesis that points are distributed randomly, Monte Carlo simulations generating permutations of the data across the area are used (146,153). Hotspots (defined as foci by the WHO) are identified using points representing the centre and the radius of the cluster size (154,155).

This method has been used to identify malaria heterogeneity in several endemic settings in Bioko Island (94), Tanzania (151), South Africa (100), and also in Indonesia (156).

Finally, spatial-temporal methods can provide more accurate predictions of malaria risk when spatial data are available at different time points. Methods incorporating temporal factors for cluster detection using SatScan software have been applied to malaria data using two different approaches. The first approach is to independently analyse each time point of dataset and visually observe any trends between the generated maps (156–159). The second approach is to use the space-time model spatial scan statistic where the moving window extends into cylindrical shape with the height reflecting the time aspect (146,160). The first approach is the most commonly used and is simpler compared to the second approach, especially if there are only few data points are analysed.

### **1.7 Malaria epidemiology in Indonesia**

Indonesia is an archipelago that is located between two continents, Asia and Australia. It is adjacent to other countries i.e. Malaysia, Singapore and Philippines in the North and Papua New Guinea and Timor Leste in the East. Indonesia has the highest burden of malaria in the South-East Asia region outside of India. It is estimated that 26% of the 255,500,000 Indonesian population live in malaria endemic areas. Of those at-risk populations, 12% live in high transmission areas and 14% in low transmission areas (16).

All species of *Plasmodium* have been reported in Indonesia including the newly emerging *P. knowlesi* (10–12,161,162). *Plasmodium falciparum* and *P. vivax* are the most prevalent species causing malaria infections in Indonesia (16). In general, the risk of transmission is



higher in eastern Indonesia than the rest of the country (17,18), with most of the endemic areas in stable transmission zones with low transmission risk. The latest estimates of age-standardised parasite prevalence for *P. falciparum* malaria in children 2-10 years old (PfPR<sub>2-10</sub>) and all-age parasite prevalence rate for *P. vivax* malaria (PvPR<sub>1-99</sub>) range broadly and are highly heterogenous (Figure 1.10 and 1.11). To date, there were several studies reporting a notable proportion of *P. knowlesi* infection in several areas in Sumatera and Kalimantan islands where the macaque populations are reported to be prevalent (10–12,162).

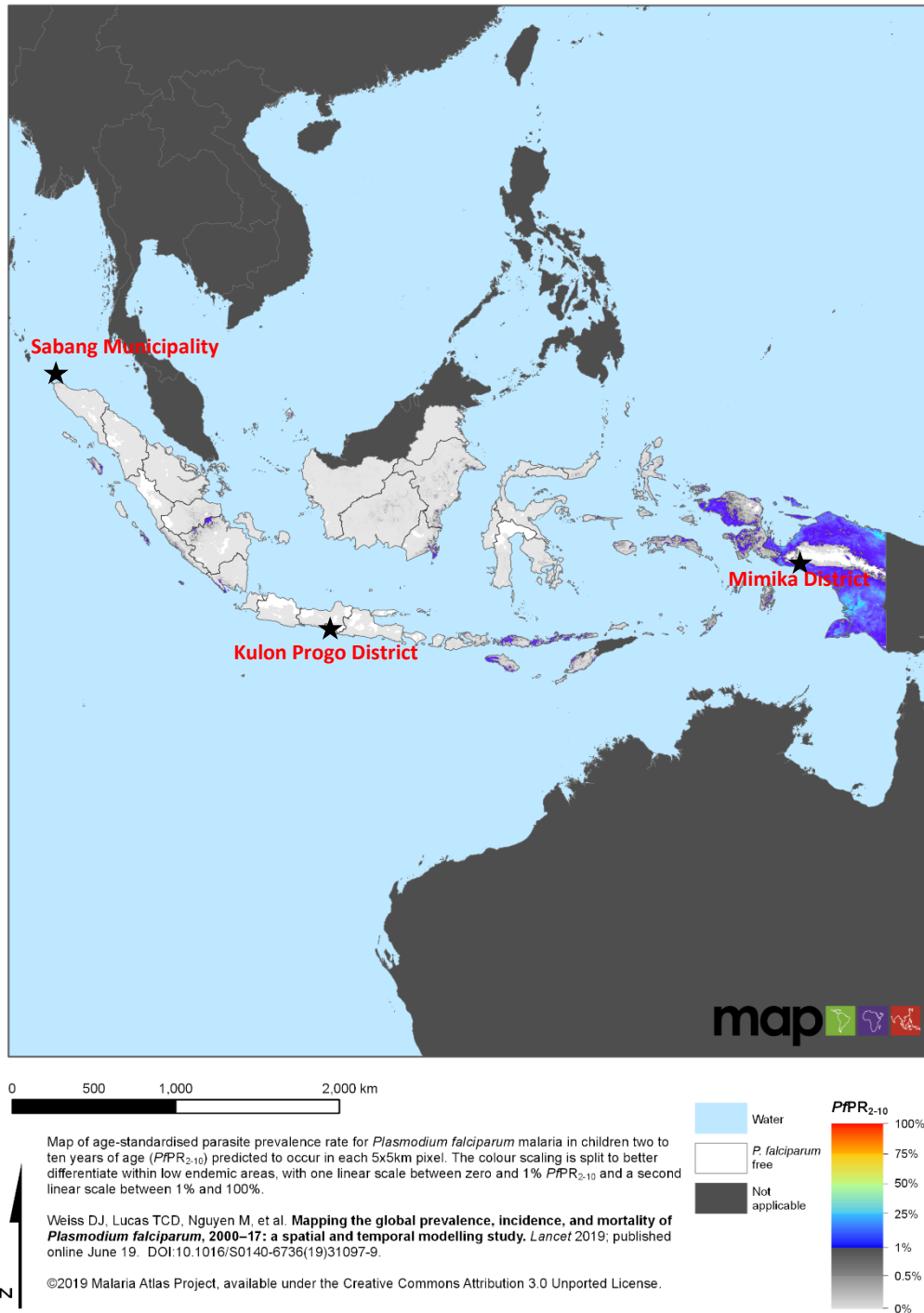
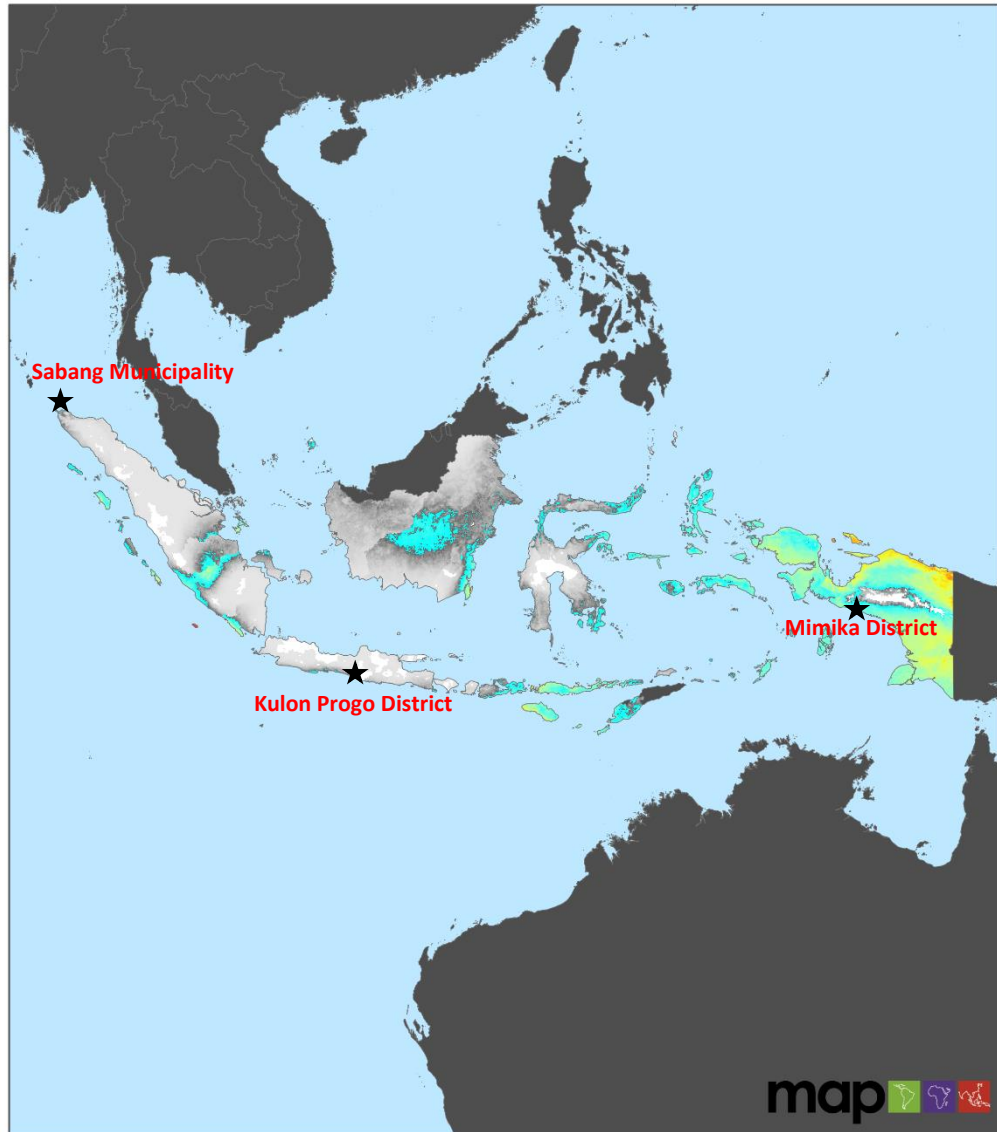


Figure 1.10 Map of age-standardised parasite prevalence for *P. falciparum* malaria in children 2-10 years old ( $PfPR_{2-10}$ ) predictions in 2017, adapted from Weiss et al. (17). Starred locations i.e. Timika District, Kulon Progo District and Sabang Municipality are 3 sites with different level of endemicity studied in this thesis.



Map of all-age parasite prevalence rate for *Plasmodium vivax* malaria ( $PvPR_{1-99}$ ) predicted to occur in each 5x5km pixel. The colour ramp is split into two linear scales to better differentiate within low endemic areas. Areas where *P. vivax* is known to be endemic, but there was not sufficient information to generate a prediction are shown in light grey.

Battle KE, Lucas TCD, Nguyen M, et al. **Mapping the global endemicity and clinical burden of *Plasmodium vivax*, 2000–17: a spatial and temporal modelling study.** *Lancet* 2019; published online June 19. DOI:10.1016/S0140-6736(19)31096-7.

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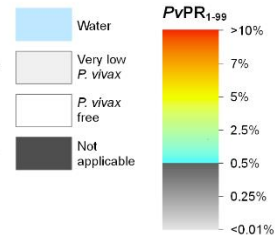


Figure 1.11 Map of all-age parasite prevalence rate for *P. vivax* malaria ( $PvPR_{1-99}$ ) predictions in 2017, adapted from Battle et al. (18). Starred locations i.e. Timika District, Kulon Progo District and Sabang Municipality are 3 sites with different level of endemicity studied in this thesis.

Indonesia's climate is typically of a high temperature (25–29°C) with relatively high humidity (76%–105%), and heavy rainfall from October to March across the country. This climate favours the survival of *Anopheles* mosquitoes and allows malaria parasites to develop in the mosquito more rapidly (163–165). There are 20 *Anopheles* species documented as malaria vectors in Indonesia with overlapping distributions in all the main islands (Figure 1.12). *Anopheles balabacensis*, *Anopheles flavirostris*, *Anopheles nigerrimus*, *Anopheles subpictus* and *Anopheles sundaicus* are the vectors found circulating in both western and eastern Indonesia. Studies suggested that these vectors were more likely to have outdoor biting habits and tend to rest outdoors in shaded locations such as in cattle shelters or under tree than inside houses (166). These vectors are especially relevant because of the higher malaria risk has been reported to be associated with forest-related work and other night outdoor activities (163,167,168).

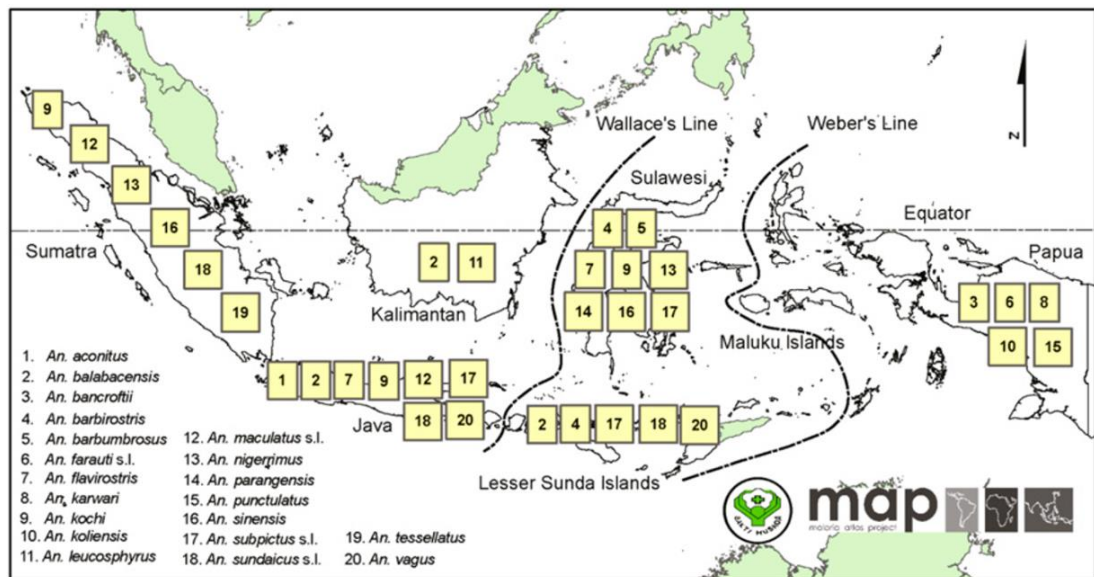


Figure 1.12 Distribution of primary *Anopheles* malaria vector in Indonesia reproduced from Elyazar et al. (166)

The majority of malaria cases (both falciparum and vivax) in Indonesia occur in adults (49,50,168–171), with the exception in high transmission areas, Papua, where the risk of *P. vivax* infection were reported to be higher in children compared to adults (171). A previous report suggested that night outdoor activity at the farm or forest e.g. sleeping in the plantation increases the risk of malaria infection (163,167,168). Human mobility becomes a serious challenge for malaria control in Indonesia. People are travelling domestically for several purposes i.e. for working, visiting relatives or just for vacation. The risk of malaria is higher for migrants since they are typically moving from densely populated Java and Bali Islands to the sparsely populated and usually highly endemic outer Islands (172). These migrants then return routinely to Java, either permanently or, more often, for family reunions and holidays (161). Mobility has been reported as one of risk factors for malaria resurgence in low transmission setting (168). However, little research has been done to investigate how significant the influence of human mobility is for reintroducing malaria transmission in low endemic area in Indonesia.

### **1.8 Intervention strategies in Indonesia**

The recent report by the Indonesian Ministry of Health provides a summary of malaria elimination strategies and the current achievements in Indonesia as illustrated in Figure 1.13 (173). The history of malaria control started with the establishment of Indonesia's National Malaria Eradication Unit in 1952. Initially, control efforts were focused on indoor residual spraying (IRS) with dichlorodiphenyltrichloroethane (DDT) and chloroquine-based treatment in Java island. In 2004, artemisinin combination therapy was introduced as first-line treatment with tightly controlled procurement and distribution of the drug. Regular therapeutic studies have shown no drug resistance (174). Microscopy confirmation

complemented by RDT is mandatory. Long-lasting insecticide-treated bed nets (LLIN) were first distributed to eastern Indonesia and parts of Sumatra in 2005, and subsequently nearly every 2 years to highly endemic districts and villages resulted in 20 million LLIN distributed in the past decade. IRS was done in villages with annual parasite incidence >20 per 1000 population and in response to outbreaks. In high-transmission areas, malaria screening for ill children was introduced into clinical management protocols. Capacity development efforts supported case management, vector control, surveillance, and case investigation. The case investigation has recently included adoption of the 1-2-5 surveillance and response protocol: case management and notification on day 1; case classification and foci investigation on day 2; and foci response and elimination by day 5. Finally, locally tailored responses have been essential for malaria elimination due to the high levels of decentralised authority.

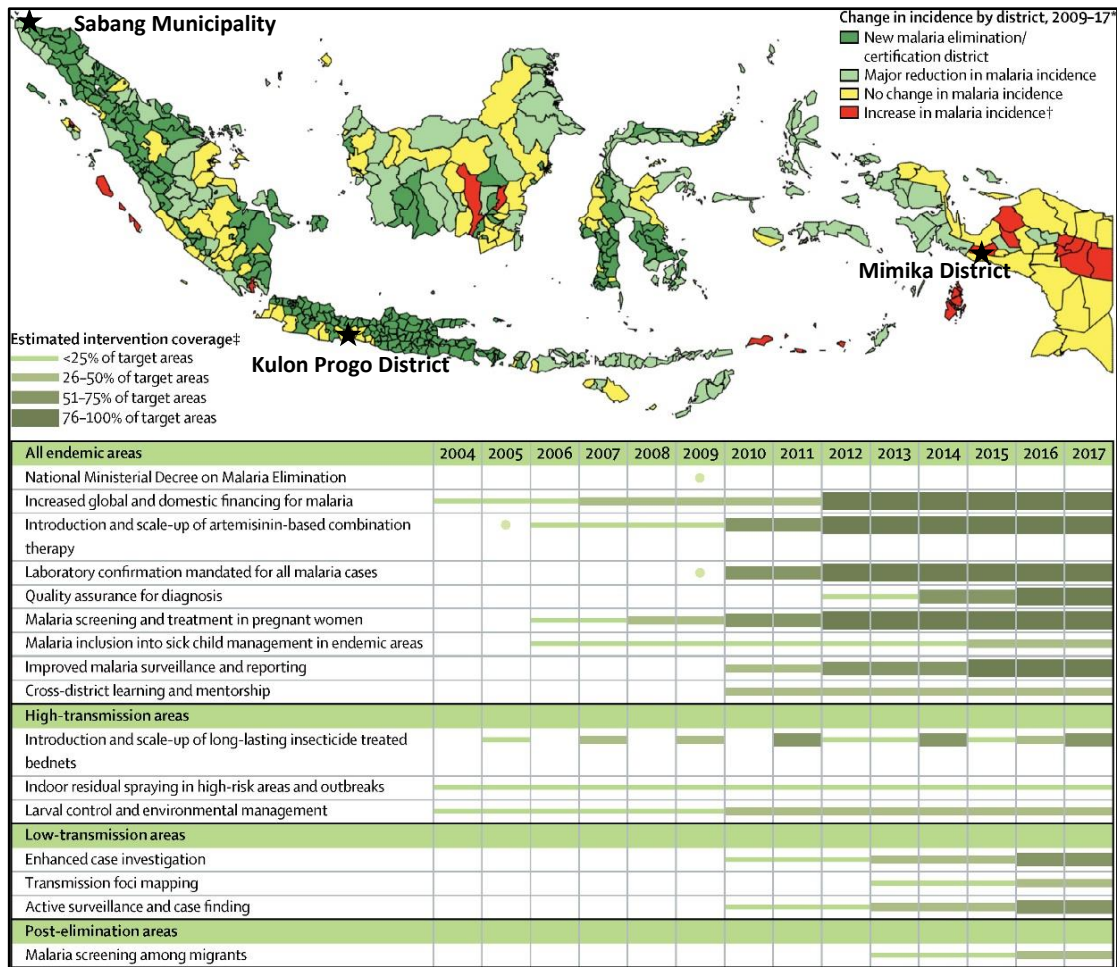


Figure 1.13 Changes in malaria incidence and intervention coverage, Indonesia 2004–2017 adapted from Sitohang et al. (173).

Starred locations i.e. Timika District, Kulon Progo District and Sabang Municipality are 3 sites with different level of endemicity studied in this thesis. \*Baseline annual parasite incidence (API) data available in 2009–10 for 90% of districts. Major reduction represents downshift in transmission strata or three-times reduction in API; no change represents same strata and less than three-times reduction in API. Increase represents an upshift in transmission strata. †Increase might reflect improved malaria surveillance. ‡Estimates based on expert consultation with the Indonesian Ministry of Health and partners.

Despite the recent success in decreasing the malaria burden from API 2.89 per 1000 in 2007 to 0.9 per 1000 population in 2017, so far, approximately only 60% of total districts and cities have been declared as malaria-free areas (173). This makes it difficult to achieve malaria elimination by 2030. Specifically, Java, the most populated island that contributes 71.6

million people (54%) of total population at risk of *P. falciparum* in the whole of Indonesia (175) has failed to achieve the target of malaria elimination by 2015. The sustained transmission of different malaria species combined with the challenge of identifying where, and in who, residual transmission is occurring, mean new strategies are needed to target transmission and reduce the burden of infection. Key to this will be improved surveillance as focus changes to include all infections, not just those that are symptomatic (169).

### **1.9 Research gaps**

Identifying persistent and intense transmission areas in a smaller geographical scales can prevent outbreaks of disease that spread from these areas and support disease elimination strategies when overall disease occurrence has declined (176–178). As malaria transmission in Indonesia is highly heterogeneous, insights into micro-epidemiological geographic variation of malaria infections in sub-national level would give more useful operational information on public health intervention planning aim to achieve local elimination. Identifying regions with higher disease burden can effectively facilitate control efforts prioritization (43,179,180).

As previously described in Section 1.4, serology has potential applications to measure population-level of transmission, confirm interruption of transmission in areas approaching zero, and characterise spatial patterns of transmission in the population. Only few (~1%) malaria parasite antigens have been studied so far (181,182), with the majority of serological data currently available at technical level focusing on responses to well-characterised blood-stage antigens. However, recent identification of alternative *P. falciparum* antigens associated with short-lived antibody responses (i.e. Etramp5.Ag1, Etramp4.Ag2, Hyp2,



GexP18, HSP40, and PfSEA-1) suggests they could be used as a potentially key indicator of very recent exposure (118). This could provide rapid, cheap estimates of malaria incidence to target and evaluate interventions. Moreover, although most serological work has been focussed on *P. falciparum*, there were some promising antigens that can be optimised as an additional application of serology for *P. vivax* (i.e. PvDBPR2, PvEBP, PvAMA-1, PvMSP-1<sub>19</sub>, PvRBP1a and PvRBP2b) to identify hypnozoite carriers for treatment; a major challenge for control programmes (119,120,134,135,138). Inclusion of these *P. falciparum* and *P. vivax* antigens in a multiplex bead-based assay could potentially expand the application of serological surveillance for malaria elimination.

Despite its potential application, feasibility of implementing serological surveillance utilising existing public health surveillance systems has not been evaluated, especially in the context of Indonesia. The majority of malaria prevalence studies, including a serological study conducted in Indonesia (183) have been based on community-based surveys which require large resources and efforts for collecting samples. Several methods have been reported as alternative sampling approaches that could provide more cost effective and efficient strategies to collect samples and data to assess population-level exposure and infection in low transmission areas. These methods target easy access groups such as school children, health facility attendees or focus on other high-risk populations such as forest workers, miners or farmers. In this thesis, we explore the implementation and evaluation of health facility-based surveys where facility attendees and their companions are sampled as an alternative sampling strategy to improve malaria surveillance capacity to monitor malaria transmission dynamics in the population.

Due to limited available data, the majority of the currently available malaria risk maps in Indonesia were based on parasitological estimates at the district level resolution (49,50), thus its utility in identifying local level hotspots is limited. Adding data collection methods that enable surveillance to remotely capture spatial patterns of transmission at the micro epidemiological level would be helpful for strategic and operational planning of control and elimination programmes. Generally, a basis for the spatial analysis of disease transmission is based on adequate address information (184,185) automatically generated by geocoding software packages that can generate accurate spatial coordinate data for a large proportion of individuals (186,187). In circumstances where formal address data are unavailable, catchment areas of, for example, community pharmacies or general practitioners have been used for describing spatial patterns in disease occurrence (34,188–190). However, this approach is likely to have less utility for resource-poor settings where formal address systems are commonly unavailable and where health-facility catchment areas are relatively large and poorly defined (191–193).

## 2. Aim and objectives

### 2.1 Aim

The main aim of this thesis is to examine approaches to optimise the operational application of serological surveillance for monitoring malaria transmission as an additional measure for the existing public health surveillance system in Indonesia. A core theme of the thesis is to evaluate the use of serology with different data collection methods to assess the additional information generated in three areas of differing endemicity.

### 2.2 Objectives

- To evaluate the use of sero-epidemiological analysis to investigate heterogeneity of transmission in an area conducting malaria elimination in Indonesia. The study presented in **Chapter 3** aimed to explore the following hypotheses:
- In the absence of active infections:
  1. Spatial analysis of serological data can identify areas at risk of malaria through identifying areas of previously high exposure.
  2. Seroconversion rate estimates can confirm low levels and historical changes in malaria transmission.
  3. Absence of seropositivity in the population under 5 years old can be used as a proxy of transmission interruption.
  4. Sero-epidemiological analysis can be used to determine factors associated with transmission.

- To evaluate the use of mobile technology-based participatory mapping approaches to geolocate health facility attendees for disease surveillance in low resource settings. The study presented in **Chapter 4** aimed to explore the following hypotheses:
  1. Participatory mapping using android tablet-based offline high-resolution maps can be used to efficiently geolocate individual residences from health facility.
  2. Open source software and maps offer potential utility to collect spatial information for research and disease surveillance purposes.
  
- To implement and evaluate use of health facility-based serological surveillance to investigate *P. falciparum* and *P. vivax* transmission dynamics in an elimination setting. The study presented in **Chapter 5** aimed to explore the following hypotheses:
  1. Estimates of population-level transmission intensity (SCR) generated from a single health facility-based survey is similar to the estimate generated from the repeated health-facility based surveys.
  2. Repeated health facility-based surveys can capture short-term changes in antibody levels over time.
  3. Spatial analysis of antibody responses to multiple malaria antigens can prospectively predict areas at high-risk of malaria outbreak.
  4. Sero-epidemiological analysis can be used to determine factors associated with transmission in elimination setting where the numbers of active infections are insufficient for conducting a risk factor analysis.

- To evaluate the use of multiple serological markers to measure transmission level and assess its association with active infections in a high transmission setting. The study presented in **Chapter 6** aimed to explore the following hypotheses:
  1. Population-level seroconversion rate estimates can confirm the high level of transmission.
  2. Species-specific serological markers are associated with *P. falciparum* and *P. vivax* infections.
  3. *P. falciparum* short-lived markers are sensitive and specific in predicting *P. falciparum* infections.

### 2.3 Thesis structure

This thesis consists of an Introduction, four scientific papers and a general Discussion summarising the main findings presented in **Chapter 2** to **Chapter 6**, discussing the thesis limitations, future directions, and implications for other infectious diseases.

The first paper (**Chapter 3**) describes how analysis of sero-epidemiological data coupled with household GPS coordinates collected through a community-based cross-sectional study can be useful in an area reporting zero cases in 3 consecutive years prior to data collection. As the absence of infections could not facilitate the identification of the population and areas at risk for malaria reintroduction in the future, the study described in this chapter was conducted to seek evidence that analysis of serological data could be an alternative tool to assess *P. falciparum* and *P. vivax* transmission level, to investigate the risk factors for transmission and to describe the heterogeneity of potential transmission in the absence of active infections detected by standard malaria diagnostic tool such as microscopy.

The second paper (**Chapter 4**) demonstrates the use of mobile technology-based participatory mapping approach for collecting geolocation data for public health research and surveillance in low resource settings. The most common mapping approaches usually require door to door visit to collect the household GPS coordinates, or use currently available data that rely on formal addresses, or remotely collect data using online-based approaches that require a stable internet connection. As many malaria endemic areas in Indonesia are usually hard to reach, have informal addresses and poor or no internet connection, the study presented in this chapter was conducted to evaluate and validate alternative approaches to remotely collect household-level spatial data from health facilities using a computer tablet-based offline high-resolution maps to support the identification of fine scale resolution of local-level disease heterogeneity.

The third paper (**Chapter 5**) describes the application of quarterly health facility-based cross-sectional surveys and epidemiological analysis of multiple antibody response data generated using bead-based multiplex serological assays coupled with household GPS coordinates collected using participatory mapping approach (validated in **Chapter 4**) in a very low transmission setting conducting elimination. As the malaria transmission was very low and only a few infections were detected by standard microscopy tests, conventional methods to measure transmission such as parasite rate and EIR are inefficient due to large sample sizes required to detect infections in human and mosquitos, respectively. This paper aimed to answer the question on how the advances in serological and mapping methods can be used to better understand *P. falciparum* and *P. vivax* transmission dynamics by utilising the existing health facility-based surveillance systems.

The fourth paper (**Chapter 6**) presents the application of analysing multiple *P. falciparum* and *P. vivax* serological markers combined with parasitological data generated by microscopy and PCR tests to better estimate malaria burden in a high transmission setting. The seropositivity to any of several new *P. falciparum* recently identified as markers of recent exposure to infection can be used as a proxy of recent malaria infection. Evaluating the sensitivity and accuracy of these serological markers in a high transmission setting will provide important information before they can be used to measure recent transmission in a lower transmission setting where active infection become rare and difficult to detect by standard parasitological diagnostics such as microscopy and RDTs. This paper aimed to provide evidence on the potential use of analysis of multiple serological markers to measure population-level transmission intensity and predict the current malaria infection. Table 2.1 presents a summary of the methods used in each paper presented in this thesis.

**Table 2.1 Summary of methods used in each paper**

Chapter	Design	Population and sample	Data sources	Outcomes
3	Community-based cross-sectional study	Communities in Sabang, Municipality, Aceh Province, Indonesia (n= 1624)	ELISA, microscopy test, paper questionnaire and handheld GPS	<ul style="list-style-type: none"> <li>- Transmission level estimates</li> <li>- Spatial patterns of household-level <i>P. falciparum</i> and <i>P. vivax</i> exposure</li> </ul>
4	Software evaluation and field testing	Households in Rizal District, Palawan Province, the Philippines (n=203) and Kulon Progo District, Yogyakarta Province, Indonesia (n=400)	Software review, tablet-based GPS and questionnaire, handheld GPS	<ul style="list-style-type: none"> <li>- Review of geolocation software</li> <li>- Accuracy of tablet-based participatory mapping approach</li> </ul>
5	Quarterly health facility-based cross-sectional study	Health facility attendees in Kulon Progo District, Yogyakarta Province, Indonesia (n=9453)	Luminex assay, microscopy test, tablet-based questionnaire and GPS	<ul style="list-style-type: none"> <li>- Transmission level estimates</li> <li>- Spatial pattern of household-level <i>P. falciparum</i> and <i>P. vivax</i> exposure</li> </ul>
6	Community-based cross-sectional study	Communities in Mimika District, Papua Province, Indonesia (n=2496)	Luminex assay, microscopy and PCR tests, paper-based questionnaire and handheld GPS	<ul style="list-style-type: none"> <li>- Transmission level estimates</li> <li>- Spatial patterns of household-level <i>P. falciparum</i> and <i>P. vivax</i> exposure</li> <li>- Predictive models of current malaria infection</li> </ul>



### 3. Analysis of serological data to investigate heterogeneity of malaria: a community-based cross-sectional study in an area conducting elimination in Indonesia

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#### RESEARCH PAPER COVER SHEET

**PLEASE NOTE THAT A COVER SHEET MUST BE COMPLETED FOR EACH RESEARCH PAPER INCLUDED IN A THESIS**

#### SECTION A – Student Details

Student	Henry Surendra
Principal Supervisor	Chris Drakeley
Thesis Title	Operational applications of serology for malaria surveillance in different transmission settings in Indonesia

***If the Research Paper has previously been published please complete section B, if not please move to Section C***

#### SECTION B – Paper already published

Where was the work published?	Malaria Journal		
When was the work published?	8 July 2019		
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion	NA		
Have you retained the copyright for the work?*	Yes	Was the work subject to academic peer review?	Yes

\*if yes, please attach evidence of retention, if no, or if the work is being included in its published format, please attach evidence of permission from the copyright holder (publisher or other author) to include this work.

#### SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published	
Please list the paper's authors in the intended authorship order.	
Stage of publication	

#### SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	First and corresponding author. I collated and analysed the data and wrote the manuscript.
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Student Signatur

Date: 30/09/2019

Supervisor Signature: \_\_\_\_\_

ate: 30/09/2019

**Analysis of serological data to investigate heterogeneity of malaria transmission: a community-based cross-sectional study in an area conducting elimination in Indonesia**

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### **3.1 Abstract**

#### **Background**

Analysis of anti-malarial antibody responses has the potential to improve characterization of the variation in exposure to infection in low transmission settings, where conventional measures, such as entomological estimates and parasitaemia point prevalence become less sensitive and expensive to measure. This study evaluates the use of sero-epidemiological analysis to investigate heterogeneity of transmission in area conducting elimination in Indonesia.

#### **Methods**

Filter paper bloodspots and epidemiological data were collected through a community-based cross-sectional study conducted in two sub-districts in Sabang municipality, Aceh province, Indonesia in 2013. Antibody responses to merozoite surface protein 1 (MSP-1<sub>19</sub>) and apical membrane antigen 1 (AMA-1) for *Plasmodium falciparum* and *Plasmodium vivax* were measured using indirect Enzyme-linked immunosorbent assay (ELISA). Seroconversion rates (SCR) were estimated by fitting a simple reversible catalytic model to seroprevalence data for each antibody. Spatial analysis was performed using a Normal model (SaTScan v.9.4.2) to identify the clustering of higher values of household antibody responses. Multiple logistic regression was used to investigate factors associated with exposure.

#### **Results**

1624 samples were collected from 605 households. Seroprevalence to any *P. falciparum* antigen was higher than to any *P. vivax* antigen, 6.9% (95% CI: 5.8-8.2) vs 2.0% (95% CI: 1.4-2.8). SCR estimates suggest that there was a significant change in *P. falciparum* transmission with no exposure seen in children under 5 years old. *Plasmodium falciparum* SCR in over 5

years old was 0.008 (95% CI: 0.003–0.017) and 0.012 (95% CI: 0.005–0.030) in Sukakarya and Sukajaya sub-districts, respectively. Clusters of exposure were detected for both *P. falciparum* and *P. vivax*, most of them in Sukajaya sub-district. Higher age, *P. vivax* seropositivity and use of long-lasting insecticide-treated bed net (LLIN) were associated with higher *P. falciparum* exposure.

### **Conclusion**

Analysis of community-based serological data helps describe the level of transmission, heterogeneity and factors associated with malaria transmission in Sabang. This approach could be an important additional tool for malaria monitoring and surveillance in low transmission settings in Indonesia.

**Keywords** Serology, epidemiology, surveillance, malaria, *P. falciparum*, *P. vivax*, elimination

### **3.2 Background**

In recent years, there has been a decline in malaria transmission in many regions, leading to optimism that malaria elimination might be achieved in numerous countries [1–8]. As transmission declines, monitoring changes in malaria transmission intensity and disease prevalence through surveillance systems becomes increasingly important to allow the evaluation of health services and control programs [9,10]. The latest World Health Organization (WHO) malaria surveillance manual confirms that improved surveillance is a major component of the WHO strategy [11]. However, conventional measures such as entomological estimates and parasitaemia point prevalence become less sensitive and relatively more expensive as transmission declines [12,13]. Disease surveillance is further compounded by difficult access to remote and isolated communities, increased risks in forest

workers and other highly mobile populations and the difficulties of tracking cross-border movements [14–20].

An additional approach to measure malaria transmission is to detect anti-malarial antibodies, which provide a marker for exposure to malaria [9]. Malaria infections generate antibodies which can be detected for several months and years after the infection has been resolved. Although serology is unlikely to be useful for diagnosing actively infected individuals because antibodies take days to develop and then persist after infection [9,13], detection of these antibodies indicates previous exposure and offers an additional, more sensitive measure of infection and transmission, particularly in low endemic settings where the sensitivity of parasitological tools is inadequate [21–25] and gold standard tests like the parasite rate and the Entomological Inoculation Rate (EIR), may have insufficient statistical power unless the sampling is intensively done [26–28]. This approach has been utilized in several countries and reported as a more sensitive tool to assess population-level malaria exposure in low-transmission settings [9,13].

Seroconversion rate (the proportion of people in the population who are expected to seroconvert each year) is a serological parameter used to understand malaria transmission dynamics. Previous studies found that seroconversion rate (SCR) provides a proxy measure for estimating the transmission intensity in a community as it was strongly correlated with the EIR and annual parasite incidence collected by the malaria surveillance programme [10,14]. Serological estimates of transmission have been utilized in many low endemic settings, including Indonesia [29,30], and have additionally been used to identify populations at higher risk of malaria exposure [9,31], foci of transmission [32,33] and to describe

historical changes in disease burden [25]. While there is great promise in this approach, it needs further refinement.

Recent studies have reported the potential use of recombinant Merozoite Surface Protein 1 (PfMSP-1<sub>-19</sub>) and Apical Membrane Antigen 1 (PfAMA-1) as serological parameters to assess malaria transmission intensity in Indonesia. First, a population-based cross-sectional study conducted in three different endemicity areas showed the potential application of these methods for detecting changes in transmission exposure, particularly in lower transmission settings and with less immunogenic antigens (such as PfMSP-1<sub>-19</sub>) [30]. Second, a cohort study of Indonesian schoolchildren found that it is possible to assess the interruption of transmission by measuring seroconversion rates from individual-level longitudinal data on antibody titres [29]. These studies suggested serological analysis has the potential to assess malaria burden and heterogeneity of infections in the Indonesian population. As antibodies to AMA-1 and MSP-1<sub>-19</sub> antigens have been reported to persist for several years after infection and in the absence of reinfection, any antibodies detected in younger children would reflect more recent infection in low transmission settings [10]. Therefore, as Indonesia aims to eliminate malaria by 2030, further implementation and evaluation of sero-epidemiological analysis in areas moving towards elimination would garner valuable information for malaria control programmes. This study explores the use of sero-epidemiological analysis for assessing the intensity and heterogeneity of malaria transmission as well as factors associated with malaria exposure in an area conducting elimination in Indonesia.

### 3.3 Methods

#### Study site

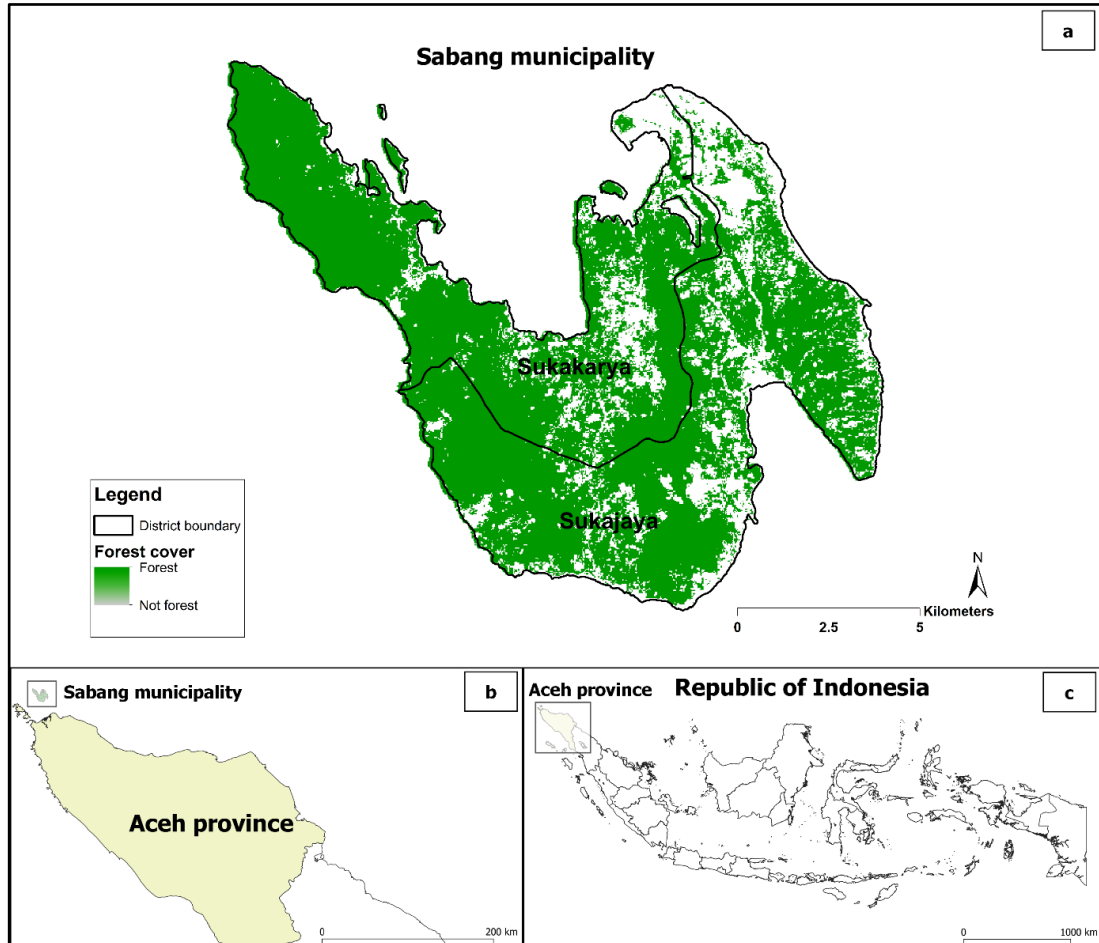


Figure 3.1 Map showing study sites in two sub-districts in Sabang municipality, Aceh province, Indonesia (a). Inset maps showing geographical location of Sabang municipality within Aceh province (b), and location of Aceh province within Indonesian archipelago (c).

The study was conducted in Sabang municipality, Aceh province, Indonesia (Figure 3.1). The municipality is located at the north-westernmost part of Indonesia and is part of Aceh province. The municipality has an area of 153 km<sup>2</sup> covering five islands but only the largest island, Weh, is permanently inhabited. The population on Weh island is approximately 30,000 and it is divided administratively into two sub-districts (Sukakarya and Sukajaya) with 18

villages. Sabang has a very low-level annual parasite incidence, 0.13 per thousand population in 2011. Based on its geographic position at the western end of the archipelago, its diverse mosquito fauna, the presence of both major malaria parasites, and its strong local government, Sabang municipality was considered as an appropriate place to pilot malaria elimination in Indonesia [34].

### **Study design and data collection**

Community-based cross-sectional surveys were performed during the malaria transmission season between October and December 2013. Households list were obtained from local authorities and were arbitrarily assigned numbers according to their geographic location. Households were randomly selected and invited to participate in the study. Households with no adult present were excluded from the survey and were replaced by the neighbouring households. Individual signed informed consent was obtained from all adults or guardians of household member under 18 years of age. Samples were collected from all household members present aged over 6 months. The minimum sample size of 439 individuals per sub-district was met to ensure the antibody SCR of 0.0036 could be estimated with a precision level of  $\pm 0.0013$  [35]. Standard microscopy blood smears were collected as per routine national diagnostic standards. Filter paper bloodspots were collected on Whatman 3M paper (Whatman, UK) as described by Corran *et al.* [36] and stored at  $-20^{\circ}\text{C}$  until transferred to the Parasitology Laboratory at Department of Parasitology, UGM, Yogyakarta. Data on age, gender, education, occupation, long-lasting insecticide-treated bed net (LLIN) use, indoor residual spraying (IRS) in last 12 months and auxiliary temperature were recorded using a short questionnaire form, and household GPS coordinates were collected using handheld GPS.



### **Laboratory methods**

Giemsa-stained thick and thin malaria films reading was performed by trained laboratory technicians to identify active infections. For serological assays, the recombinant proteins *Plasmodium falciparum* MSP-1<sub>-19</sub>, *P. falciparum* AMA-1, *Plasmodium vivax* MSP-1<sub>-19</sub> and *P. vivax* AMA-1 were used as antigens in indirect enzyme-linked immunosorbent assay (ELISA) as described in [9]. Briefly, antigens were coated on 96 well plates at the concentration of 0.5 µg/mL in coating buffer and incubated at 4°C overnight. The plates were washed in phosphate buffered saline with tween (PBST) and blocked with 1% (w/v) skimmed milk solution for 3 hours. After washing, samples were added in duplicate at a final dilution of 1:1000 to each plate using a pool of hyperimmune serum as a positive control and the plates were incubated overnight at 4°C. The plates were washed and 50µl of HRP-conjugated rabbit anti Human IgG (DAKO, #P0214) were added into each well and incubated for 3 hours. After a further series of washes substrate solution (OPD, Sigma #P8287, in PBS) was added and the reaction was allowed to develop for 15-20 minutes before addition of stopping solution (2M H<sub>2</sub>SO<sub>4</sub>). The optical density was read using ELISA reader at 450nm. All serology was performed by trained laboratory technicians at the Department of Parasitology, UGM, Yogyakarta.

### **Statistical analysis**

All statistical analyses were conducted in Stata IC 15 (Stata Corp, College Station, TX, USA). Infants under 1 year of age were excluded from each dataset to remove any influence of maternally derived antibodies [10]. Raw OD measurements were averaged and normalized against the positive control curve on each plate. A cut-off for seropositivity was determined for each antigen by calculating the mean plus 3 standard deviation values of OD values from

serum samples of 40 Javanese individuals who had no history of travel to malaria endemic areas in Indonesia. Cut-offs were generated separately for each antigen [13]. Individuals were categorized as seropositive for *P. falciparum* if their antibody responses were above the cut-off for PfAMA-1 and/or PfMSP-1-19 and seropositive for *P. vivax* if their antibody responses were above the cut-off for PvAMA-1 and/or PvMSP-1-19. Seroconversion rates were estimated by fitting a simple reversible catalytic model to seroprevalence data for each antibody [10]. Models with two SCRs allowing detection of changes in SCR were fitted and a likelihood test ratio was performed to decide the most appropriate model. Bivariate and multivariable analysis were performed to identify potential factors associated with *P. falciparum* (and *P. vivax*) exposure among study participants. Logistic regression models were performed to estimate odds ratios (ORs) of factors associated with being seropositive to *P. falciparum* or *P. vivax*, respectively. Adjusted odds ratios (aORs) were obtained using a multivariable model, including the following covariates: age, gender, seropositivity to *P. vivax*, education status, employment status, LLIN use, IRS in last 12 months, fever status, and altitude. Samples from participants aged under 18 years old were excluded from analysis of education and employment status. Statistically significant variables ( $p < 0.05$ ) detected in bivariate analysis were included in a multivariable model. The final model was developed using the forward stepwise approach which compared multivariable models to the most significant bivariate model using p-values calculated from likelihood ratio tests. Scatter plots matrix and coefficient correlation analysis were done to assess potential cross-reactivity between *P. falciparum* and *P. vivax* antigens.

## **Spatial analysis**

The spatial software SaTScan (v.9.4.2) was used to detect clusters of higher than average age-adjusted antibody responses to PfAMA-1, PfMSP-1-19, PvAMA-1 and PvMSP-1-19. The Normal model was used to detect clusters of households with higher than average age-adjusted antibody responses to PfAMA-1, PfMSP-1-19, PvAMA-1, PvMSP-1-19 antibody responses. This method has been previously utilized in several studies investigating malaria transmission heterogeneity in low endemic setting [25,37]. Antibody responses data were first log<sub>10</sub> transformed and then adjusted for age. The residuals from linear regression (log titre regressed against age in years, performed in Stata IC 15) were used to determine whether antibody responses were higher or lower than expected for any given age assuming a homogeneous distribution of risk. Residuals less than zero represent individuals whose responses were lower than or average for their age group whilst residuals above zero represent individuals whose responses were higher than average. These data were then averaged per household and categorized, based equally around the median, as 'lower than average', 'average', 'slightly higher than average', 'higher than average', and 'much higher than average' to generate an antibody response heat map. The scan statistic was set to calculate non-overlapping, statistically significant ( $p < 0.05$ ) clusters with a maximum set radius of 3 km and with minimum 2 observations detected in a cluster. Data generated from SaTScan were then plotted using ArcGIS software (v10.5).

### 3.4 Results

#### Study population

General characteristics of the sampled population is presented in Table 3.1. There were 1624 samples collected in the surveys from 605 households. The average number of people sampled per household was 3 (SD: 1.64). The proportion of females sampled (61%) was slightly higher than males. The majority of the samples came from Sukajaya sub-district (63%), and the median age of participants was 22 years (IQR: 9-38). Educational attainment was high, with only 0.34% of adults  $\geq 18$  years old who had not completed primary education. More than half (57%) of the working-age population ( $\geq 18$  years old) were unemployed. The population LLIN coverage was 60%, with 68% of those who owned nets reporting to have slept under it the night before. Only 15% of study households had received IRS in the previous 12 months. 9% of the population had fever with body temperature reading  $> 37.5^{\circ}\text{C}$ . Examination of microscopy slides found no malaria infections.

#### Seroprevalence and associated factors

Seropositivity to *P. falciparum* antigens was higher than seropositivity to *P. vivax* antigens, with seroprevalence 6.89% (95% CI: 5.76-8.24) and 1.97% (95% CI: 1.39-2.77), respectively. Seroprevalence ranged from 1.2 to 11.4 % for *P. falciparum* and 0.5 to 2.8% for *P. vivax* across age groups. Notably, there were no seropositive individuals aged under 5 years old identified for either *P. falciparum* or *P. vivax* (0/210). Seroprevalence to each antigen can be found in Additional file 1.

Multivariable analysis in Table 3.1 shows that age, seropositivity to *P. vivax* and use of LLINs were significantly associated with *P. falciparum* seropositivity, after controlling for other covariates. As would be expected, seroprevalence increased with age. Adults were more likely to be seropositive compared to children under 15 years old, with adjusted OR 5.69 (95% CI: 2.43-13.37), 12.05 (95% CI: 5.59-25.94) and 10.27 (95% CI: 4.74-22.27) for age group 16-24, 25-40 and over 40 years old, respectively. Seropositivity to *P. falciparum* was also significantly associated with higher proportion of LLIN use, with adjusted OR 1.80 (95%: 1.20-2.72). In addition, people who were seropositive to any *P. vivax* antigen were 3 times more likely to be seropositive for *P. falciparum*, with adjusted OR 3.47, (95% CI: 1.48-8.12). Other factors such as gender, residence, education, employment, IRS, fever and altitude were not significantly associated with *P. falciparum* seropositivity. Multivariable logistic regression revealed that there were no factors significantly associated with *P. vivax* seropositivity (Additional file 2).

**Table 3.1 Demographic characteristics and factors associated with *P. falciparum* transmission in Sabang, Indonesia, 2013**

Variable (n = 1624)	Total	<i>P. falciparum</i> seropositive		Multivariable
	N (%)	n	% (95% CI)	aOR (95% CI)
Age (years)				
<15 years old	656 (40.39)	8	1.2 (0.6-2.4)	1
16-24 years old	270 (16.63)	19	7.0 (4.5-10.8)	5.69 (2.43-13.37)**
25-40 years old	347 (21.37)	45	13.0 (9.8-16.9)	12.05 (5.59-25.94)**
>40 years old	351 (21.61)	40	11.4 (8.5-15.2)	10.27 (4.74-22.27)**
<i>P. vivax</i> seropositive				
No	1592 (98.03)	104	6.5 (5.4-7.9)	1
Yes	32 (1.97)	8	25.0 (12.9-42.9)	3.47 (1.48-8.12)**
LLIN use				
No	1098 (68.28)	63	5.7 (4.5-7.3)	1
Yes	510 (31.72)	48	9.4 (7.2-12.3)	1.80 (1.20-2.72)**

\* p value < 0.05 \*\* p value < 0.01. Individual level data: age, gender, education status, employment status and fever. Household level data: LLIN use, IRS in last 12 months and altitude

### ***Plasmodium falciparum* and *Plasmodium vivax* transmission intensity**

Figure 3.2 describes the SCR estimates for *P. falciparum* and *P. vivax* in Sukakarya and Sukajaya sub-districts, Sabang municipality, Indonesia in 2013. The SCR estimates suggested that there was a significant change in *P. falciparum* transmission in both Sukakarya and Sukajaya sub-districts, with no exposure seen in children under 5 years old. The data suggested that the *P. falciparum* transmission intensity in people aged over 5 years old was SCR 0.008 (95% CI: 0.003–0.017) and SCR 0.012 (95% CI: 0.005–0.030) in Sukakarya and Sukajaya, respectively. The SCR estimates for *P. vivax* (Fig. 3.2c and 3.2d) also suggested a very low level of transmission, SCR 0.001 (95% CI: 0.000-0.005) and 0.002 (95% CI: 0.001-0.006), respectively. There was no evidence for a difference in SCR between people aged under 5 and over 5 years old in either Sukakarya or Sukajaya. Overall, these model SCR estimates suggested that the magnitude of transmission in population level was likely to be similarly very low for *P. falciparum* and *P. vivax*.

### **Heterogeneity of *P. falciparum* and *P. vivax***

Spatial analysis of higher than average age-adjusted antibody responses identified 5 significant clusters for PfAMA-1 and 3 clusters for PfMSP-1-19. All 5 of the PfAMA-1 clusters were seen in Sukajaya (Figure 3.3a), whilst 2 of 3 PfMSP-1-19 clusters seen in Sukajaya and spatially overlapped with the PfAMA-1 clusters (Figure 3.3b). One additional PfMSP-1-19 cluster was identified in Sukakarya. The analysis of age adjusted antibody responses to *P. vivax* antigens identified 2 clusters for PvAMA-1 and 3 clusters for PvMSP-1-19 in Sukajaya (Figure 3.4). The clusters identified for PvAMA-1 spatially overlapped the PvMSP-1-19 clusters. All of these *P. vivax* clusters were seen in Sukajaya, whilst no clusters were identified in

Sukakarya. Overall, the clusters identified for *P. falciparum* and *P. vivax* were seen in the same areas.

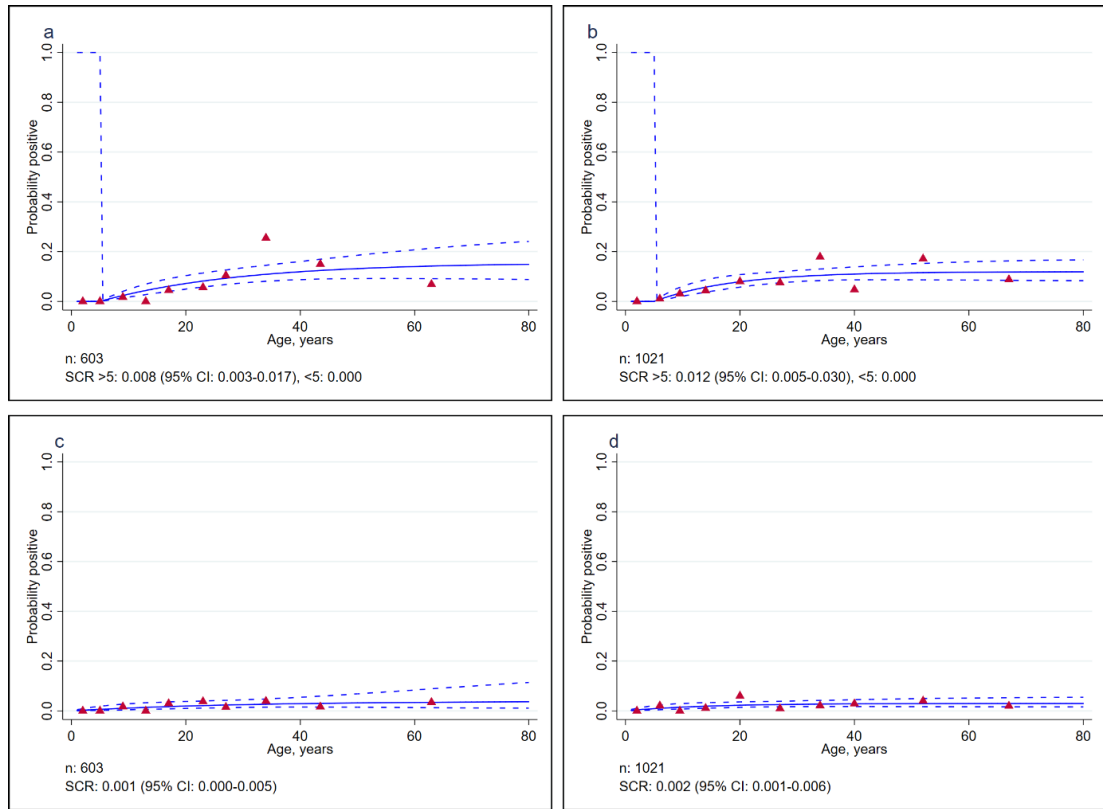


Figure 3.2 Age-seroprevalence plots for *P. falciparum* in Sukakarya (a), Sukajaya (b), for *P. vivax* in Sukakarya (c) and in Sukajaya (d), 2013. Solid lines represent the fitted probability for being seropositive to either MSP-1-19 or AMA-1 antigen, dashed lines represent the 95% confidence interval of these fits and red triangles represent the observed proportion of seropositive per age decile. SCR value represent the average annual rate at which the population become seropositive to any of *P. falciparum* or *P. vivax* antigen.

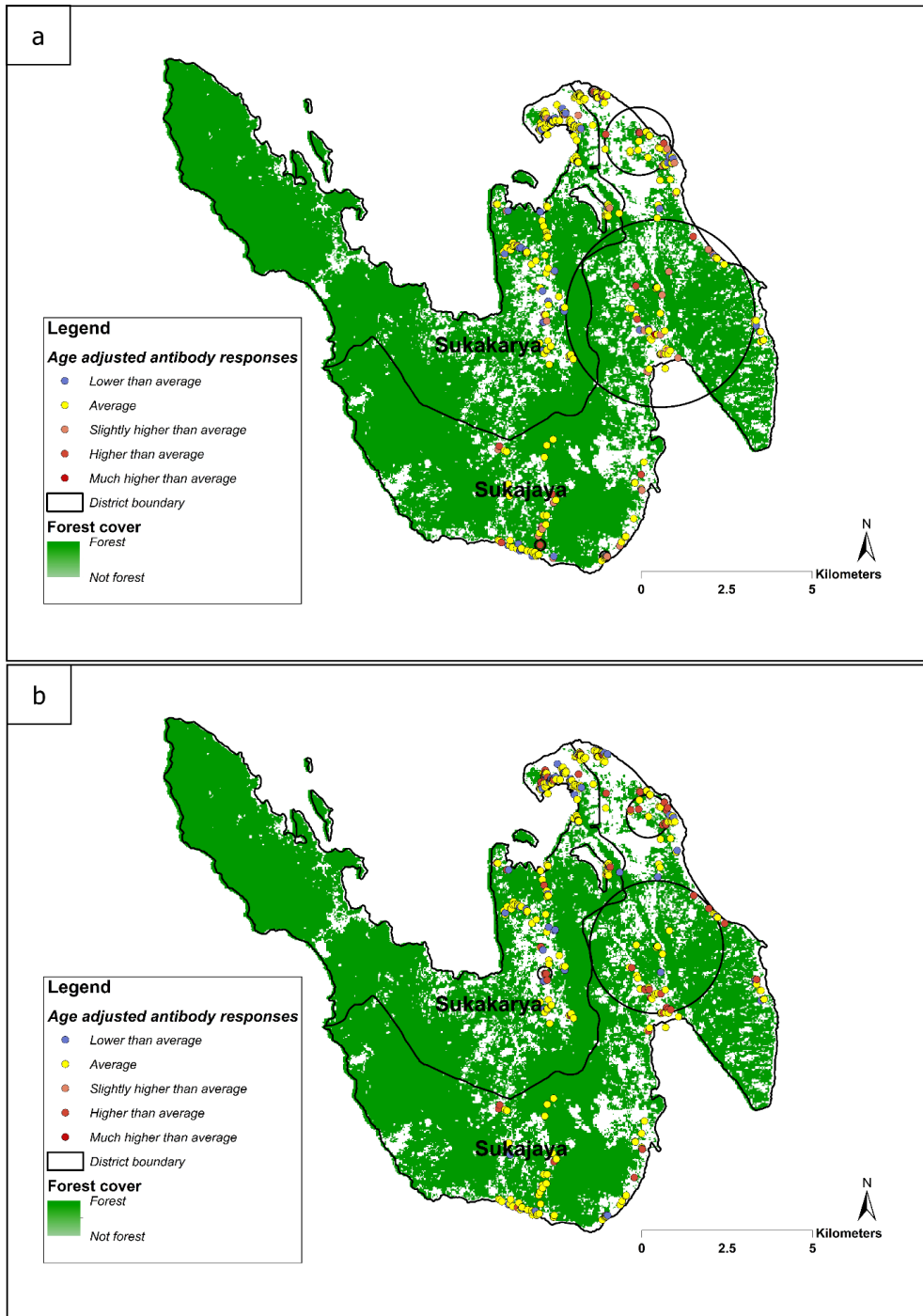


Figure 3.3 Spatial distribution of household-averaged, age-adjusted antibody responses to **a)** PfAMA-1 and to **b)** PfMSP-1-19 in Sukakarya and Sukajaya sub-districts, Sabang, Indonesia. The resultants residual values were categorised as: 'lower than average' (-4.326 to -0.499), 'average' (-0.500 to 0.500), 'slightly higher than average' (0.501 to 1.000), 'higher than average' (1.001 to 1.500 ) and much higher than average (1.501 to 2.842). Black circle indicates a cluster of significantly higher than expected antibody responses detected using SaTScan ( $p$  value < 0.05).



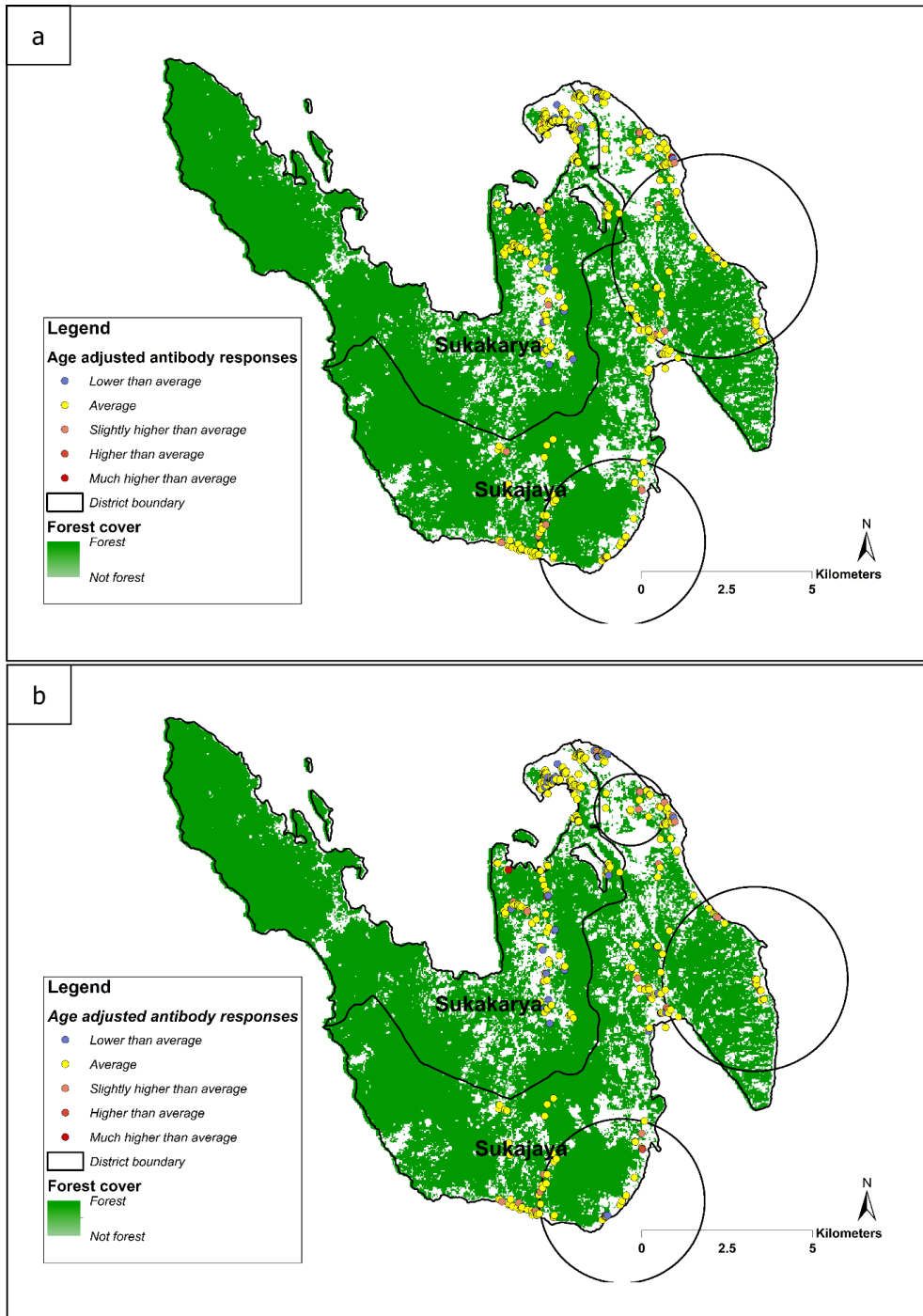


Figure 3.4 Spatial distribution of household-averaged, age-adjusted antibody responses to **a)** PvAMA-1 and to **b)** PvMSP-1-19 in Sukakarya and Sukajaya sub-districts, Sabang, Indonesia. The resultants residual values were categorised as: 'lower than average' (-4.933 to -0.499), 'average' (-0.500 to 0.500), 'slightly higher than average' (0.501 to 1.000), 'higher than average' (1.001 to 1.500 ) and much higher than average (1.501 to 2.117). Black circle indicates a cluster of significantly higher than expected antibody responses detected using SaTScan ( $p$  value < 0.05).

### 3.5 Discussion

This study describes the analysis of community-based serological data to investigate malaria transmission dynamics in a low transmission setting, Sabang, Indonesia. The seroprevalence and SCR data represent exposure to infection and demonstrate that the population level of transmission intensity were similarly very low for both *P. falciparum* and *P. vivax*. The seroprevalence in children under 15 years old was negligible, 1.2% and 0.5% for *P. falciparum* and *P. vivax*, respectively. The spatial analysis of household-level data on antibody responses to any of the antigens tested describe the heterogeneity of both *P. falciparum* and *P. vivax* exposure in the study area. These results supported previous utilization of sero-epidemiological analysis in assessing population-level transmission intensity and differentiating between areas of different endemicity in Indonesia [30]. Moreover, multivariable analysis utilizing serological and epidemiological data collected through community-based survey identified that age, *P. vivax* seropositivity and LLIN use were significantly associated with *P. falciparum* seropositivity. These associations are likely related to historical exposure as *P. falciparum* seroprevalence was estimated to be low and parasite screening found no active infections detected by microscopy. Although sub-microscopic infections might present in the community, a previous study suggested that the proportion of sub-microscopic infections detected via PCR (polymerase chain reaction) was very low 0.07% (11/16,229) in the region [34]. However, though PCR is very sensitive, a smaller proportion of individuals with sub-microscopic parasitaemia will be detected if smaller volumes of samples such as on filter paper bloodspot were used. One ml of whole blood is often used for DNA extraction and PCR for detecting sub-microscopic parasitaemia.

The *P. falciparum* SCR estimates suggest that there was no exposure seen in children under 5 years old in both sub-districts in Sabang municipality. These results could represent a step change in *P. falciparum* transmission due to the successful impact of malaria control programme implemented in the study area, evidenced by lower antibody prevalence in children born after the intervention scale-up. This finding was supported by a previous study documenting a significant drop in malaria cases after the launch of the control program in 2004. Malaria cases in Sabang declined from 88 cases per 1000 population in 2004 to 1 per 1000 by 2010. The decline in malaria transmission in Sabang is likely related to an extensive IRS programme immediately following the tsunami in 2004, large scale LLIN distribution, and a change in malaria treatment policy to artemisinin-based combined therapy as first-line treatment for uncomplicated malaria [34]. Sabang was certified as a malaria-free region by the Indonesian Government as a result of successfully maintaining zero cases since the last locally transmitted case reported in 2011. Since then, the surveillance system detected 12 imported cases consisting of 6 *P. vivax*, 4 *P. falciparum* and 2 mixed *P. vivax* and *P. falciparum* infections from 2011 to 2013, with no local transmission. However, the surveillance system detected 15 PCR confirmed *Plasmodium knowlesi* infections that classified as an outbreak in 2014 [38].

Consistent with the higher *P. falciparum* SCR estimates in people over 5 years old, multivariable analysis revealed that adults were more likely to be seropositive compared to children under 15 years old. This is likely the result of higher exposure by staying overnight in high-risk areas. A recent study revealed that the clusters of malaria (*P. knowlesi*) infections in Sabang was associated with people who had a history of staying overnight in the forest, without protection from mosquitoes, in an area where macaques are common [38].

Unfortunately, data on travel behaviour and occupation in these surveys were not recorded to enable testing of these hypotheses. Future research would need to include more detailed questions regarding travel behaviour, occupation and other essential risk factor data such as travel history to high-risk areas, night outdoor activities, sleeping in plantation or forest, housing, personal protection, etc. Several programme initiatives, for example a multi-country study on vector control tools to address outdoor transmission and project management quality improvement for national malaria program workforce carried out under the Asia Pacific Malaria Elimination Network would be beneficial for the malaria elimination effort in the region. In addition, the use of LLIN was almost two times higher in area where *P. falciparum* seroprevalence was higher. Consistent with previous report suggesting high coverage of LLINs (over 75%) in six malaria focal villages in Sabang, this finding suggests that people living in higher risk of exposure were aware of the importance of LLIN to prevent malaria transmission in those areas [34].

The estimated age-seroprevalence curves and SCR value suggested that age was not associated with *P. vivax* transmission in either sub-district in Sabang. *Plasmodium vivax* seroprevalence was very low (2.0%) and, therefore, the absence of any associations is likely due to the statistical limitations of the low number of seropositive samples. The other possible explanation is that *P. vivax* infections may induce lower antibody responses or shorter-lived responses which the current assay may miss. Work is ongoing to identify *P. vivax* antigens that elicit short-term responses for easy identification of very recent exposure [39,40]. The need for testing more potential *P. vivax* antigens is supported by a previous study showing that the number of *P. vivax* cases tend to be higher than the number of *P. falciparum* cases in Sabang [34].

The spatial analysis of age-adjusted antibody responses to either antigen (AMA-1 or MSP-1<sub>19</sub>) identified significant clusters of higher exposure (hotspots) for both *P. falciparum* and *P. vivax* exposure across the study areas. Although multivariable risk factors analysis found there was no significant association between residence and higher seroprevalence to *P. falciparum* and *P. vivax*, the spatial analysis suggested that the risk of malaria transmission in the study setting is heterogeneous with people experiencing higher exposure in Sukajaya sub-district. The spatial analysis also suggest that the clusters identified for *P. falciparum* and *P. vivax* were seen in the same areas. Being able to characterize the micro-epidemiology of malaria exposure could assist malaria control programme to better allocate resources and target the intervention to achieve their goal of elimination. Targeting hotspots could be a highly efficient way to reduce malaria transmission at all levels of transmission intensity [41]. Although this study identified potential high-risk areas using historical data collected in 2013, being able to identify areas which had the most recent exposure is useful for malaria surveillance. A recent study suggested that one of two clusters of *P. knowlesi* infections in Sabang were identified in similar high-risk areas identified in this study [38]. As suggested in the latest WHO malaria surveillance manual [11], maintaining surveillance activities in the most receptive areas could be useful to prevent potential reintroduction or resurgence of the disease in the future. Therefore, utilizing antibody responses data to identify recent or historical hotspots of transmission could be a powerful alternative approach where gaining direct evidence of an increased exposure to infectious mosquito bites is no longer ideal in low transmission settings.

Finally, people who were seropositive to any *P. vixax* antigen were 3 times more likely to be *P. falciparum* seropositive, after controlling for age, gender, residence, employment,

education, IRS, fever status, and altitude. In addition, clusters of high antibody responses suggest that *P. falciparum* and *P. vivax* receptive areas were seen in the same areas. As there was no cross-reactivity evident from the serological data (Additional file 3), these findings could suggest that people were historically exposed to both infections, potentially due to the presence of efficient vectors in those identified areas.

Findings in this study are based on community-based samples and data collected during the malaria transmission season. Although this study describes the potential use of serological data analysis in estimating malaria transmission intensity, heterogeneity and factors associated to disease exposure, the results generated would need to be carefully interpreted. Previous studies suggested that malaria transmission in other areas of Indonesia was affected by seasonality [30,34,42–44] and behavioural factors such as farm or forest-related night outdoor activity (e.g. sleeping in forest gardens) [45,46] and domestic travel to higher endemic areas [47]. However, due to limited data collected, our study could not examine the effect of behavioural factors such as forest-related activities or recent travel history to high-risk areas outside Sabang. Therefore, future studies measuring population level antibody responses coupled with collecting more data that could describe behavioural factors associated to higher risk of exposure would be more epidemiologically informative to assist malaria surveillance and control programme to achieve elimination in the region.

### **3.6 Conclusion**

In conclusion, these data add to the body of evidence that sero-epidemiological analysis of community-based surveys are an important additional tool to investigate malaria

transmission dynamics in area aiming for elimination in Indonesia. Recent identification of alternative antigens associated with short-lived antibody responses suggests a potentially key indicator of very recent exposure which would be a very important information for public health surveillance [48]. The addition of a novel panel of *P. knowlesi* antigens [49] would enhance understanding of malaria transmission dynamics as recent studies reported that although laboratory identification of *P. knowlesi* in Indonesia is challenging [50], surprisingly, there were two clusters of *P. knowlesi* cases detected in Sabang after the municipality successfully eliminated *P. falciparum* and *P. vivax* cases [38]. Moreover, another recent study also reported there was a considerable proportion of *P. knowlesi* infection in another western part of Indonesia, in North Sumatera province [51]. Exploratory work employing techniques such as multiplex fluorescent magnetic bead-based serological assay to investigate and validate a panel of potential antigens for these applications is underway [40,52]. The development and validation of a standardized serological sample and data collection methods utilizing existing public health surveillance system, for example as described in [53] will also facilitate the optimization of serological surveillance in understanding transmission dynamics to support malaria control programme in achieving elimination.

## **Abbreviations**

CI: confidence interval; EIR: entomological inoculation rate; IRS: indoor residual spraying; LLIN: long-lasting insecticide-treated bed nets; PfAMA-1: *P. falciparum* apical membrane antigen 1; PfMSP-1-19: *P. falciparum* merozoite surface protein 1; PvAMA-1: *P. vivax* apical membrane antigen 1; PvMSP-1-19: *P. vivax* merozoite surface protein 1; SCR: seroconversion rates; WHO: world health organization.

## **Ethics approval and consent to participate**

This study was approved by the Institutional Review Board of the Medical Health Research Ethics Committee, Gadjah Mada University, Indonesia (KE/FK/927/EC) and the Research Ethics Committee of the London School of Hygiene and Tropical Medicine (11944). Written informed consent was obtained from all participants in this study.

## **Consent for publication**

Not applicable

## **Availability of data and materials**

The datasets used and analysed during this study are not publicly available due to the inclusion of identifying information on individuals but are available from the corresponding author on reasonable request and approval from relevant ethics committees.

## **Competing interests**

The authors declare that they have no competing interests.



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## **Authors' contributions**

HS collated and analysed the data and wrote the manuscript; MAW contributed to study design, data collection and reviewed the manuscript; EHM contributed to blood samples collection and supervised the serological assay, MES, NFL and WAH contributed to the study design and field work supervision; I, TY, M and H carried out field work; JC and CD provided the antigens, standardized serological assay protocols, interpreted findings and reviewed the manuscript, S designed the study, coordinated field data collection, supervised serological assay and reviewed the manuscript. All authors read and approved the final manuscript.

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### 3.8 Supplementary information

**Additional file 1.** Seroprevalence for each antigen studied

Antigen	N	Number positive	% (95% CI)
PfAMA-1	1615	85	5.3 (4.3-6.5)
PfMSP-1 <sub>-19</sub>	1624	58	3.6 (2.8-4.6)
PvAMA-1	1624	19	1.2 (0.8-1.8)
PvMSP-1 <sub>-19</sub>	1624	17	1.2 (0.7-1.7)

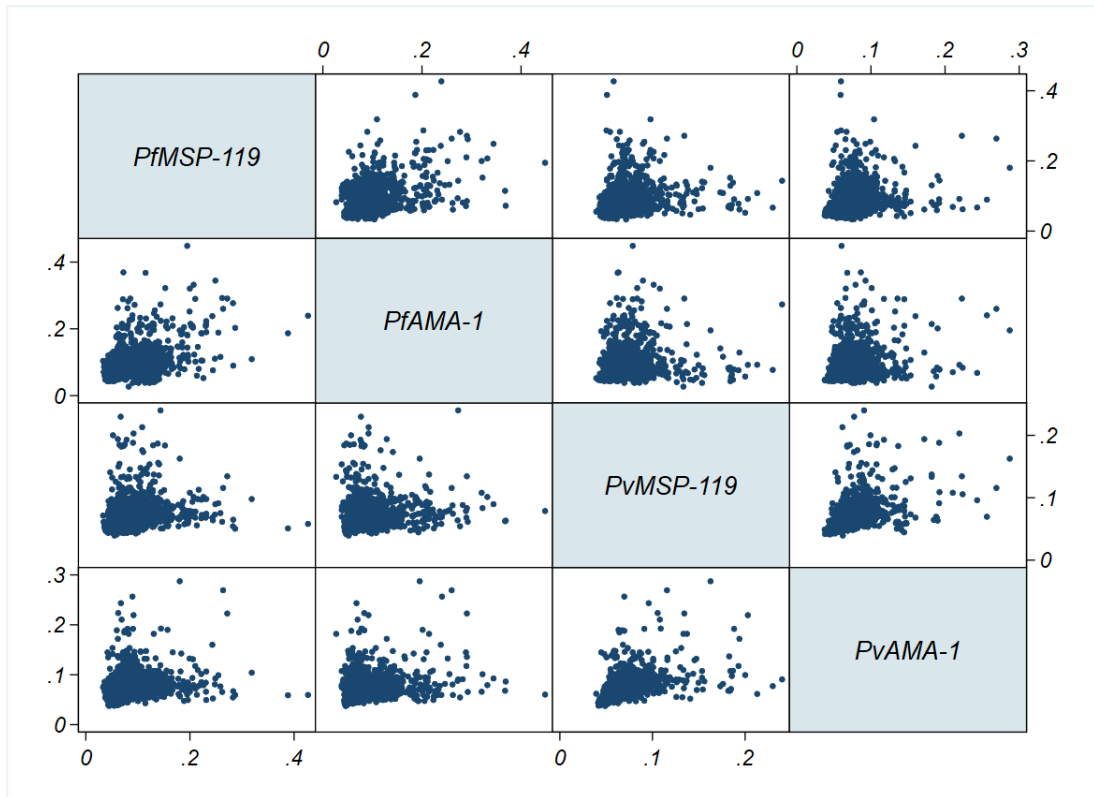


**Additional file 2.** Demographic characteristics and factors associated with *P. vivax* transmission in Sabang, Indonesia, 2013

Variable (n = 1624)	Total	<i>P. vivax</i> seropositive		Bivariate	<i>p</i>
	N (%)	n	% (95% CI)	OR (95% CI)	
Age (years)					
<15 years old	656 (40.39)	3	0.5 (0.1-1.4)	1	
16-25 years old	270 (16.63)	11	4.1 (2.3-7.2)	9.24 (2.56-33.41)	0.001
25-40 years old	347 (21.37)	8	2.3 (1.2-4.5)	5.14 (1.35-19.49)	0.016
>40 years old	351 (21.61)	10	2.8 (1.5-5.2)	6.38 2 (1.75-23.35)	0.005
Gender					
Female	984 (60.55)	23	2.3 (1.6-3.5)	1	
Male	641 (39.45)	9	1.4 (0.7-2.7)	0.59 (0.27-1.29)	0.190
Residence					
Sukakarya	603 (37.13)	11	1.8 (1.0-3.3)	1	
Sukajaya	1021 (62.87)	21	2.1 (1.3-3.1)	1.13 (0.54-2.36)	0.745
Education					
None	3 (0.34)	1	0.4 (0.1-2.6)	1	
Primary education	764 (86.33)	27	2.2 (1.5-3.2)	6.14 (0.83-45.38)	0.075
Higher education	118 (13.33)	3	2.5 (0.8-7.5)	6.97 (0.72-67.75)	0.094
Employment					
Unemployed	516 (57.33)	20	2.5 (1.6-3.8)	1	
Non-office-based job	215 (23.89)	4	1.8 (0.7-4.8)	0.73 (0.25-2.16)	0.572
Office-based job	105 (11.67)	2	1.9 (0.5-7.1)	0.73 (0.17-3.19)	0.681
Student	64 (7.11)	6	1.2 (0.6-2.7)	0.48 (0.19-1.22)	0.123
LLIN use					
No	1098 (68.28)	19	1.7 (1.1-2.7)	1	
Yes	510 (31.72)	13	2.5 (1.5-4.3)	1.48 (0.73-3.03)	0.278
IRS last 12 months					
No	1376 (84.83)	25	1.8 (1.2-2.7)	1	
Yes	246 (15.17)	7	2.8 (1.4-5.9)	1.58 (0.68-3.70)	0.290
Fever					
No	1483 (91.26)	31	2.1 (1.5-3.0)	1	
Yes	142 (8.74)	1	0.7 (0.1-4.8)	0.33 (0.45-2.45)	0.280
Altitude (meter)					
< 120	716 (50.46)	13	1.8 (1.1-3.1)	1	
> 120	703 (49.54)	10	1.4 (0.8-2.6)	0.78 (0.34-1.79)	0.559

Individual level data: age, gender, education status, employment status and fever.

Household level data: LLIN use, IRS in last 12 months and altitude



**Additional file 3.** Scatter plots matrix of antibody responses (optical density) to *P. falciparum* and to *P. vivax* antigens tested in the study describing the absence of cross-reactivity between the *P. falciparum* and *P. vivax* antigens

#### 4. Use of mobile technology-based participatory mapping approaches to geolocate health facility attendees for diseases surveillance in low resource settings

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#### SECTION A – Student Details

Student	Henry Surendra
Principal Supervisor	Chris Drakeley
Thesis Title	Operational applications of serology for malaria surveillance in different transmission settings in Indonesia

***If the Research Paper has previously been published please complete section B, if not please move to Section C***

#### SECTION B – Paper already published

Where was the work published?	International journal of health geographics		
When was the work published?	18 June 2018		
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion	NA		
Have you retained the copyright for the work?*	Yes	Was the work subject to academic peer review?	Yes

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#### SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published	
Please list the paper's authors in the intended authorship order.	
Stage of publication	

#### SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	Shared first-author with Kimberly M Fornace. Both authors contributed equally to study design, data collection, all analysis and writing.
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Student Signature: [Redacted]

Date: 30/09/2019

Supervisor Signature: [Redacted]

Date: 30/09/2019

**Use of mobile technology-based participatory mapping approaches to geolocate health facility attendees for disease surveillance in low resource settings**

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**†Kimberly M. Fornace and Henry Surendra contributed equally to this work**

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## **Authors' contributions**

KMF and HS designed this study, analysed data and wrote the manuscript. KMF, HS, TRA, RR, MLMM and GS identified and evaluated software programs. HS, RR, MLMM, JL, FE, RAA and S collected the data. All authors read and approved the final manuscript.

## **4.1 Abstract**

**Background:** Identifying fine-scale spatial patterns of disease is essential for effective disease control and elimination programmes. In low resource areas without formal addresses, novel strategies are needed to locate residences of individuals attending health facilities in order to efficiently map disease patterns. We aimed to assess the use of Android tablet-based applications containing high resolution maps to geolocate individual residences, whilst comparing the functionality, usability and cost of three software packages designed to collect spatial information.

**Results:** Using Open Data Kit GeoODK, we designed and piloted an electronic questionnaire for rolling cross sectional surveys of health facility attendees as part of a malaria elimination campaign in two predominantly rural sites in the Rizal, Palawan, the Philippines and Kulon Progo Regency, Yogyakarta, Indonesia. The majority of health workers were able to use the tablets effectively, including locating participant households on electronic maps. For all households sampled (n = 603), health facility workers were able to retrospectively find the participant household using the Global Positioning System (GPS) coordinates and data collected by tablet computers. Median distance between actual house locations and points collected on the tablet was 116 m (IQR 42–368) in Rizal and 493 m (IQR 258–886) in

Kulon Progo Regency. Accuracy varied between health facilities and decreased in less populated areas with fewer prominent landmarks.

**Conclusions:** Results demonstrate the utility of this approach to develop real-time high-resolution maps of disease in resource-poor environments. This method provides an attractive approach for quickly obtaining spatial information on individuals presenting at health facilities in resource poor areas where formal addresses are unavailable and internet connectivity is limited. Further research is needed on how to integrate these with other health data management systems and implement in a wider operational context.

**Keywords:** Electronic data collection, mHealth, Geographical information systems, Surveillance, Mobile technology, Participatory mapping

## 4.2 Background

Infectious disease risks can be highly heterogeneous at fine spatial scales due to environmental, social and biological factors [1]. As infectious disease control programmes move towards elimination, it is increasingly important to identify and target foci of transmission areas and understand the factors that may contribute to disease persistence in these locations [2–4]. Disease reports aggregated at coarser spatial scales, such as district or regional levels, may not capture these differences in micro-epidemiology [5, 6].

Numerous studies have utilised global positioning system (GPS) technology to develop fine-scale maps of disease infection and exposure (e.g. [7, 8]), identify hotspots of disease transmission (e.g. [9, 10]) and target control measures (e.g. [11, 12]). These studies typically use population-based cross-sectional surveys including GPS coordinates for patient

households or frequently visited locations to map disease risks. Alternatively, when household surveys are not feasible, convenience sampling approaches targeting easy access groups can be used to estimate risks in a population. Examples of these approaches include school-based surveys (e.g. [13, 14]) and surveys of clinic attendees (e.g. [15]). These methods may not fully capture risks in the wider population but are substantially more cost effective to implement and may be more feasible in low resource settings.

A key limitation of convenience sampling approaches is that the interviewer does not visit the patient household and therefore cannot collect GPS coordinates at the site. If formal address information is available for a region, the patient address can be used to identify the GPS coordinates. However, this type of information is often not available for many countries or high-risk groups, such as migrant or mobile populations. In these situations, other methods can be used to estimate locations of patient households, such as identifying the nearest landmark, clinic or school catchment area or using participatory mapping techniques in which the patient identifies the location of their house on a paper map [16, 17]. These methods can be used to yield maps of relatively high spatial accuracy however, digitising maps and data management may be time consuming.

To address this issue, we assessed the use of tablet- based applications to geo-locate patient households remotely. Tablets are widely used to administer questionnaires and collect health information electronically as well as to scan barcodes and track samples [18–21]. Digital data collection can improve data quality and completeness as well as increase efficiency of data cleaning and analysis [22]. While these applications are frequently used to record GPS coordinates of the current location, the utility for participatory mapping for

health surveys has not currently been assessed. We evaluated multiple software programs for use in rural resource poor settings with no internet connectivity as part of a malaria elimination research project. As such, a core requirement was the ability to load satellite images for use offline. We aimed to (1) identify appropriate tablet-based applications and assess the functionality, cost and technical expertise required to set up and use the programs; and (2) assess the accuracy of data collected using offline maps for the selected application.

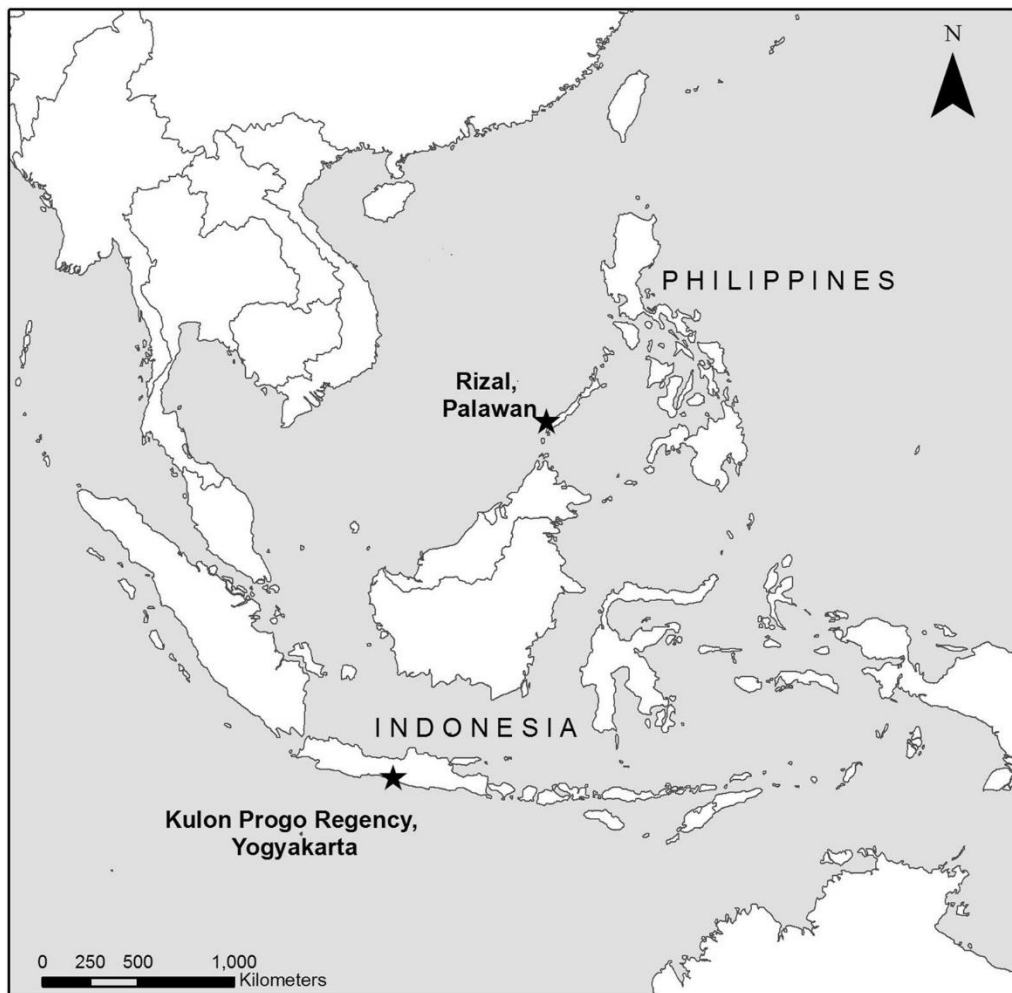
### **4.3 Methods**

#### **Study areas**

We evaluated different software programs for use in malaria surveillance of clinic attendees in two rural sites in Southeast Asia: Rizal Municipality, Palawan, Philippines (1256 km<sup>2</sup>, estimated population 50,100, 15 health facilities) and Kulon Progo Regency, Yogyakarta, Indonesia (586 km<sup>2</sup>, estimated population 430,500, 8 health facilities). These sites are targets of on-going research projects to enhance surveillance for malaria elimination aiming to establish, integrate and evaluate combinations of laboratory, clinical and epidemiological data collected during health facility surveys to estimate the magnitude and heterogeneity of malaria transmission. Kulon Progo Regency is the site of one of the few remaining foci of malaria transmission in Java Island, Indonesia and was chosen as epidemiologically representative of a pre-elimination area where researchers and local control programmes are actively working towards elimination for Indonesia's national strategic plan for malaria. Rizal, Palawan was selected as representative of an area in the Philippines transitioning from reduction of disease burden to malaria elimination. Samples were collected from patients



and companions attending health facilities and microscopy, molecular and serological methods were used to identify infections and characterise transmission intensity. Both sites had multiple health facilities with poor or no internet connectivity. For each site, significant landmarks such as clinics, mosques, churches and schools were identified by local personnel and geolocated using a handheld GPS (Garmin, USA). Other spatial data, such as locations of roads and administrative boundaries, were assembled from available sources including government departments, freely accessible geospatial databases and open source GIS platforms such as OpenStreet Map ([www.openstreetmap.org](http://www.openstreetmap.org)) and Global Administrative Areas (GADM; [www.gadm.org](http://www.gadm.org)).



*Figure 4.1 Study areas*

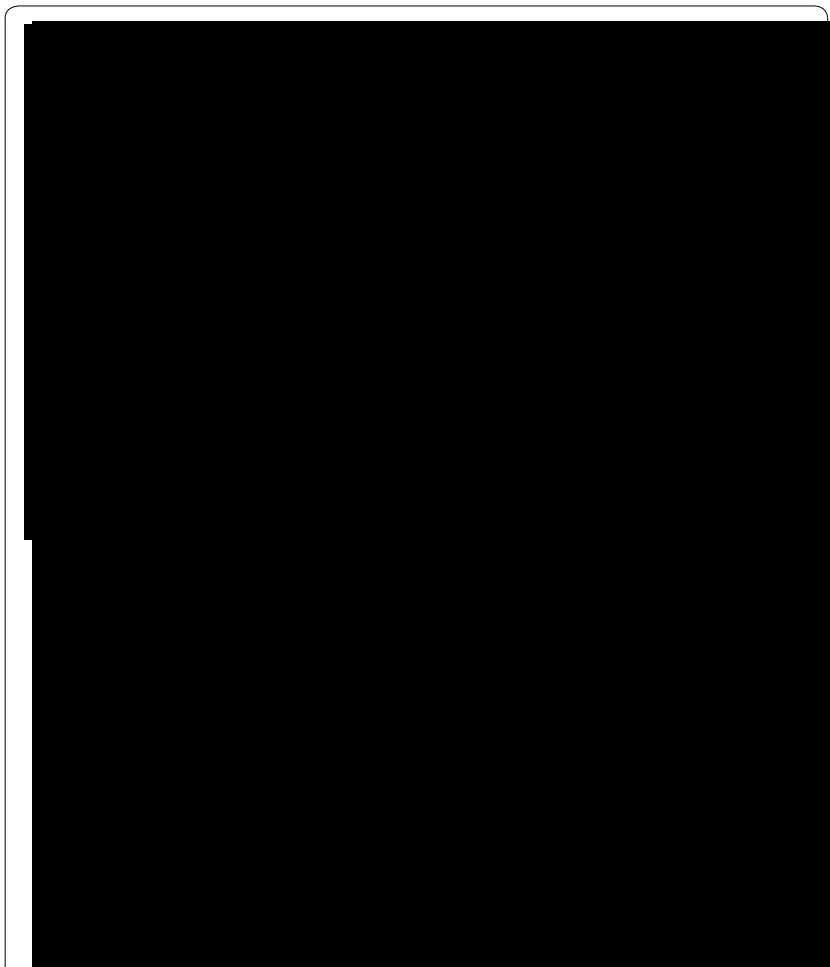
#### **Data collection methods and survey**

To develop data collection methods for these activities, we first evaluated multiple mobile-based data collection systems with the capacity to collect questionnaire data, GPS coordinates, and to take photographs of rapid diagnostic test results and scan barcodes used for sample tracking. For each software program, we set up a questionnaire as well as an offline map using best available satellite and GPS data for the health facility catchment area (Additional file 1). These questionnaires were tested by project staff in each site. All

questionnaires were set up on Android tablets with 8 GB of internal memory and additional memory on external SD cards. Based on initial testing and map development, final data collection tools were designed using GeoODK and trialled in health facility surveys in the Philippines and Indonesia (Figure 4.1). Maps were produced in Mapbox Studio, including high resolution satellite data, administrative boundaries and key landmarks and available census data.

For multiple health facilities in each site, we conducted rolling cross sectional surveys of clinic attendees as part of larger malaria surveillance projects. During these surveys, consenting clinic attendees participated in a short questionnaire survey in Tagalog or Bahasa Indonesia and were asked to geo-locate their household using the digital maps provided. Initial 2 days training sessions were conducted for health facility personnel, followed by routine field supervision during the first week and regular meetings to identify any outstanding issues. Questionnaires were administered by the trained health facility personnel using Android-based tablets (Figure 4.2). Data collected was checked for completeness and field and data management staff were interviewed on the ease of use and any issues with questionnaire or map data. Multiple health facilities from each site (3 facilities in Rizal and all 8 in Kulon Progo Regency) were selected to be representative of the data collected in each region, including the main regional health facility and several smaller satellite facilities in more remote areas. As this survey had an opportunistic sampling design, this population is not representative of the wider population in the study areas but rather individuals attending these health facilities. To assess the accuracy of reported GPS points, randomly selected households reporting to selected health facilities were followed up in both sites and GPS points of actual house locations were recorded using a handheld GPS.

Although accuracy of the handheld GPS units could be impacted by poor satellite signal or high canopy or building coverage, the mean accuracy of these devices was within 5 m of the recorded household location and we considered this measurement the actual location of the household. Root mean square error of the Euclidean distance in meters between the actual and reported household locations was calculated to assess accuracy of participant's estimates collected by tablets and identify factors affecting this accuracy.



*Figure 4.2 A health facility attendee identifies house location on a tablet*

#### **4.4 Results and discussion**

##### **Software programs and characteristics**

We initially identified three data collection programs capable of using offline maps: GeoODK (University of Maryland and International Institute for Applied Systems Analysis, College Park, USA), Survey123 for ArcGIS (Esri, Redlands, USA) and ePAL (Tripod Software, Salford, UK) (Table 4.1). GeoODK is an Android-based open source platform for form design, mobile data collection and data management system. Survey123 is a mobile data collection application which integrates into the Esri ArcGIS platform. ePAL is a custom-built application interfacing with other open source data collection systems (ODK and CommCare) to add capacity to use offline maps. While GeoODK was freely available, there were some developer costs for ePAL and ArcGIS Survey123 required the purchase of a software licence. Correspondingly, software programs had varying levels of technical support available. For GeoODK, part of the ODK open data kit, tutorials and manuals were available online in addition to active web forums for software developers and users. Product developers provided quick responses to technical queries and, in one instance, reviewed our questionnaires and maps to assist with troubleshooting. ArcGIS also had extensive user guides and tutorials available online. The software licence purchased included access to Esri technical support as well as online forums. While no formal documentation or support was available for ePAL, the software developers were available to address questions.

**Table 4.1 Characteristics of software applications assessed**

	<b>GeoODK</b>	<b>ArcGIS Survey123</b>	<b>ePAL</b>
Types of spatial data collected	Points, polygons and GPS tracks	Points, lines and polygons	Point
Able to load background maps offline	Yes	Yes	Yes
Format of offline maps	MBTile	ArcGIS Tile Package	PNG format tile dataset
Access to satellite imagery	External data only	Access to World Imagery and other ArcGIS layers	External data only
Storage space for imagery	Tablet internal memory	Tablet memory or external storage	Tablet memory or external storage
Questionnaire set up	XLS form	XLS form	Integrates with other data collection software
Integration	Integrates with barcode scanner and other software	Limited	Integrates with CommCare and ODK
Downloading data	Upload to server or download as XML file offline	Upload to server or download as SQLite database offline	Download as part of data from CommCare or ODK
Technical knowledge required	Some programming required to create MBTiles	Basic GIS knowledge only	Basic GIS knowledge only
Analysis	Summary statistics available from data on online server	Summary statistics available from data on online server	None
Cost and licencing	Free, open source software	Purchase of licence required (over USD 5000 for multiuser licence)	Limited purchase costs (less than USD 5000 developer costs)

### **Sources of satellite imagery and spatial data**

In order for individuals to geo-locate their households, base maps must be assembled with sufficient spatial data on local geography and key landmarks. Freely available high resolution satellite data, such as Google Earth ([www.google.com/earth](http://www.google.com/earth)), OpenStreetMap ([www.openstreetmap.org](http://www.openstreetmap.org)) and Bing Maps ([www.bing.com/maps](http://www.bing.com/maps)), are increasingly used in public health to develop sampling frames [23, 24], collect spatially referenced disease data [25, 26] and target interventions [12, 27]. These data are usually of sufficient resolution to allow identification of individual houses and may contain further data on nearby points of interest. However, although these data can be freely accessed online, exporting imagery to raster datasets or other formats required for offline use is frequently covered by intellectual property agreements and may require user agreements or payments. Additionally, high resolution data is not always available in remote, sparsely populated areas and available data may not be temporally accurate, presenting a challenge in areas with high rates of change or following natural disasters.

Alternatively, very high-resolution imagery is available through aerial photography or commercial satellite-based remote sensing sources, such as SPOT 6-7, Quickbird and IKONOS ([www.digitalglobe.com](http://www.digitalglobe.com)). These data have resolutions of 1.5 m per pixel or less and have accurate data on the time of collection or can be tasked to collect data following significant changes. However, collecting these data can be prohibitively expensive in many low income settings and processing and usage requires significant technical expertise. High resolution data may also be available through licensed software, for example Esri imagery through ArcGIS. Although accessing this imagery requires purchase of a software licence, high resolution imagery from aerial and satellite-based remote sensing is available for most of the world for offline use. These data are pre-processed and available in easy to use formats including metadata on the

date of collection and temporal accuracy. Selecting the most appropriate imagery depends on the rates of land use change and development and availability of data for a particular region as well as the resources and technical expertise available.

In addition, the inclusion of geo-referenced information on key landmarks can help participants identify their houses or neighbourhoods [16]. This may include spatial point data on schools, clinics and other points of interest in addition to line or polygon data on roads, rivers and administrative districts. These datasets may be assembled from a range of sources such as government mapping departments, open source spatial data platforms (e.g. OpenStreet Map or GADM) or through collecting GPS data on the ground. In some instances, where previous community-based surveys have been conducted or censuses have collected GPS coordinates, point data may be available for individual houses. For each site, we used all available vector data, including any household head names, emphasising labels for commonly identified features.

### **Setting up questionnaires and imagery**

All software programs trialled used XLS forms to design questionnaires or, for ePal, integrated with other data collection software using XLS forms. However, each program required a different format for offline maps. ArcGIS Survey123 was the most user-friendly option, allowing tile packages to be exported directly from ArcGIS with only basic GIS knowledge required. Both ePAL and GeoODK required additional processing time and expertise; ePAL required the creation of tiled PNG (Portable Network Graphics) datasets and GeoODK required MBTiles, a format storing tiled map data in SQLite databases which is commonly used by Android mapping applications. Production for both file types could be done using open source software such as



Quantum GIS ([www.qgis.org](http://www.qgis.org)) and Mapbox Studio ([www.mapbox.com](http://www.mapbox.com)) however MBTiles required some programming knowledge to correctly format maps.

For all formats, there were trade-offs between map resolution and speed. Producing high resolution maps resulted in large file sizes and consequently increased times to open maps on tablets. While both ArcGIS Survey123 and ePAL could store map files on either internal tablet memory or external SD cards, GeoODK could only use files stored on internal memory, limiting the possible size. For sites with more detailed spatial data relating to households and other landmarks, we reduced the resolution of the satellite data. If limited vector data were available, we increased the resolution but created multiple tiled datasets with smaller geographic areas to optimise rendering of maps on mobile devices.

### **Field testing of data collection method**

Based on the initial questionnaire testing, we chose to use GeoODK due to better integration of barcode scanners and other functionalities as well as faster loading of maps. Accuracy was not assessed for all software as all had similar map interfaces and accuracy was primarily dependent on the quality of the maps and the participant and interviewer abilities to use geographic information. GeoODK questionnaires and maps were set up on all tablets in the office while connected to the internet and data management staff were trained on setting up the questionnaire and downloading data offline. Training sessions were conducted to introduce fieldworkers to the use of the tablet and questionnaire; these field workers included community health workers and clinic staff, many of whom had not used electronic data collection methods or tablets prior to this work. Most fieldworkers were able to use the software effectively, although a few reported still preferring previously used paper data collection forms. Although

there were some technical issues, such as forms freezing or crashing, the majority of data (over 99%) was complete and collected without any problems. Despite the inclusion of satellite imagery, most participants relied on names of household owners included on maps or labelled local landmarks rather than satellite imagery to locate the participant's households. In some cases, when clinics were busy and maps were slow to load, fieldworkers did not wait for maps to load and fully zoom into an area, resulting in less accurate household geolocation; this issue was addressed by including maps with lower resolutions or smaller geographical areas which were faster to load.

An additional consideration is the availability of electricity; as not all clinics surveyed had reliable access to electricity or generators, we used external batteries or solar chargers in areas without constant power supplies. This did not result in the loss of any data but should be accounted for in budgeting and planning. Although data could be uploaded to an online server if an internet connection was available, the internet connection was poor and intermittent, resulting in the loss of data when the connection was interrupted during upload. Instead, all data was downloaded offline by copying XML files from the tablet memory to office computers. While GeoODK had functions to quickly produce summary statistics from data uploaded to the online server, we used R statistical programming language to read, merge and produce summary statistics for XML files (R statistical software, [www.R-project.org](http://www.R-project.org)).

### **Accuracy of tablet-based geo-location strategies**

To assess the accuracy of reported coordinates, we manually traced and recorded GPS points for 203 households in Rizal, Palawan and 400 households in Kulon Prugo Regency, Yogyakarta (Figure 4.3). All households could be identified by fieldworkers using the name and locations

collected by tablet and all households were located to their correct logistical unit used for interventions by the malaria control programme (sitios in the Philippines and desain Indonesia). Within these selected households, participants included 112 women and 91 men with a median age of 11 (range under 1–84 years) in Rizal, Palawan and 259 women and 143 men with a median age of 42 (range under 1–80 years) in Kulon Progo. In Rizal, 59 individuals had fever and 3 individuals were identified as malaria positive by microscopy while 34 individuals were febrile and 5 microscopy positive malaria cases were identified in Kulon Progo.

The median distance between house locations and points recorded by the tablet was 116 m (IQR 42–368) in Rizal and 493 m (IQR 258–886) in Kulon Progo Regency. Root mean squared error was 895 and 702 m for Rizal and Kulon Progo Regency respectively. While most locations recorded by tablet were fairly accurate, a minority of points (6% in Rizal and 5% in Kulon Progo Regency) were over 2 km away from the actual house, primarily in areas where few landmarks were recorded. Although there was no clear relationship between accuracy of reported house locations and distance from the health facility, data collected on households over 2 km from the health facility were less accurate overall (Figure 4.4). As geo-referenced point data was not available for all landmarks, we assessed whether areas with higher population density (places likely to have more distinct landmarks) were associated with accuracy of reported points. Gridded population density at 100 m resolution was obtained from WorldPop [28]; population density was not correlated with accuracy of reported points ( $p$  value = 0.11). These data may be improved by the inclusion of higher resolution maps or improved spatial information on remote areas. However, despite these limitations, data collected was of sufficient quality to identify houses of all sampled health facility attendees and enabled accurate fine-scale mapping of participants for these areas.

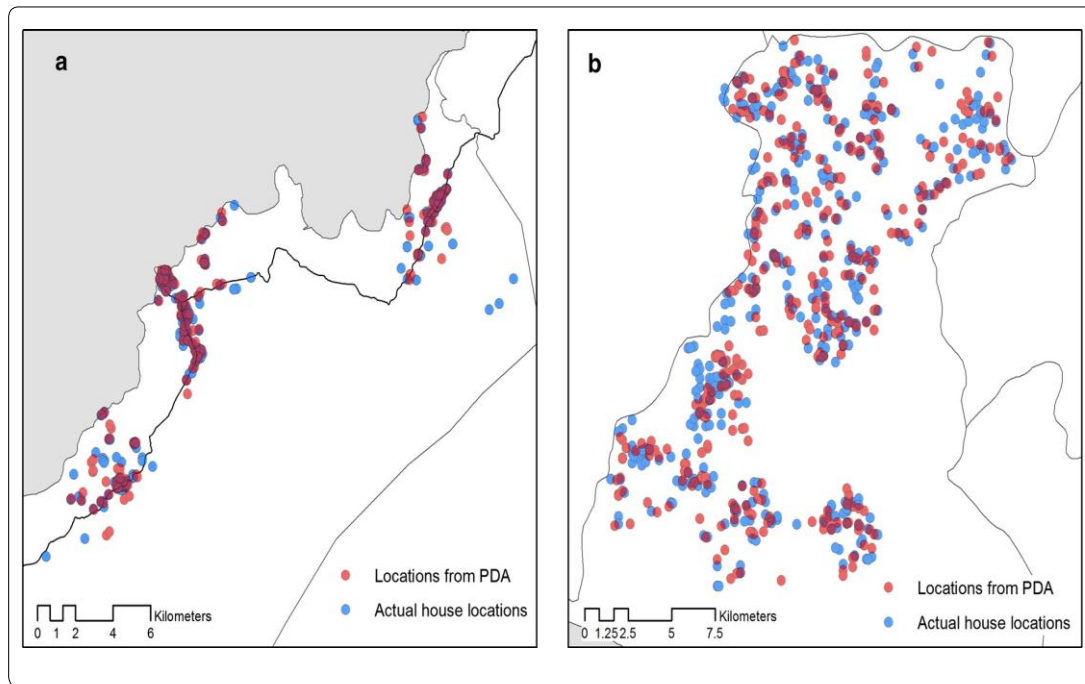


Figure 4.3 Comparison between house locations collected by tablet and actual house locations in **a** Rizal, Palawan **b** Kulon Progo

To assess the variability in accuracy between different health facilities in the same site, we compared spatial accuracy in records collected at all 8 facilities in Kulon Progo Regency. The accuracy of the mapping exercise varied within the 8 health facilities, with the closest accuracy measured in Samigaluh 1 (RMSE 353 m), and the least accuracy found in Girmulyo 2 (RMSE 817 m). Moreover, the exercise was able to locate 50.3% (95% CI 45.3–55.2%) and 78.3% (95% CI 74.2–82.3%) of households within an accuracy of  $\leq 500$  and  $\leq 1000$  m, respectively. The highest proportion of households that were located within  $< 1000$  m were Samigaluh 1 (97.9%, 95% CI 93.8–100%), whilst the lowest proportion of households correctly located were Kokap 2 (53.1%, 95% CI 38.9–67.2). Of households that were not located within 1000 m ( $n = 86$ ), 40.7% were in Kokap district, 31.4% in Girmulyo, 11.6% in Samigaluh and 8.1% in Kalibawang and 8.1% in Pengasih district. While accuracy of GPS points was not significantly correlated with distance

from the health facilities for the Regency overall ( $p$  value = 0.98), distance from the health facility was associated with decreased accuracy in the catchment area of Kokap 2 ( $p$  value = 0.003) (Figure 4.5). This area is heavily forested and less densely populated, with very limited landmarks. Data suggests reported household locations in Kokap 2 were more accurate if they lived closer to the health facility or in close proximity to other landmarks such as mosques, schools or shops that were available on the map. In addition, the accuracy was higher in more populated health facility catchment areas where more landmarks were available.

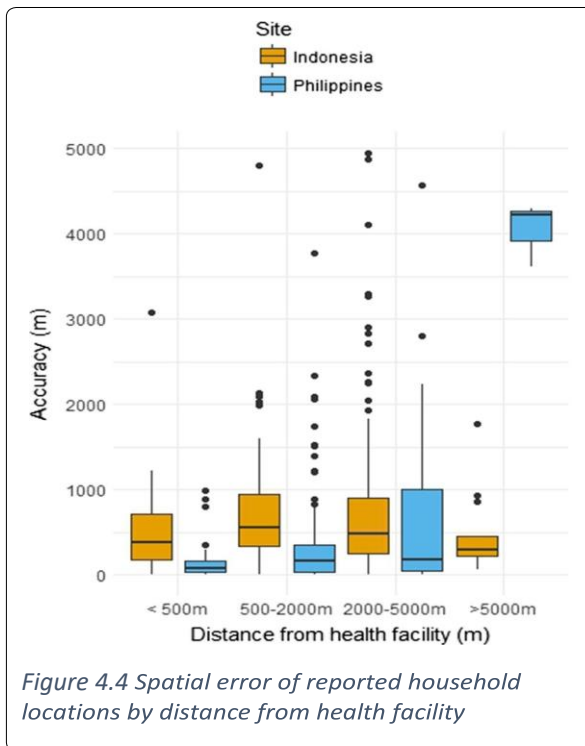
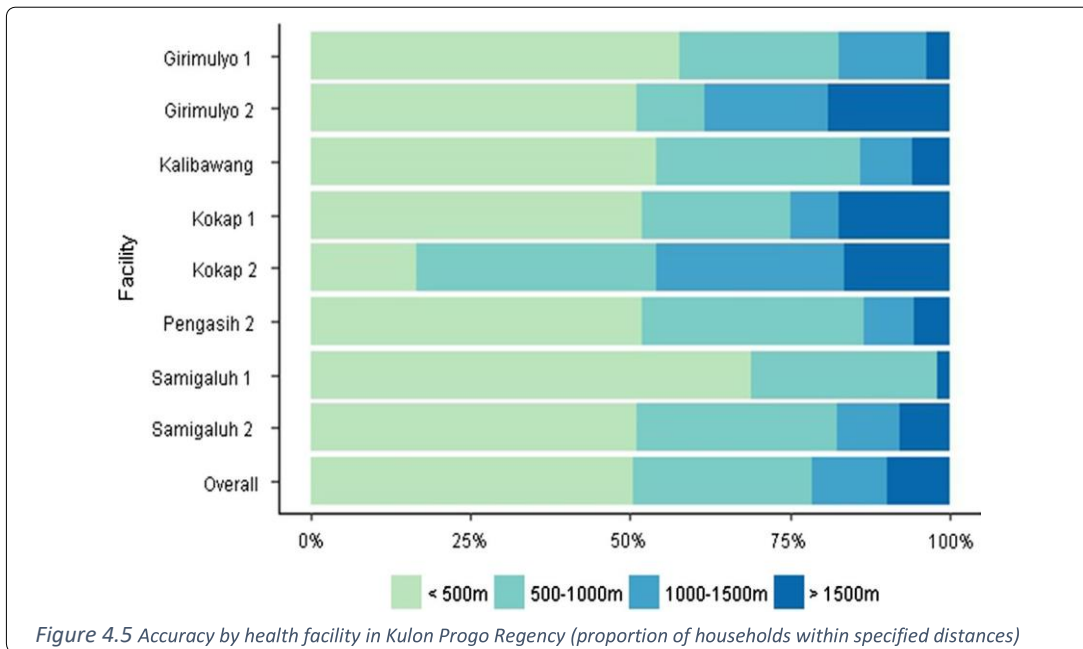


Figure 4.4 Spatial error of reported household locations by distance from health facility



#### 4.5 Conclusions

Tablet-based applications are an effective method of geolocating participant households when it is not feasible to visit individual households. While numerous software platforms are available, selection should be based on the setting and resources available. Field testing of this software in the Philippines and Indonesia suggests data collection is sufficiently accurate to identify most households and would be appropriate for monitoring fine-scale spatial patterns of disease. Implementing this strategy could extend health facility capacity to remotely collect spatial information and monitor areas where infections are most regularly occurring. The rapid assessment of spatial representation of the population and any foci of infection or exposure can prevent spread of disease and support health programs to better target disease control and elimination activities.

The choice of software and spatial data to include should be guided by availability of data, technical expertise, required data resolution and resources. For sites with a stable internet connection and good coverage by Google Earth or other free imagery, software can be used with free online imagery. If no internet coverage is available, software such as GeoODK, ArcGIS Survey123 and ePAL can be used to incorporate offline maps. Licenced software such as ArcGIS Survey123 provides good access to high resolution imagery, technical support and requires only basic GIS knowledge to set up; however, this software requires the purchase of licences and may require additional costs. Alternatively, if other spatial data is available, open source software, such as GeoODK, or applications designed to interface with open source software, such as ePAL, can be used to include custom designed maps. This involves limited to no software costs but requires more technical expertise and may require additional costs for purchase of satellite imagery. The types of data to be collected, such as spatial points, line or polygon data, barcodes or images, should also be evaluated. For example, programmes with a sampling unit at an individual level is likely to require higher resolution point household locations while programmes targeting larger administrative units may require lower resolution polygon data. An additional consideration is whether geo-location data collection software will need to integrate with other data management systems, such as larger national health data management systems. As technology continues to develop, the functionalities of these programs as well as additional new software applications may continue to expand.

The geolocation strategy tested in our study offers an alternative approach for obtaining spatial information from health-facility attendees in a setting that is typical for much of rural Southeast Asia and other parts of the world. The accuracy of the strategy in this setting improved in areas where more landmarks were available. This method could also be employed with other EAG

(Easy Access Group) surveys such as school-based surveys that have been reportedly able to identify geographical variation in malaria transmission in different settings [13, 29– 31]. Moreover, the GIS data collected in this study can be incorporated into a database that enables the display of information in the form of a basic map to enable reactive surveillance and other public health activities. In addition, this data should be linked to other environmental and spatial data so statistical analysis can identify associations between disease and environmental factors [4]. This can facilitate the identification of transmission hotspots are occurring and be used to target interventions [2].

Tablet-based geolocation strategies provide an important method of collecting spatial data in low resource settings when it is not feasible to visit patient households to directly collect GPS data and no formal address system is available. We have applied this approach in two settings in Southeast Asia, this approach is also being utilised in the Caribbean and African settings for both malaria and tuberculosis and therefore is applicable globally. While further research is needed to investigate the utility and feasibility of this method in a range of settings before implementing in a broader operational context, this study highlights the tools available and how these may be employed in low resource settings.

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### **Competing interests**

The authors declare that they have no competing interests.

### **Availability of data and materials**

The datasets used and analysed during this study are not publicly available due to the inclusion of identifying information on individuals but are available from the corresponding author on reasonable request and approval from relevant ethics committees.

### **Ethics approval and consent to participate**

This study was approved by the Institutional Review Board of the Research Institute for Tropical Medicine, Philippines, the Medical Health Research Ethics Committee, Gadjah Mada University, Indonesia (KE/FK/0290/EC/2017) and the Research Ethics Committee of the London School of Hygiene and Tropical Medicine (11597 and 11944). Written informed consent was obtained from all participants in this study.

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## 4.7 Supplementary information

**Table SI1:** Example questionnaire and associated data types

Field ID	Question	Data type
date_consultation	1) Date of Consultation:	Date/ time
barcode	2) Participant's Barcode:	Barcode reader
<b>I. TYPE AND LOCATION OF HEALTH FACILITY</b>		
type_facility	1) Type of health facility: <i>(Select one)</i>	Field-list
oth_facility	1-a) Other health facility: <i>(Select one)</i>	String
province	2) Province: <i>(Select one)</i>	Field-list
municipality	3) Municipality: <i>(Select one)</i>	Field-list
barangay	4) Barangay: <i>(Select one)</i>	Field-list
sitio	5) Sitio: <i>(Select one)</i>	Field-list
other_sitio	5-a) Other Sitio:	String
<b>II. PARTICIPANT'S PROFILE</b>		
participant_select	1) Patient or Companion of Patient? <i>(Select one)</i>	Field-list
surname_name	2) Last Name:	String
firstname_name	First Name:	String
middlename_name	Middle Initial:	String
suffix	Name Suffix, if any <i>(Jr. Sr., I, II, III, etc.)</i> :	String
gender	3) Gender: <i>(Select one)</i>	Field-list
age	4) Age	
age_year	4-a) Year:	Integer
age_months	4-b) Months:	Integer
contact_number	5) Contact Number: <i>(Optional)</i>	String
ethnicity	6) Ethnicity: <i>(Select one)</i>	Field-list
oth_ethnicity	6-a) Other name of Ethnic Group:	String
education	7) Educational Attainment <i>(Select one)</i> :	Field-list
occupation	8) Current occupation (by industry): - Select all that apply	Field-list
oth_occupation	8-a) Other occupation	String
<b>III. RESIDENCE AND GPS COORDINATES</b>		
stay_length	1) How long have you been living at your present address? <i>(Select one)</i>	Field-list
barangay_2	1-a) Barangay: (Answer without abbreviating)	Field-list
sitio_2	1-b) Sitio: <i>(Answer without abbreviating)</i>	String
gps_tag	1-c) Has the Location of Primary Residence been identified?	Yes/ No
residence_primary	1-d) Location of Primary Residence:	GPS coordinates
other_residence	2) Do you have any other residence?	Yes/ No
visited_secondary	2-a) If YES, Have you visited your other residence these past 4 weeks?	Yes/ No
barangay_3	2-b) Barangay: (Answer without abbreviating)	Field-list

sitio_3	2-c) Sitio: <i>(Answer without abbreviating)</i>	String
residence_secondary	2-d) Location of Secondary Residence:	GPS coordinates
IV. TRAVEL, AND ACTIVITIES		
travel	1) Have you traveled anywhere in the past 4 weeks?	Yes/ No
places_visited1	1-a) If YES, what places have you visited?	Field-list
places_visited2	Other places:	String
places_visited3	Other places:	String
activities1	2) What activities did you do these past 2 weeks?	String
activities2	Other activities:	String
activities3	Other activities:	String
V. BEDNET OWNERSHIP		
bednet_use	1) Do you own a bed net?	Yes/ No
bednet_ins	1-a) If YES, was the bednet treated with insecticide?	Yes/ No
bednet_use	1-b) If YES, do you use the bednet?	Yes/ No
VI. HEALTH INFORMATION		
temp_fever	1) What is your temperature on the day of consultation? <i>(Select one)</i>	Field-list
axillary_temperature	1-a) After getting you axillary temperature, does it indicate of having a fever?	Yes/ No
fever_length	1-b) If <b>YES</b> , how many day/s has it been since the onset of your fever? <i>(Select one)</i>	Field-list
fever_length_meds	1-c) Did you take any medicine for your fever?	Field-list
fever_drugs	1-d) If YES, what fever medicine did you take?	Field-list
oth_fdrugs	Other fever medicine:	Field-list
oth_symptoms_select	2) Are you experiencing any other symptom?	Yes/ No
symptoms_select	2-a) If YES, what other symptoms are you experiencing? <i>(Select all that apply)</i>	Field-list
symptoms_select_other1	Other symptoms:	String
symptoms_select_other2	Other symptoms:	String
symptoms_select_other3	Other symptoms:	String
symptoms_length	2-b) How long have you had these symptoms? <i>(Select one)</i>	Field-list
VII. DIAGNOSTIC TESTS		
filter_paper	1) Sample specimen for Filter Paper collected?	Yes/ No
bsmp	2) Sample specimen for Blood Film collected?	Yes/ No
rdt	3) Was Malaria Rapid Diagnostic Test (RDT) performed?	Yes/ No
VIII. RAPID DIAGNOSTIC TEST RESULT		
blood_examined	1) Date of Blood Examined:	Date/ time
rtd_type	2) Type of RDT Kit used:	Field-list
rdt_result	3) RDT Result:	Field-list
rdt_positive	3-a) Type of Malaria Infection:	Field-list
image_RDT	3-b) Take a picture of the RDT kit result.	Image
rdt_treatment	4) Treatment for Malaria-infected individual:	field-list
referred	5) Reason for referral:	field-list

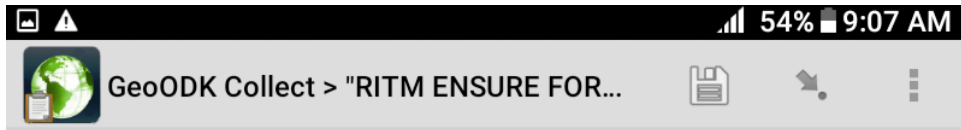


Figure SI1: Screenshots of GeoODK questionnaire on Android tablet

a. Offline map loaded on GeoODK questionnaire



- b. GPS coordinates collected using offline map



III. RESIDENCE AND GPS COORDINATES

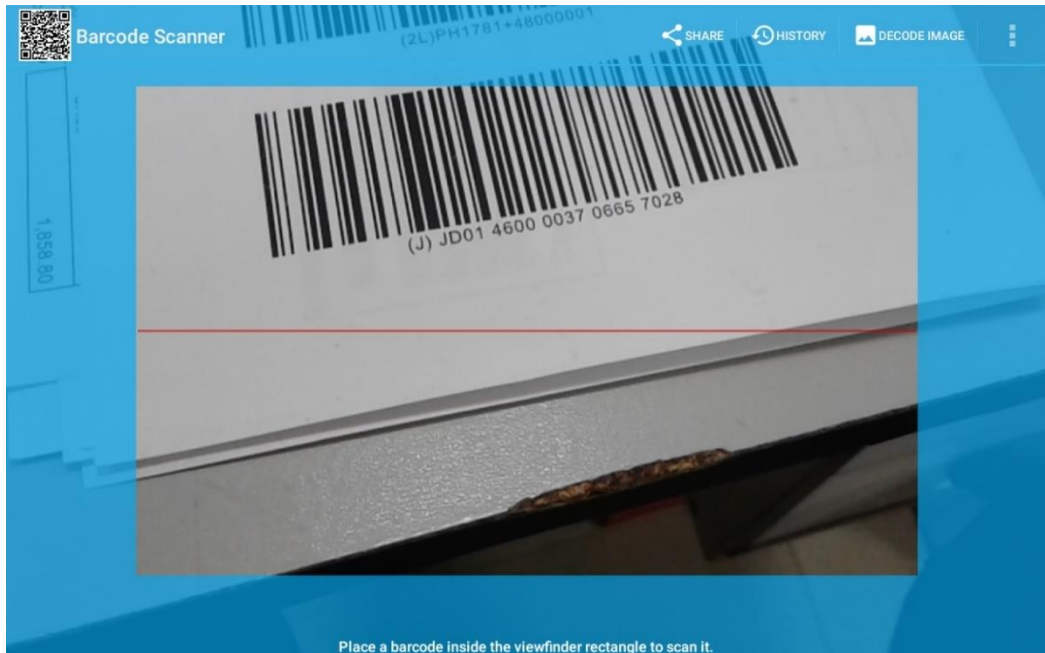
1-d) Location of Primary Residence:

*GPS coordinates can only be collected when outside.*

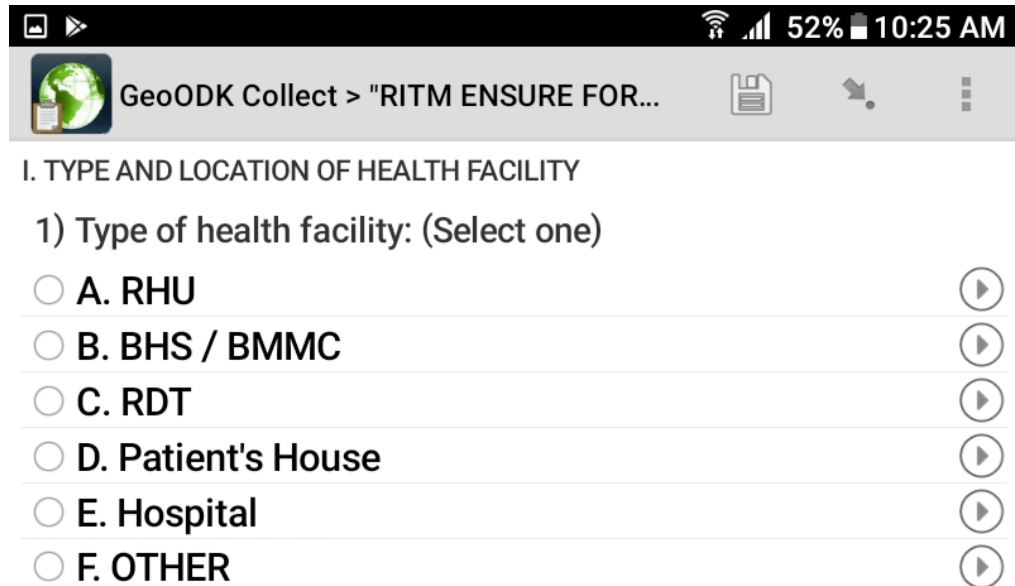
**View or Change Location**

8.927575 117.559719 0.0 0.0

- c. Barcode scanner used to scan sample barcode labels



d. Example of collection of multiple choice questionnaire data



I. TYPE AND LOCATION OF HEALTH FACILITY

1) Type of health facility: (Select one)

A. RHU

B. BHS / BMMC

C. RDT

D. Patient's House

E. Hospital

F. OTHER

## 5. Using health facility-based serological surveillance to predict receptive areas at risk of malaria outbreaks in elimination areas

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Student	Henry Surendra
Principal Supervisor	Chris Drakeley
Thesis Title	Operational applications of serology for malaria surveillance in different transmission settings in Indonesia

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Student Signature: \_\_\_\_\_

Date: 30/01/2020

Supervisor Signature: \_\_\_\_\_

Date: 30/01/2020

**Using health facility-based serological surveillance to predict receptive areas at risk of malaria outbreaks in elimination areas**

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## 5.1 Abstract

**Background:** In order to improve malaria burden estimates in low transmission settings more sensitive tools and efficient sampling strategies are required. This study evaluated the use of serological measures from repeated health facility-based cross-sectional surveys to investigate *P. falciparum* and *P. vivax* transmission dynamics in an area nearing elimination in Indonesia.

**Methods:** Quarterly surveys were conducted in 8 public health facilities in Kulon Progo District, Indonesia, May 2017 to April 2018. Demographic data were collected from all clinic patients and their companions, with household coordinates collected using participatory mapping methods. In addition to standard microscopy tests, bead-based serological assays were performed on finger-prick bloodspot samples from 9453 people. Seroconversion rates (SCR, i.e. the proportion of people in the population who are expected to seroconvert per year) were estimated by fitting a simple reversible catalytic model to seroprevalence data. Mixed effects logistic regression was used to examine factors associated with malaria exposure and spatial analysis was performed to identify areas with clustering of high antibody responses.

**Results:** Parasite prevalence by microscopy was extremely low (0.06% (95% confidence interval: 0.03-0.14, n=6) and 0 for *P. vivax* and *P. falciparum*, respectively). However, spatial analysis of *P. vivax* antibody responses identified high-risk areas that were subsequently the site of a *P. vivax* outbreak in August 2017 (62 cases detected through passive and reactive detection systems). These areas overlapped with *P. falciparum* high-risk areas and were detected in each survey. General low transmission was confirmed by the SCR estimated from a pool of the four surveys in people aged 15 years old and under (0.020 (95% confidence interval: 0.017–0.024) and 0.005 (95% confidence interval: 0.003–0.008) for *P. vivax* and *P. falciparum*, respectively). The SCR

estimates in those over 15 years old was 0.066 (95% confidence interval: 0.041-0.105) and 0.032 (95% confidence interval: 0.015-0.069) for *P. vivax* and *P. falciparum*, respectively.

**Conclusions:** These findings demonstrate the potential use of health facility-based serological surveillance to better identify and target areas still receptive to malaria in an elimination setting. Further implementation research is needed to enable integration of these methods with existing surveillance systems.

**Keywords:** serology, surveillance, mapping, malaria, elimination

## 5.2 Background

Transforming malaria surveillance into a core intervention is one of the three pillars of the WHO global technical strategy for malaria elimination [1]. As transmission declines, malaria risk becomes more heterogeneous and is often clustered in specific localities or populations [2,3]. Identifying areas of ongoing infection or areas at risk of outbreaks is important to ensure that control strategies can be deployed in the most efficient manner [4–6]. In many South East Asian settings, surveillance becomes more challenging with the presence of multi-species infections combined with the difficulty of identifying where, and in which populations, residual transmission might be occurring [7,8].

In many countries, surveillance has focused on passive case detection performed via health facilities [9,10]. However, innovative additional strategies are needed in countries nearing elimination as malaria cases become increasingly rare and disproportionately affect high-risk populations, who may not utilise public health facilities [10]. Studies suggest that passive surveillance will miss a large proportion of asymptomatic and sub-microscopic infections

present in the community [8,11,12] and may also not optimally capture imported infections occurring in temporary visitors who may be unable or unlikely to visit a health facility. Effectively targeting both of these groups is likely to hasten progress toward elimination.

Resurgence of malaria is often associated with imported infections and/or *P. vivax* relapsing infections in areas that remain highly receptive to malaria [13–16]. Studies have demonstrated the usefulness of spatially referenced entomological data to characterise the heterogeneity of malaria receptivity in areas approaching elimination to prevent outbreaks in the future [17–19]. However, entomological surveillance can often be logistically challenging in low transmission areas due to the difficulty of catching meaningful numbers of mosquitoes. An alternative approach is to identify areas where the population show evidence of current or previously high malaria exposure. This can be done using serological markers of infection and identifying populations with higher than average anti-malaria antibodies [20–23]. Serological measures are a sensitive tool to estimate current and previous transmission intensity in a population and their use has been particularly well validated in low transmission areas where the sensitivity of parasitological tools is inadequate [24–27]. However, these studies used community-based cross-sectional surveys that often require large resources to visit households for collecting samples and household global positioning system coordinates to map the transmission risk. In order to further reduce logistical constraints, convenience sampling approaches targeting health facility attendees can be used to estimate and map risks in a population when household surveys are not feasible [28] and has been shown to be a good proxy for malaria transmission in the community [29]. Moreover, the simple addition of a geolocation approach to remotely record the residence of health facility attendees in the survey [30] allows for rapid assessment of the



micro-epidemiology of malaria cases in the community and could help to identify geographical foci of exposure.

Indonesia is one of countries facing challenges in eliminating both *P. falciparum* and *P. vivax* infections. Previous studies in Indonesia suggest that the current diagnostic sensitivity (microscopy and rapid diagnostic test (RDT)) and timeliness of transmission measurement are not sufficient to describe and predict decreasing numbers of cases and potential outbreaks in low transmission areas striving for elimination [31–33]. The risk of outbreaks is high where there are larger numbers of migrants or travellers [31,34–36] and/or where residents with asymptomatic infections are not actively seeking treatment for malaria [37–45]. Therefore, surveillance systems need to be improved to better locate and target infections and further reduce transmission [32,46]. This study evaluated the use of serology, geolocation tools, and repeated health facility-based surveys for capturing malaria transmission dynamics in conjunction with existing surveillance system in an area conducting elimination in Indonesia.

### **5.3 Methods**

#### **Study setting**

Indonesia has the second highest burden of malaria in the South-East Asia region, with an estimated 16 million people (~6% of the population) living in high-risk areas [47]. All species of *Plasmodium* have been reported in Indonesia with the majority of infections caused by *P. falciparum* and *P. vivax* [35,48–51]. Malaria transmission is highly heterogenous [52,53], with large areas being transmission free, leading to a governmental target of achieving malaria elimination across the country by 2030 [46]. This study was conducted in Kulon Progo District,

Yogyakarta Province, Indonesia, located on the south coast of Java Island. Kulon Progo is one of the few remaining foci of malaria transmission on Java Island, Indonesia (Figure 5.1). The study site consists of 12 sub-districts (586 km<sup>2</sup> in total) with a population of approximately 430,500 people in 2016. Each district has at least one public health facility (21 in total). Malaria transmission is concentrated in the forested hillside area that border with other endemic areas of Central Java Province [54]. Transmission occurs during the wet season between August to December, with very low or zero cases during the other months. Based on routine passive data recorded in local health facilities, there was a significant decline in malaria annual parasite incidence from 0.48 per 1000 population in 2012 to 0.22 per 1000 population in 2016. Eight health facilities in 5 sub-districts where *P. falciparum* and/or *P. vivax* transmission was ongoing were chosen as study sites. *An. maculatus* and *An. balabacensis* are the main malaria vectors in Kulon Progo [55].

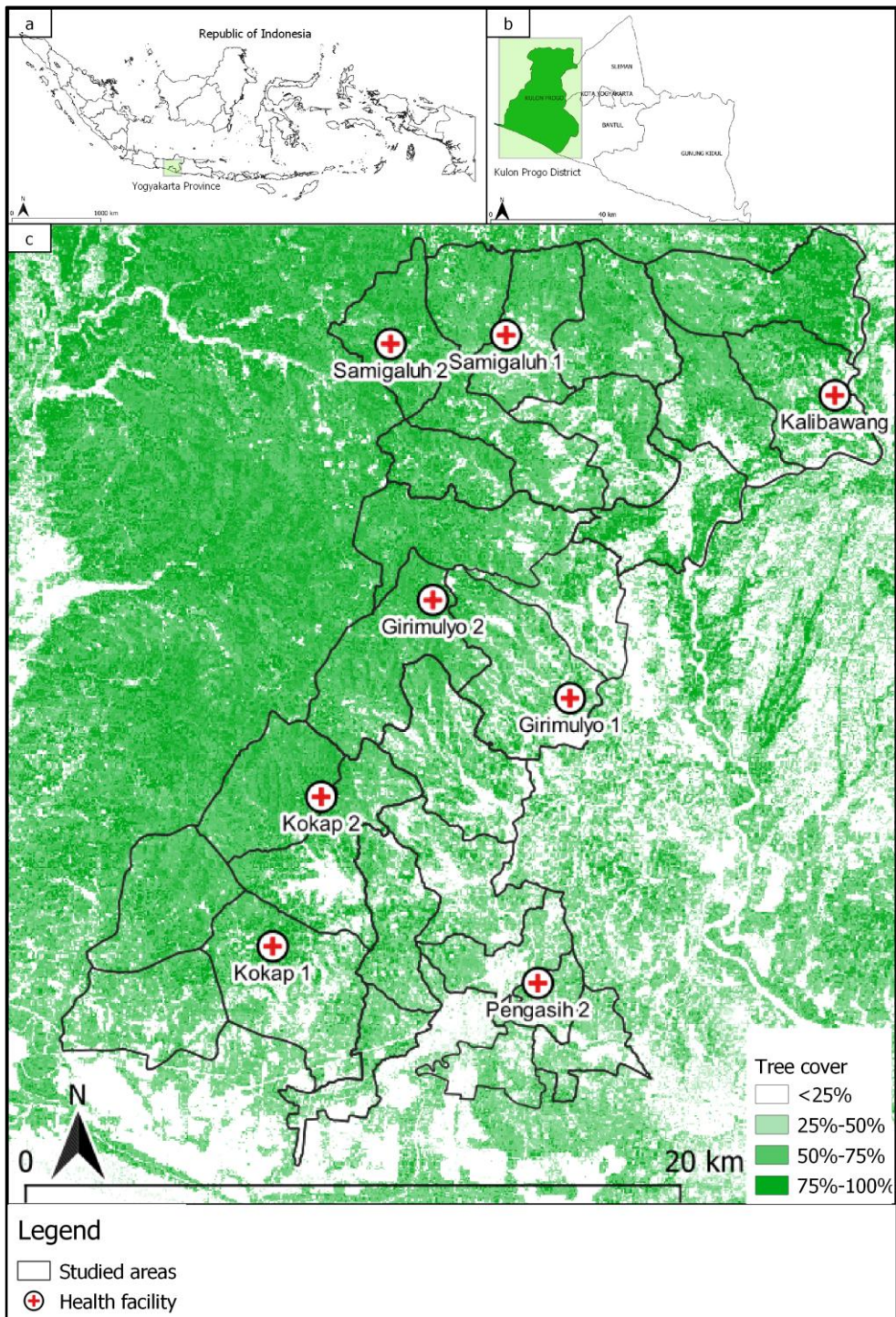


Figure 5.1 Maps showing the location of Yogyakarta Province in Indonesia (a), location of Kulon Progo District in Yogyakarta Province (b), and the location of 8 studied health facilities in Kulon Progo District (c). Tree cover data, derived from classified Landsat imagery at 30 metres resolution, were obtained from Hansen et al. [56]

## Survey design and data collection

The study population included all attendees of the 8 selected public health facilities. Surveys were conducted quarterly during the period of May 2017 to April 2018. Each survey continued until the minimum sample size was met. The sample size calculation was performed using methods specific for estimating antibody seroconversion rates (SCR, i.e. the proportion of people in the population who are expected to seroconvert per year) [56]. The SCR to either *P. falciparum* apical membrane antigen 1 (PfAMA1) or merozoite surface protein 1 (PfMSP-1<sub>-19</sub>) in Kulon Progo was expected to be lower than the SCR reported in the neighbouring pre-elimination setting, Purworejo District, Indonesia (SCR 0.019 (95% CI: 0.015-0.022)). Therefore, a minimum sample size of 248 individuals per facility was set to ensure an antibody SCR of 0.0036 could be estimated with a precision level of +/- 0.0018.

Finger prick blood samples were collected as dried bloodspots together with thick and thin blood smears from all consenting participants attending the facilities. Patients who were very ill and required urgent care, and children <6 months of age were excluded. Data on age, gender, axillary temperature, patient (versus accompanying person) status, permanent residence, travel behaviour, occupation, bed net use and current symptoms or reasons for attending the clinic were collected. Fever status was defined as having axillary temperature >37.5 °C and/or reported having fever in the previous 24 hours of sample collection. Participants were asked to geolocate their household using high-resolution digital offline maps via the open source GeoODK. The validation of this mapping approach was performed at the beginning of our first survey and has been reported in Fornace et al. [30]. All data were collected via interview using open data kit [57] on tablets (Samsung Galaxy Tab 3 SM-T210). Demographic data on reported cases, surveillance (passive and reactive case findings) and control programme activities were collected

from the District Health Office of Kulon Progo in between every serological survey. Data on the age distribution of the population in the study area was obtained from the 2016 census published by the Central Agency on Statistics of Kulon Progo. Tree cover data, derived from classified Landsat imagery at 30 meter resolution, were obtained from Hansen et al. [58].

### **Laboratory methods**

Thick and thin blood smears were read by trained health facility lab technicians at each facility. Bloodspot samples were tested against a panel of *P. falciparum* and *P. vivax* antigens including apical membrane antigen 1 (PfAMA1; PvAMA-1), merozoite surface protein 1 (PfMSP-1<sub>-19</sub>; PvMSP-1<sub>-19</sub>), erythrocyte binding protein (PvEBP), reticulocyte binding protein 1a [amino acids 160–1170] (PvRBP1a) and reticulocyte binding protein 2b [amino acids 161–1454] (PvRBP2b) using a bead-based assay as described by Wu et al. [59] and read using Luminex MAGPIX<sup>®</sup> (Luminex Corp, Austin, TX). For serological data analysis, infants under 1 year of age were excluded from each dataset to remove any influence of maternally derived antibodies [60]. Antibody responses measured as median fluorescence intensity (MFI) values were normalised against the MFI values of the positive control run on each plate. For each plate, the percentage of plate-to-reference standard MFI difference was calculated and used to adjust the median MFI values.

### **Statistical analysis**

All statistical analyses were conducted in Stata IC 15 (Stata Corp, College Station, TX, USA). A cut off for seropositivity was determined based on finite mixture models according to the mean of log MFI values plus three standard deviation of the seronegative population. Separate cut off

values were generated for each antigen [61]. Individuals were categorised as seropositive for each species if their antibody responses were above the cut-off for either of the two or five antigens for *P. falciparum* and *P. vivax*, respectively. SCR were estimated by fitting a reverse catalytic model to seroprevalence data for each species [60]. Models allowing two forces of infection in SCR were fitted if deemed a better fit, using likelihood ratio methods. Mixed effects logistic regression models were performed to examine risk factors associated with being seropositive to *P. vivax*. Variables with evidence of an association ( $p < 0.05$ ) in bivariate analysis were included in a multivariable model. Health facility was treated as a random effect variable in both bivariate and multivariable models.

### **Spatial analysis**

The 'Normal model' in the spatial software SaTScan (v.9.4.2) was used to detect clusters of individuals with higher than average age-adjusted antibody responses to each antigen per survey. In order to obtain age-adjusted values, the MFI data were log<sub>10</sub> transformed and the residuals from linear regression were used to determine whether antibody responses were higher or lower than expected for any given age assuming a homogeneous distribution of risk across age. Firstly, residuals were categorised into 4 categories i.e. below 25<sup>th</sup> percentile, 25<sup>th</sup>–75<sup>th</sup> percentile, 75<sup>th</sup>–90<sup>th</sup> percentile and above 90<sup>th</sup> percentile for each antigen. Individuals were then assigned score 4 (highest) if they had residual values above the 90<sup>th</sup> percentile, 3 (higher than average) for 75-90<sup>th</sup> percentile, 2 (average) for 25-75<sup>th</sup> percentile and 1 (low) for residual below the 25<sup>th</sup> percentile to any of the two or five antigens for *P. falciparum* or *P. vivax* antigen, respectively. The residual scores were then used to calculate non-overlapping, statistically significant ( $p < 0.05$ ) clusters of higher than average age-adjusted antibody responses with a

maximum radius of 3 km, minimum 2 observations detected in a cluster using the Purely Spatial scan. The analysis was run separately for each survey to ascertain spatial pattern at each survey time point. Clusters identified from SatScan were then plotted in QGIS software (v.3.6.3) to identify the potentially receptive areas. Spatial autocorrelation for each survey time point was assessed using Moran's I in ArcGIS (v.10.5) using the age-adjusted antibody residuals from the regression model.

## **5.4 Results**

### **Study enrolment and population demographics**

A total of 9453 individuals were sampled during four repeated cross-sectional surveys performed in 8 health facilities in Kulon Progo District, Yogyakarta Province, Indonesia during the period of May 2017 to April 2018 (Table 5.1). Blood smears and dried bloodspot samples were collected from >98% of attendees and their companions. Participation rates were above 90% for all surveys, ranging from 82 to 100% across facilities. Study participants were mostly female (65%), the median age was 42 years old (IQR: 27–55), and the majority attended the facilities as patients (78.6%). Children were underrepresented in the sample, in comparison to the general population. Approximately 30% of the study population were forest workers involved in coconut/palm tapping, fruit farming, logging, and other related jobs. 42% of the study population reported having at least one bed net in their house, resulting in overall usage of 27% in the study population. Only 16% of the population reported recent travel; with the highest proportion of travel recorded during quarter 1 and 2 (May to October 2017). Approximately 5% of the study population were febrile or reported having fever in the previous 24 hours.

**Table 5.1 Number of samples, participation rates and general characteristics of health facility attendees per survey**

	Quarter 1 (May-July)	Quarter 2 (August-October)	Quarter 3 (November-January)	Quarter 4 (February-April)	Total
Sample size, n	2363	2370	2379	2341	9453
n per facility					
Kokap 1	299	300	286	300	1185
Kokap 2	298	298	297	301	1194
Samigaluh 1	298	300	298	299	1195
Samigaluh 2	300	297	300	280	1177
Kalibawang	296	300	298	263	1157
Girimulyo 1	285	299	300	299	1183
Girimulyo 2	300	276	300	299	1175
Pengasih 2	287	300	300	300	1187
Participation rates					
Mean %	95	96	91	96	94
Range*	91–99	90–99	82–99	90–100	91–96
Female, n (%)	1578 (66.8)	1527 (64.4)	1530 (64.3)	1502 (64.2)	6137 (64.9)
Age, median (IQR)	40 (25–54)	41 (27–54)	42 (27–55)	43 (30–57)	42 (27–55)
Patients, n (%)	1803 (76.3)	1939 (81.8)	1878 (78.9)	1812 (77.4)	7432 (78.6)
Occupation, n (%)					
Forest workers	655 (27.7)	709 (29.9)	620 (26.1)	800 (34.2)	2784 (29.5)
Non-forest workers	685 (29.0)	647 (27.3)	738 (31.0)	678 (29.0)	2748 (29.1)
Not working	1023 (43.3)	1014 (42.3)	1021 (42.9)	859 (36.8)	3917 (41.5)
Lives in a house with bed net, n (%)	1091 (46.2)	1132 (47.8)	999 (42.0)	777 (33.3)	3999 (42.3)
Slept under the bed net, n (%)	710 (30.1)	685 (28.9)	666 (28.0)	527 (22.5)	2588 (27.4)
Recent travel, n (%)	595 (25.2)	581 (24.6)	211 (8.9)	111 (4.7)	1498 (15.9)
Fever, n (%)	127 (5.4)	116 (5.0)	146 (6.1)	93 (4.0)	484 (5.2)

\* Range of health facility level summaries

### Data captured by routine passive surveillance during the study period

The routine passive and reactive case detection in the study area detected 72 *P. vivax* and 8 *P. falciparum* microscopy positive infections out of 15,067 slides read in 2017, with the majority of infections found in males (70.2%) and adults over 15 years old (89.0%). All *P. falciparum*



infections were classified as imported. The majority of the *P. vivax* infections (86.1%, n=62) were found in Kokap 1 health facility catchment area in quarter 2 (74%, n=46). Of all of the infections detected, 39% (n=24) were detected passively at the health facility, with the rest being detected via door to door active case detection performed by the village malaria workers (i.e. screening of suspected cases based on clinical signs). The *P. vivax* cases found through active case detection in Kokap 1 area were classified as a malaria outbreak by local authorities as there had been no indigenous case reported in the area since 2016, with only 2 *P. vivax* relapsed cases reported in July 2017.

#### **Health-facility based serological surveillance**

Few microscopy positive infections were detected; 6/9356 (0.06%, 95% CI: 0.03-0.14) for *P. vivax* and no *P. falciparum* positive individuals were identified. All infections were found in Kokap 1 health facility, with 5 infections detected in quarter 2 and 1 in quarter 4. Of these infections, 1 was from a companion, and 5 were from patients not suspected of having malaria. Most of the infections were asymptomatic (66.7%) (i.e. afebrile). Seroprevalence to *P. vivax* antigens was higher than seroprevalence to *P. falciparum* antigens in all surveys (Table 5.2). As expected, the seroprevalence increased with age for both species and varied between health facilities and over time. The highest overall seroprevalence was found in quarter 2 (August to October 2017), 46.3% (95% CI: 44.2-48.3) and 23.9% (95% CI: 22.2-25.7) for *P. vivax* and *P. falciparum*, respectively with similar patterns observed according to proportion of higher than average age-adjusted antibody responses to multiple antigens (Figure 5.2).

**Table 5.2 Seroprevalence to *P. vivax* and *P. falciparum* at quarterly surveys**

	Quarter 1 (May-July)		Quarter 2 (August-October)		Quarter 3 (November-January)		Quarter 4 (February-April)	
	Number positive	Seroprevalence % (95% CI)	Number positive	Seroprevalence % (95% CI)	Number positive	Seroprevalence % (95% CI)	Number Positive	Seroprevalence % (95% CI)
<b><i>P. vivax</i></b>								
Age group								
1-15 years old	44	12.1 (8.0-17.8)	24	11.2 (7.6-16.2)	26	17.6 (12.2-24.6)	9	10.5 (5.5-18.9)
>15 years old	1014	41.0 (38.9-43.1)	1000	50.1 (47.9-52.3)	906	41.6 (39.6-43.7)	918	41.8 (39.8-43.9)
All ages	1058	38.8 (36.8-40.8)	1024	46.3 (44.2-48.3)	932	40.1 (38.2-42.1)	927	40.7 (38.7-42.7)
<b><i>P. falciparum</i></b>								
Age group								
1-15 years old	6	3.4 (1.6-7.5)	8	3.7 (1.9-7.3)	5	3.4 (1.4-7.9)	1	1.2 (0.2-7.8)
>15 years old	405	18.8 (17.3-20.6)	521	26.1 (24.2-28.1)	489	22.6 (20.9-24.4)	504	23.0 (21.3-24.8)
All ages	411	17.7 (16.2-19.3)	529	23.9 (22.2-25.7)	494	21.4 (19.8-23.1)	505	22.1 (20.5-23.9)

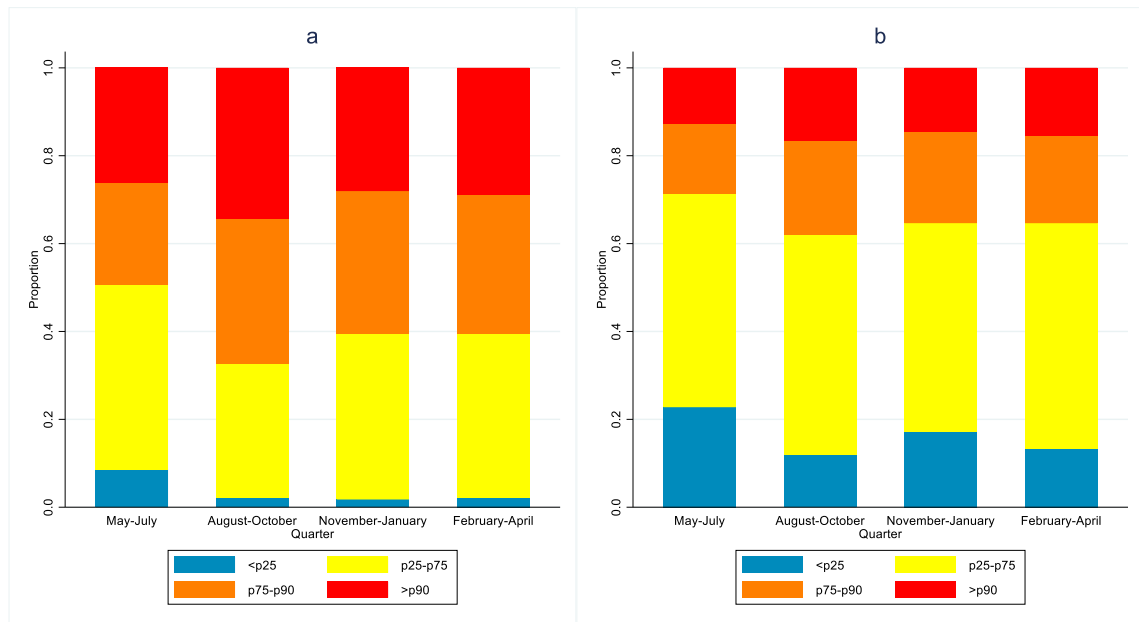


Figure 5.2 Proportion of level of age-adjusted antibody responses to multiple (a) *P. vivax* and (b) *P. falciparum* antigens by survey time. Blue represent proportion of individuals with the highest antibody below the 25<sup>th</sup> percentile, yellow 25<sup>th</sup>–75<sup>th</sup> percentile, orange 75<sup>th</sup>–90<sup>th</sup> percentile and red above 90<sup>th</sup> percentile.

### Transmission intensity and factor associated with transmission

Based on the population-level SCR values, and consistent with microscopy and routine reporting data, the transmission intensity was higher for *P. vivax* than *P. falciparum*. The SCR model estimates (Figure 5.3) suggested that there was evidence for two forces of infection. The *P. vivax* SCR was 0.020 person-year (95% CI: 0.017–0.024) and 0.066 person-year (95% CI: 0.041–0.105) for  $\leq 15$  and over 15 years old, respectively. The *P. falciparum* SCR was 0.005 person-year (95% CI: 0.003–0.008) and 0.032 person-year (95% CI: 0.015–0.069) for  $\leq 15$  and over 15 years old, respectively. At health facility-level, *P. vivax* SCR model estimates (Figure 5.4) showed evidence for two forces of infection only in two health facilities where active cases were identified. However, number of samples were low in the youngest age groups which may have influenced the fitting and estimates. Multivariable analysis found, gender, occupation, time of survey and bed net use were significantly associated with being *P. vivax* seropositive, after controlling for

other covariates factors (Table 5.3). The odds of being seropositive was higher in males (aOR 1.3, 95% CI: 1.2-1.5), forest goers (aOR 1.2, 95% CI: 1.0-1.3), those reporting sleeping under a bed net (aOR 1.2, 95% CI: 1.1-1.3) and during quarter 2 (aOR 1.5, 95% CI: 1.3-1.6).

**Table 5.3 Factors associated with *P. vivax* transmission in Kulon Progo District, Indonesia, 2018**

Variable	Total n (%)	<i>P. vivax</i> seropositive		Bivariate		Multivariable	
		n	% (95% CI)	OR <sup>a</sup> (95% CI)	<i>p</i>	aOR <sup>b</sup> (95% CI)	<i>p</i>
Age (years)							
≤15	623 (6.8)	80	12.9 (10.5-15.7)	1		1	
16-30	2108 (23.1)	750	35.6 (33.6-37.6)	4.6 (3.6-5.9)	0.000	5.1 (3.9-6.6)	0.000
31-45	2531 (27.7)	1115	44.1 (42.1-46.0)	6.5 (5.0-8.3)	0.000	6.6 (5.1-8.7)	0.000
>45	3880 (42.4)	1836	47.3 (45.8-48.9)	7.5 (5.9-9.7)	0.000	7.7 (5.9-10.0)	0.000
Gender							
Female	5945 (65.0)	2309	38.8 (37.6-40.1)	1		1	
Male	3206 (35.0)	1476	46.0 (44.3-47.8)	1.3 (1.2-1.4)	0.000	1.3 (1.2-1.5)	0.000
Status							
Accompanying	1960 (21.4)	895	45.7 (43.5-47.9)	1		1	
Patients	7191 (78.6)	2889	40.2 (39.0-41.3)	0.9 (0.8-1.0)	0.028		
Occupation							
Non-forest goers	2653 (29.0)	1023	38.6 (36.7-40.4)	1		1	
Forest goers	2685 (29.4)	1393	51.9 (50.0-53.8)	1.6 (1.4-1.8)	0.000	1.2 (1.0-1.3)	0.012
Unemployed	3810 (41.6)	1368	35.9 (34.4-37.4)	0.9 (0.8-1.0)	0.011	1.0 (0.9-1.1)	0.446
Survey time							
Quarter 1	2324 (25.4)	903	38.9 (36.9-40.9)	1		1	
Quarter 2	2217 (24.2)	1024	46.2 (44.1-48.3)	1.4 (1.2-1.5)	0.000	1.5 (1.3-1.6)	0.000
Quarter 3	2328 (25.4)	930	40.0 (38.0-42.0)	1.1 (0.9-1.2)	0.348	1.0 (0.9-1.2)	0.524
Quarter 4	2283 (24.9)	928	40.7 (38.6-42.7)	1.1 (1.0-1.2)	0.196	1.0 (0.9-1.2)	0.799
Bed net use							
No	6650 (72.7)	2556	38.4 (37.3-39.6)	1		1	
Yes	2502 (27.3)	1229	49.1 (47.2-51.1)	1.2 (1.1-1.3)	0.000	1.2 (1.1-1.3)	0.001
Fever							
No	8640 (94.9)	3604	41.7 (40.7-42.8)	1		1	
Yes	465 (5.1)	164	35.3 (31.1-39.7)	0.6 (0.5-0.8)	0.000		
Recent travel							
No	7681 (84.1)	3171	41.3 (39.3-44.4)	1		1	
Yes	1457 (15.9)	609	41.8 (40.2-42.4)	0.9 (0.8-1.1)	0.243		

**a** bivariate OR adjusted by correlation at health facility level

**b** multivariable OR adjusted by other covariates with bivariate *p* value < 0.05, and correlation at health facility level

Quarter 1: May-July 2017, Quarter 2: August-October 2017, Quarter 3: November 2017-January 2018, Quarter 4: February-April 2018

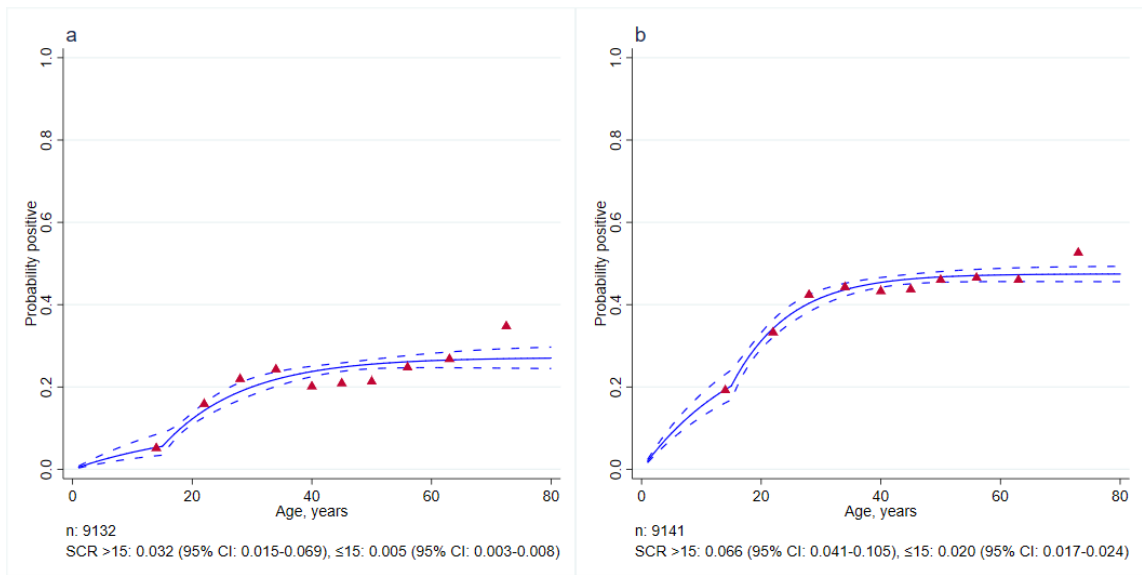


Figure 5.3 Age-seroprevalence plots for *P. falciparum* (a) and for *P. vivax* (b). Solid lines represent the fitted probability for being seropositive to either of the two or five antigens for *P. falciparum* and *P. vivax*, respectively. Dashed lines represent the 95% confidence interval of these fits and red triangles represent the observed proportion of seropositive per age decile. SCR value represent the average annual rate at which the population become seropositive to any of the *P. falciparum* or *P. vivax* antigen, respectively.

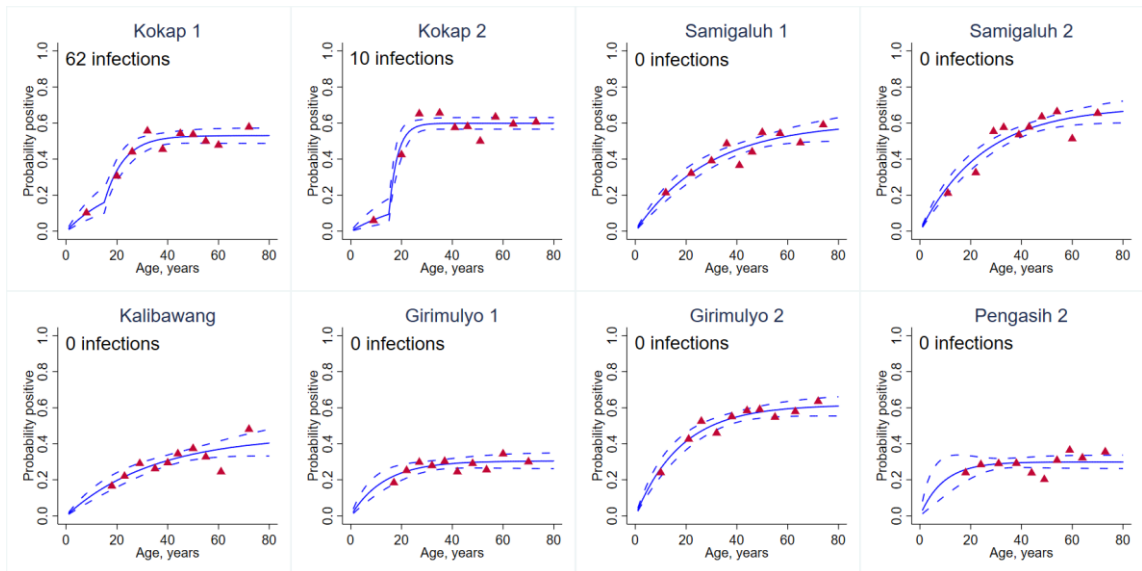


Figure 5.4 *P. vivax* age-seroprevalence plots and total number of *P. vivax* microscopy infections per health facility. Solid lines represent the fitted probability for being seropositive to either of the five *P. vivax* antigens. Dashed lines represent the 95% confidence interval of these fits and red triangles represent the observed proportion of seropositive per age decile.

### **Heterogeneity of transmission**

Moran's I suggested significant spatial autocorrelation for both species at each time point. The spatial analysis of higher than average age-adjusted antibody responses to multiple *P. vivax* antigens (Figure 5.5) identified the same village in the Kokap 1 catchment area prior to when the *P. vivax* outbreak occurred during the quarter 2 (outbreak started in early August 2017, in between the first and second survey). The analysis consistently identified significant clusters of *P. vivax* exposure in catchment areas of Kokap 1 and Kokap 2 in each survey. These catchments were areas where active infections were detected by the existing surveillance in quarter 1, 2 and 3, with no cases in quarter 4. Significant clusters were also identified in Samigaluh 2 in quarter 2 and 4, and in Girimulyo 2 in quarter 4. The same areas were also identified using *P. falciparum* antigens (Additional file 1). In addition, the spatial analysis suggest that the *P. vivax* clusters identified were also the place where the majority of fever cases were seen in quarter 2 when the outbreak occurred (Additional file 2).

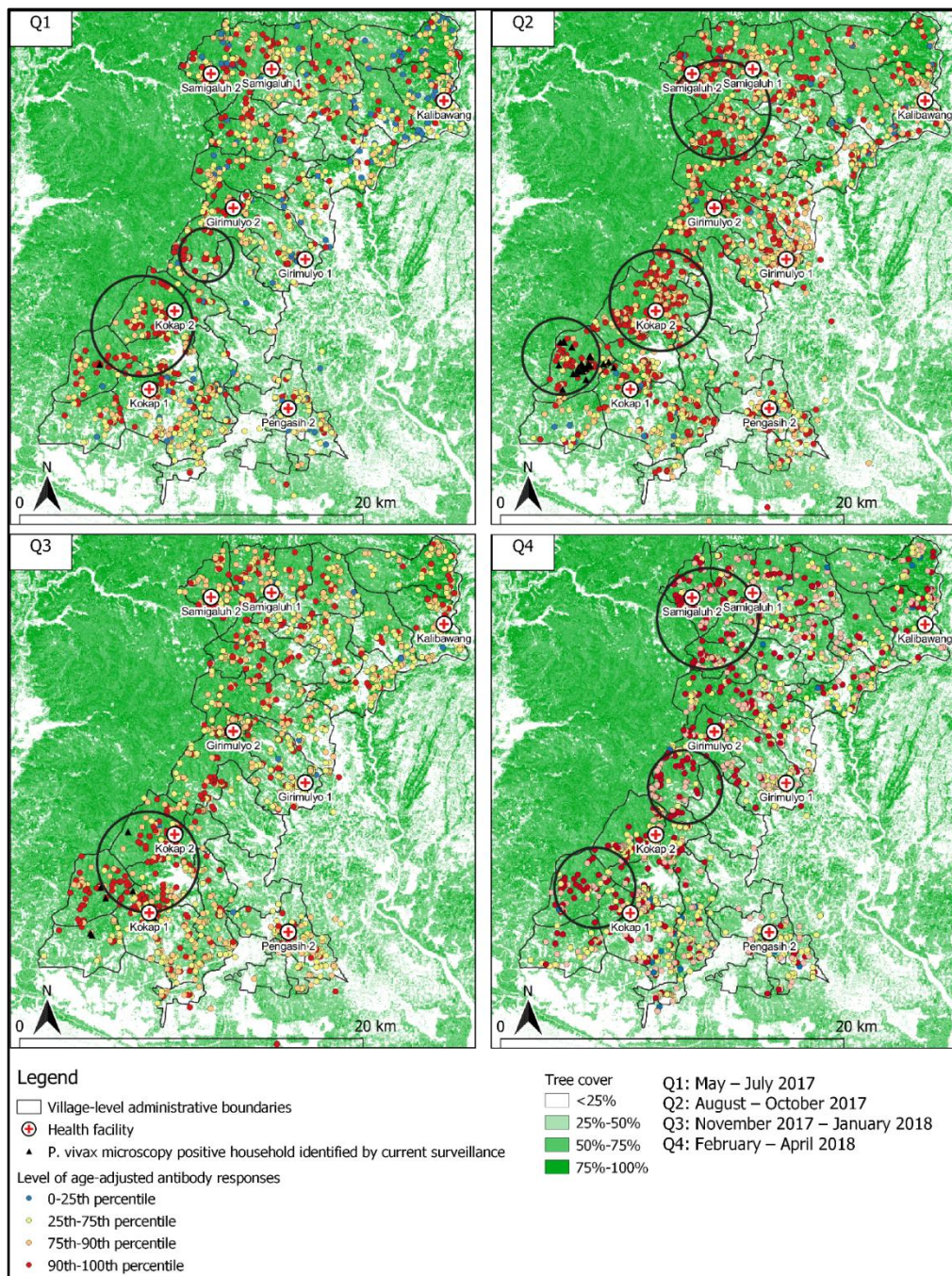


Figure 5.5 Spatial distribution of age-adjusted antibody responses to multiple *P. vivax* antigens over time of surveys overlaid with *P. vivax* microscopy infections captured by the current surveillance systems. Black triangles represent *P. vivax* microscopy positive households. Black circle indicates a cluster of significantly higher than expected antibody responses detected using SaTScan ( $p$  value < 0.05).

## 5.5 Discussion

The study found that analysing serological and spatial epidemiological data collected via health facilities in quarterly cross-sectional surveys was a useful supplement to passive data collection and could potentially be used to identify and target areas that remain receptive to malaria, and therefore at risk of outbreaks (Additional file 3, 4, 5 and 6). Consistent with the parasitological data, the population-level SCR estimates suggest very low level of transmission in the  $\leq 15$  years old population (current transmission). The SCR's equate to 5 per 1000 and 20 per 1000 people seroconverting per year for *P. falciparum* and *P. vivax*, respectively. For comparison, the SCR's in adults over 15 years old (historical transmission), were 32 per 1000 and 66 per 1000 people for *P. falciparum* and *P. vivax*, respectively. Moreover, spatial analysis of age adjusted antibody responses identified clusters of high antibody responders in areas which subsequently report *P. vivax* cases. These findings support the potential utility of serological tools to improve malaria surveillance in the absence of active cases, and their incorporation in malaria elimination programmes. Multivariable analysis suggests that surveillance could potentially prioritise targeting males and forest goers as they were the high-risk populations who might reintroduce infections to a community in the future.

Although the accuracy of the mapping exercise varied within the 8 health facilities (353-817 meters), the addition of a relatively simple tablet-based participatory mapping approach with a short questionnaire administered during facility attendees' interviews allowed the collection of fine-scale spatial variation of malaria infections and exposure. If employed, this approach could iteratively improve spatial accuracy of public health mapping at the local level [30]. Integrating spatial data with age adjusted antibody responses to a panel of malaria antigens identified health



facility catchment areas with significantly higher antibody responses than the population average. These clusters of high antibody responses were detected in the same areas across all 4 surveys for both species and were the location for a malaria outbreak during the study period. Importantly, the serological outcomes highlighted the area prior to the outbreak and had this area been subject to targeting with interventions or more in-depth surveillance, the outbreak may have been prevented. Areas that were most recently receptive to transmission could be targeted with interventions as these are places that may be most susceptible to outbreaks and this strategy is likely to be more efficient than untargeted approaches to reduce transmission in low transmission settings [5]. Two other clusters in Girimulyo 2 and Samigaluh 2 were identified, suggesting that other high-risk areas are located in the most forested areas of the region which also bordered with another malaria higher endemic setting with ongoing transmission [62].

Whilst the microscopy data collected during the repeated surveys identified very few infections, and therefore could not be utilised to identify risk factors, the numbers of serological positives enabled the examination of risk factors for exposure to infection within the population. Our analysis found that people who were *P. vivax* seropositive were 3-fold more likely to be *P. falciparum* seropositive. As there was no cross-reactivity evident from the serological data, this suggests that the population have been exposed to infections with both species, although this exposure could have been historical. This implies that both species are transmitted in similar areas and that these places are, or were, particularly receptive to the transmission of malaria. Risk factor analysis for *P. vivax* seropositivity confirmed that people aged over 15 years old, males and forest-related activities were associated with higher exposure to malaria. These findings are consistent with findings from previous studies in the area suggesting that malaria

infection is expected to be less common among children compared to adults most likely due to a different level of behavioural risk (night outdoor activities and forest-related jobs such as loggers, coconut/palm tapper, fruit farmer, etc.) which leads to higher exposure among males and adults [7,10,31,35]. Interestingly, higher exposure was also associated with bed net use. The coverage and usage of bed nets was relatively low in this study setting and may be indicative of people living in higher risk areas being more likely to use a net, potentially due to the presence of more mosquitoes. The data suggest that people  $\leq 15$  years old were more likely to be sleeping under a bed net compared to adults over 15 years old. This finding may also suggest that bed net is no longer effective to prevent transmission in the studied population. Therefore, an alternative intervention such as targeted repellent distribution for adults or impregnated hammocks for forest workers could be useful to reduce transmission in the future.

*P. vivax* seroprevalence was highest during the period of August to October. This overlaps with the expected high transmission season (August to December) and was also the period when people in the study were most likely to report recent travel. However, our analysis suggested that the clusters of high exposure identified in this study were not necessarily the place where recent travel from was reported. A possible explanation of these findings is that the transmission occurred after Ramadhan where people were more likely to return to their region after several days or weeks of traveling to areas of higher endemicity to gather and celebrate Eid day with their family. Previous studies indicated migration and high rates of imported cases from higher transmission areas as factors that linked to malaria resurgence and outbreaks in low transmission settings [10,13,31,63]. A study in Zanzibar estimated that residents travelling to other endemic settings contribute 1 to 15 times more imported cases than visitors, highlight the importance of strengthening surveillance to capture infection in travellers in countries nearing

elimination [64]. However, the investigation conducted by the surveillance program did not identify if there was a link between migration during or after Ramadhan with the outbreak occurred in the period. These findings suggest that surveillance needs to be intensified in periods with high population movement such as during and/or after Ramadhan and during fruit (i.e. durian) harvesting time which often coincides with the wet season in the region, to enable early detection and responses to prevent transmission in the future, particularly in receptive areas identified in the study.

Our findings suggest that serological analysis can be used to estimate heterogeneity of *P. falciparum* and *P. vivax* transmission and predict high-risk areas from a single health facility-based cross-sectional survey. This sampling approach could be a more efficient surveillance strategy as the serological sampling is performed (in addition to parasitological diagnosis) in well-established health infrastructures therefore allowing rapid treatment and surveillance response if clinical cases are detected. On the other side, the repeated surveys might potentially be more useful in informing short-term changes in malaria exposure in other endemic settings where malaria transmission is still ongoing and more intense.

Although the health facility surveys provide sufficient samples to estimate burden of infection and transmission level in the population, there were several limitations to be considered when implementing the methods. Firstly, we found that the facility survey approaches captured only a small proportion of children under 15 years of age compared to the general population. Whilst we have observed risk is significantly higher in adults and the underrepresentation of children may not be an issue for malaria in this setting, it could limit the approach for general disease surveillance. Routine data collected by the district health office surveillance suggest that this

could be due to the low proportion of children attending public health facilities in some areas where private health facilities may be easier to access. This phenomenon might not be the case in many other countries where often young children are the most common demographic to attend health facilities. Future studies in Indonesia could consider attendees to private health facilities as an easy access group to improve the facility-based sampling approach. In addition, surveys based in facilities are likely to miss asymptomatic infections, as well as those occurring in people who choose not to use public facilities. This is indicated by our finding suggesting that majority of cases (61%) were captured by the active case surveillance. Secondly, people living further from facilities may be less likely to attend health facilities resulting in the methods being less likely to detect clusters of high exposure further from facilities. However, it is conceivable that iterative refinements of the maps over time with clinical and demographic data would improve this. Inclusion of a mapping exercise in active surveillance performed by community health workers would be useful to capture heterogeneity in areas further from the facilities or those not seeking care. It may also help to identify if there are any spatial aspects to specific movement and behaviours. Recent travel was not significantly associated with increased seropositivity but being male and working in the forest were and whilst there was some evidence of spatial autocorrelation in the data, this was not accounted for in the regression modelling meaning estimates are likely to be over-precise. There are potential benefits to understanding the spatial context for risk behaviours which may be influenced by season for farming or harvesting and for traditional and religious holidays. The fourth limitation is in the analysis and interpretation of the serological data. Whilst outwardly the multiplex assay for serological screening is attractive in increasing the number of antigenic targets to both reduce the likelihood of missing individuals non-responsive to specific antigens and simultaneously screen for multiple species, the best analytical approaches in combining data are still relatively undeveloped and

validated. Using standard approaches based on seroprevalence, SCR and regression analysis has generated important observations but in future it will be important to combine these into more readily usable metrics and/or platforms such as serological lateral flow devices that offers more rapid test [65].

## 5.6 Conclusion

The health facility-based serological surveillance implemented and evaluated in this study provide an alternative approach for quickly obtaining parasitological, serological, geolocation and risk factor data. A single survey is efficient in supplementing the existing surveillance in very low endemic areas approaching zero cases, although the repeated surveys might be more useful in informing short-term changes in exposure in other higher endemic settings. Combining these methods with novel multiplex serological techniques could improve malaria surveillance capacity and result in a better understanding of transmission dynamics, in the absence of infection detected by standard diagnostic tools such as microscopy. Future work could expand the use of multiplex bead-based assays to include a panel of other species of *Plasmodium* antigens as well as to other available neglected tropical diseases (NTDs) antigens such as soil transmitted helminths, filariasis, etc. to similarly improve surveillance of these infections. How this approach is incorporated as a practical tool into programmes will requires significant technological and operational refinement [66] and financial assessment of the potential benefit. However, the argument for serological surveillance is particularly strong for *P. vivax* as there are no current diagnostics to detect latent hypnozoites and this is what the approach described in the manuscript has detected. Finally, reliability of implementing these methods would need to be evaluated in other areas aimed at eliminating malaria. Future works will need to assess the

bottleneck of implementing these methods to allow further integration into existing surveillance systems.

### **Abbreviations**

CI: confidence interval; PfAMA1: *P. falciparum* apical membrane antigen 1; PfMSP-1<sub>-19</sub>: *P. falciparum* merozoite surface protein 1; PvAMA-1: *P. vivax* apical membrane antigen 1; PvEBP: *P. vivax* erythrocyte binding protein; PvMSP-1<sub>-19</sub>: *P. vivax* merozoite surface protein 1; PvRBP1a: *P. vivax* reticulocyte binding protein 1a [amino acids 160–1170]; PvRBP2b: *P. vivax* reticulocyte binding protein 2b [amino acids 161–1454]; SCR: seroconversion rates; WHO: World Health Organization.

### **Ethics approval and consent to participate**

This study was approved by the Medical Health Research Ethics Committee, Universitas Gadjah Mada, Indonesia (KE/FK/0290/EC/2017) and the Research Ethics Committee of the London School of Hygiene and Tropical Medicine (11944). Written individual informed consent was obtained from all participants in this study.

### **Consent for publications**

Not applicable

### **Availability of data and materials**

The datasets used and analysed during this study are not publicly available due to the inclusion of identifying information on individuals but are available from the corresponding author on reasonable request.

**Competing interests**

The authors declare that they have no competing interest.

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**Authors' contributions**

HS designed and coordinated the study, performed serological assay, analysed data and wrote the manuscript. HS, JC and CD supervised the data collection and lab work. RAK TBR, SYD performed data collection. KKAT and CC produced the antigens. KKAT developed the serological assay. S, RAA, GS, JC and CD interpreted findings and reviewed the manuscript. All authors read and approve the final manuscript.

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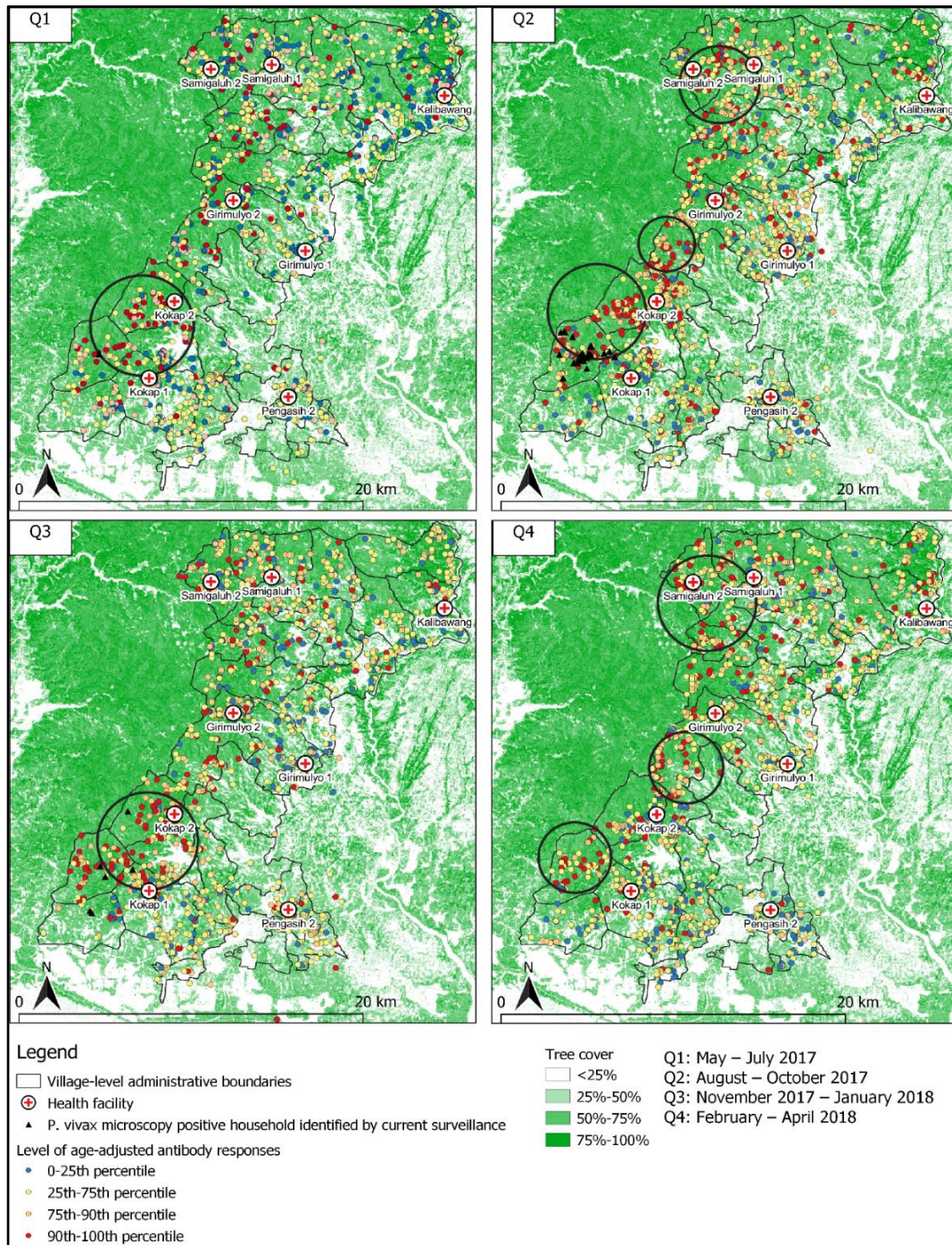
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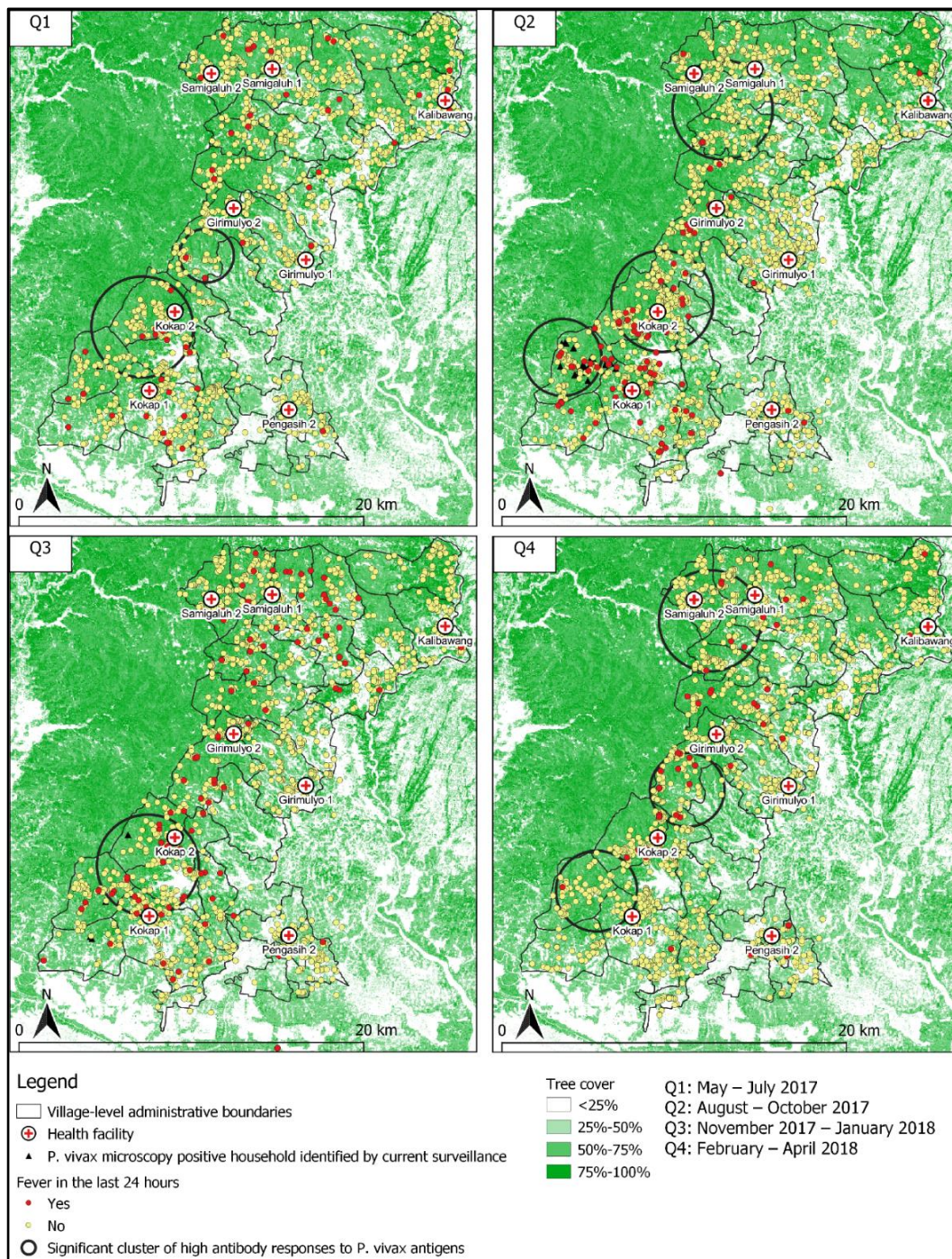
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## 5.8 Supplementary information

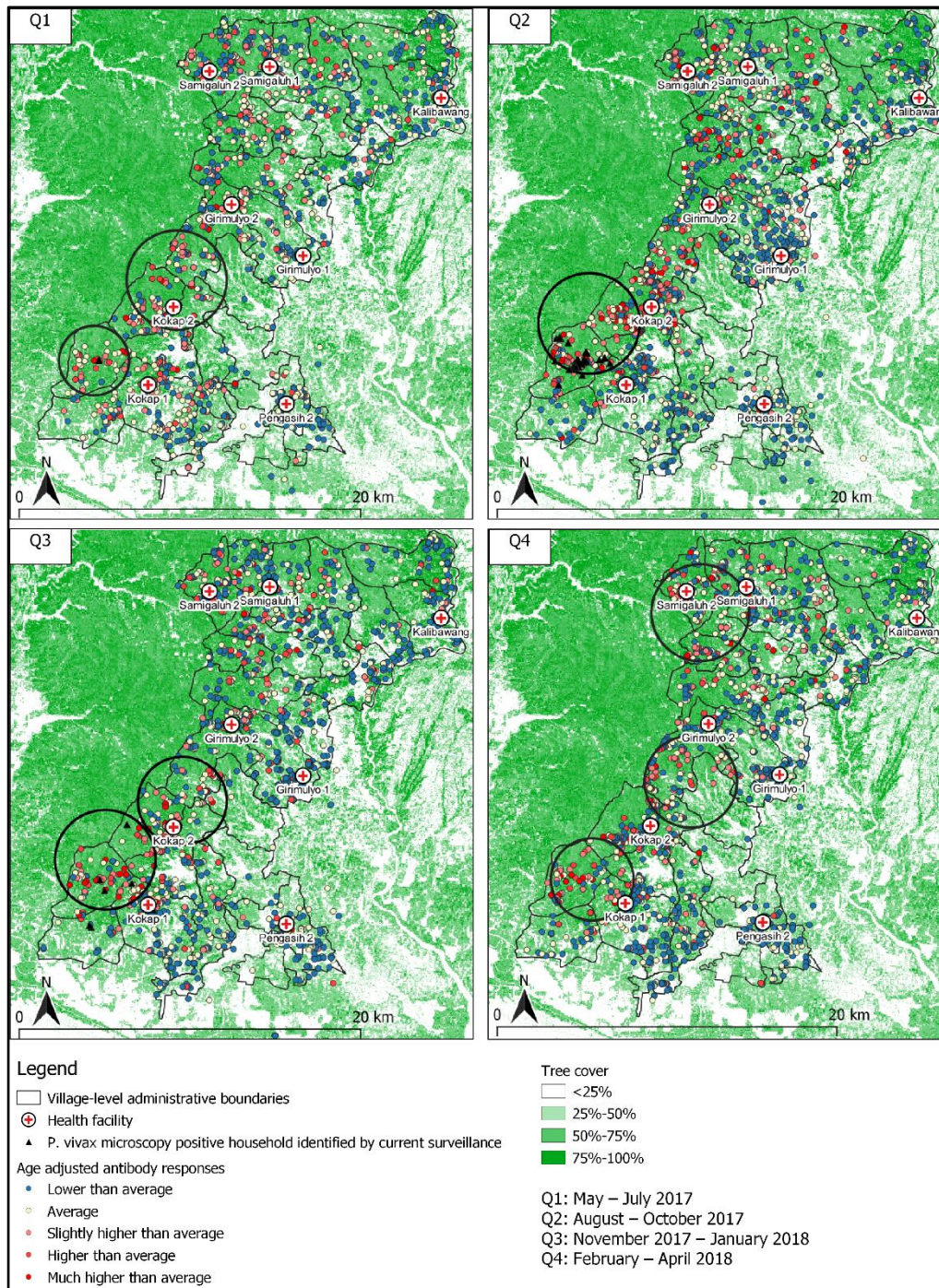


**Additional file 1.** Spatial distribution of age-adjusted antibody responses to multiple *P. falciparum* antigens over time of surveys overlaid with *P. vivax* microscopy infections captured by the current surveillance systems. Black triangles represent *P. vivax* microscopy positive households. Black circle indicates a cluster of significantly higher than expected antibody responses detected using SaTScan ( $p$  value < 0.05).

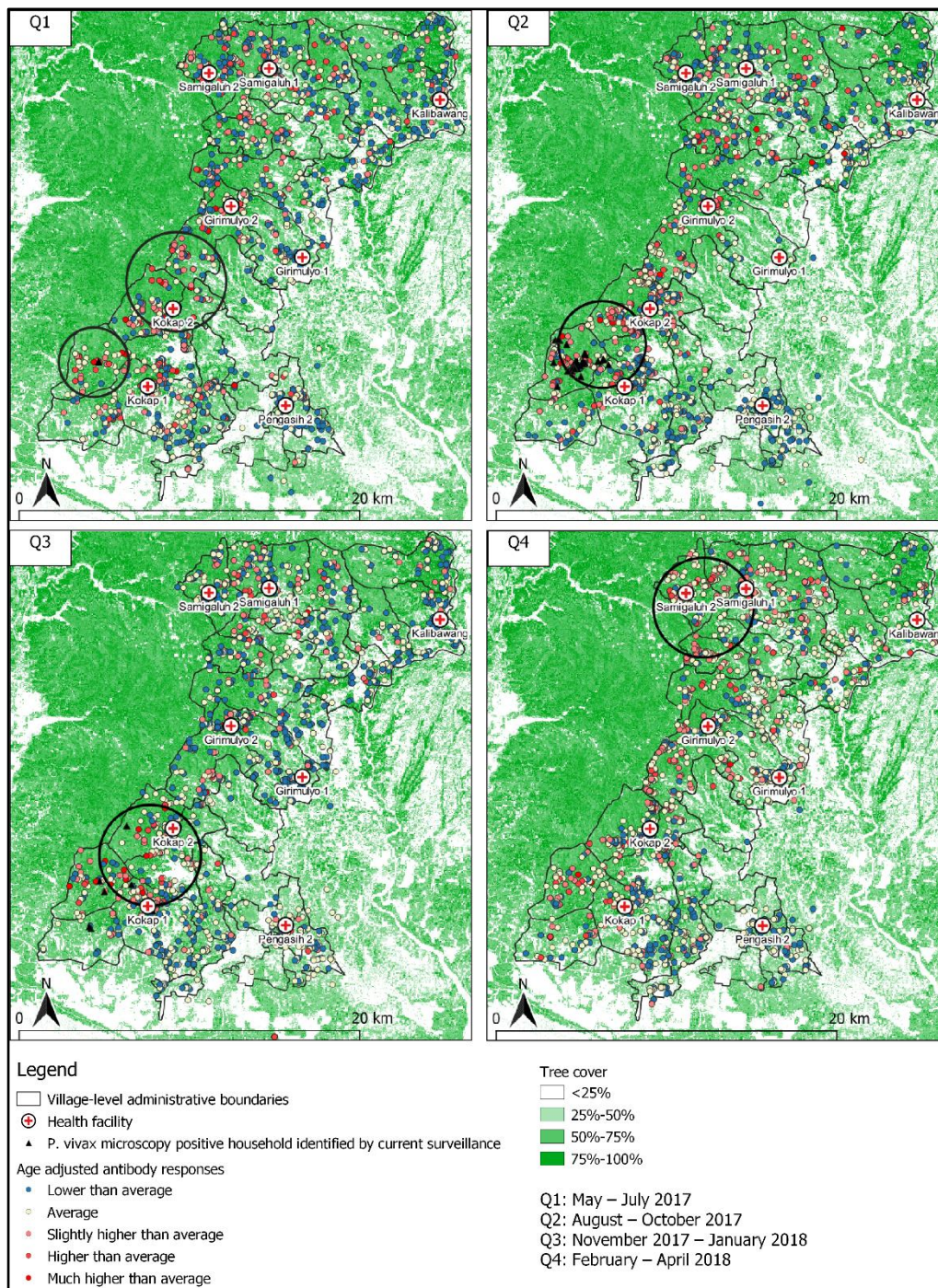




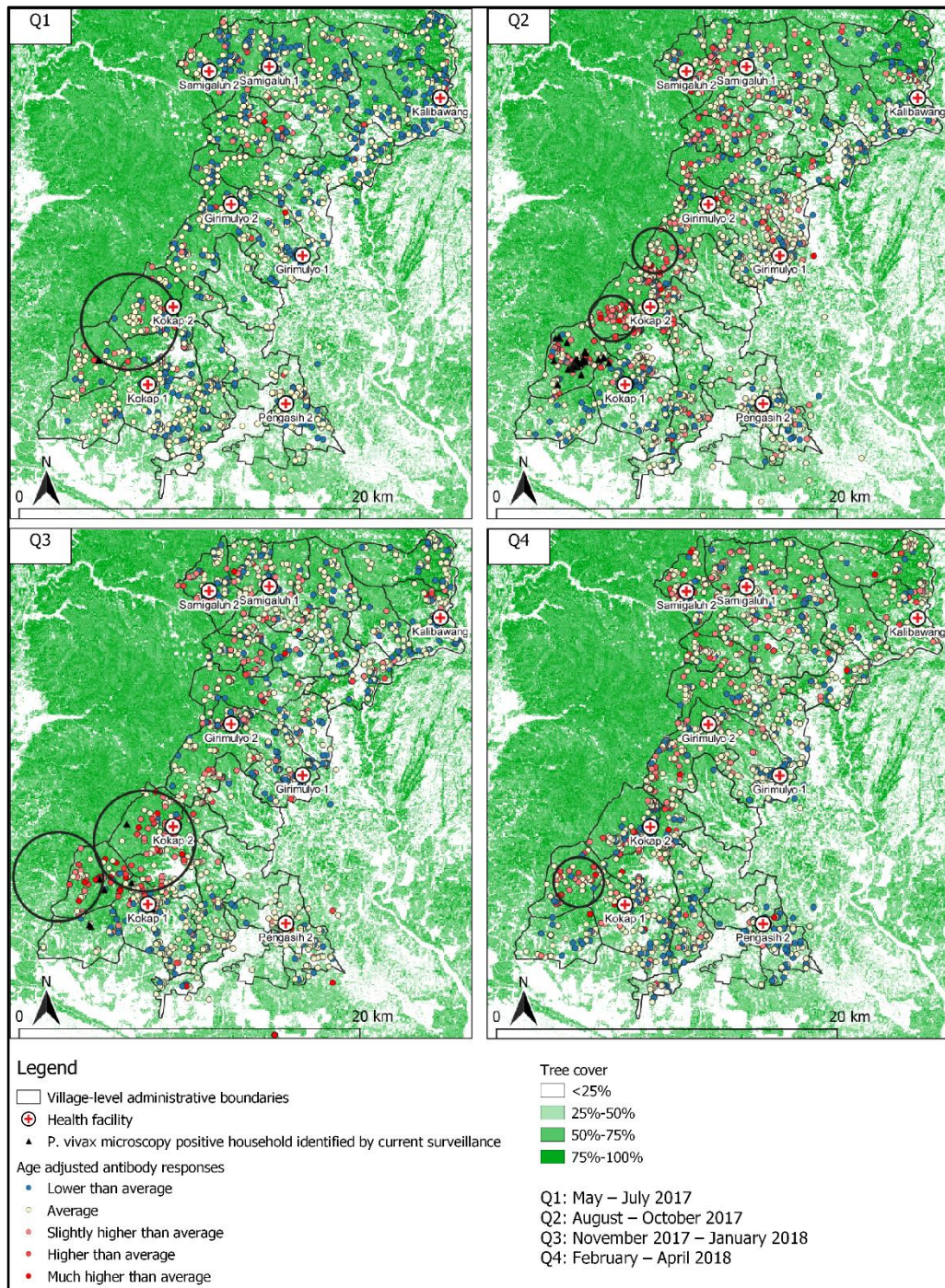
**Additional file 2.** Maps showing cluster of significantly higher than expected antibody responses to multiple *P. Vivax* antigens over time of surveys overlaid with fever status and *P. vivax* microscopy infections captured by the current surveillance systems.



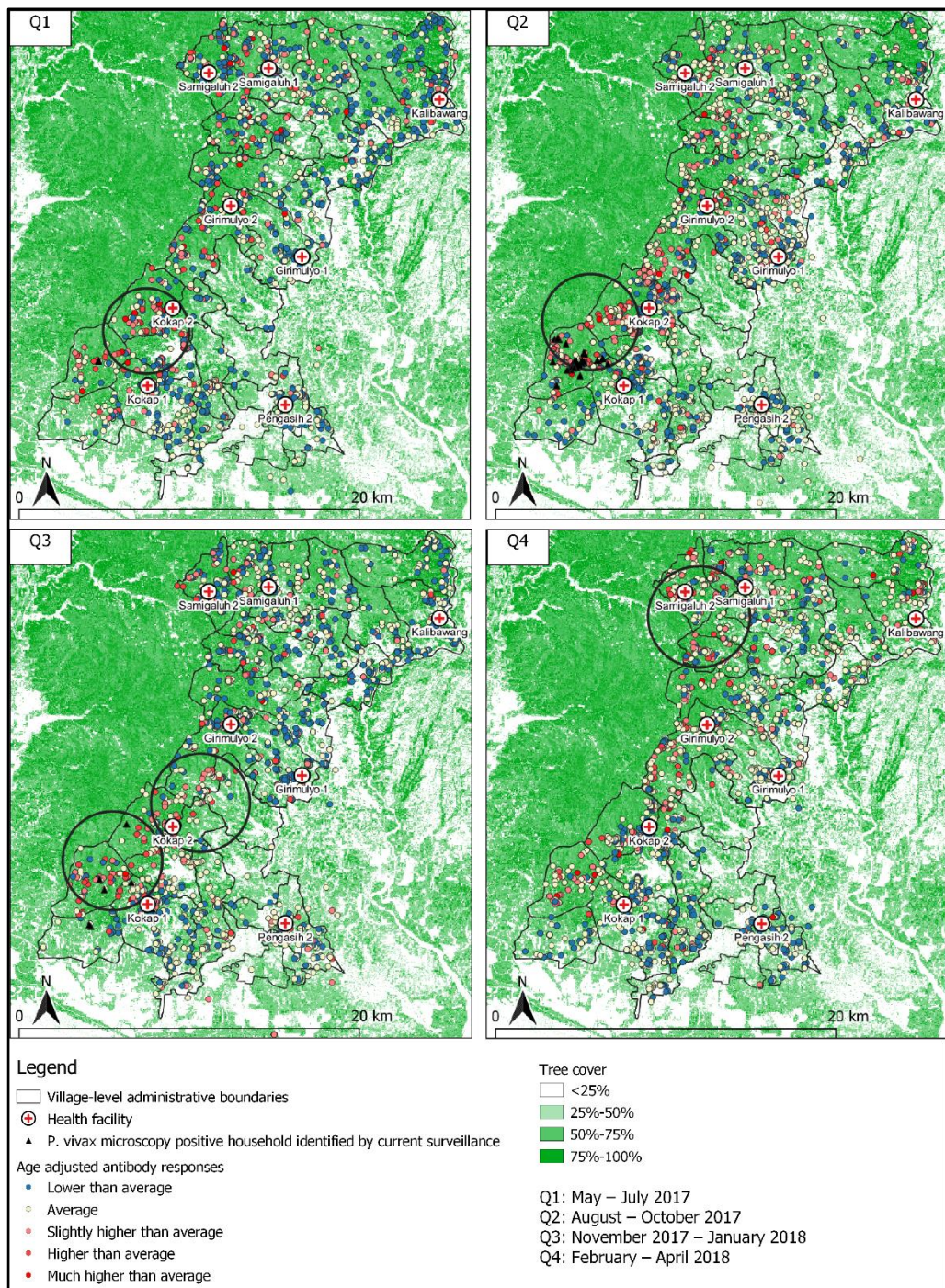
**Additional file 3.** Maps showing cluster of significantly higher than expected antibody responses to PvAMA-1 antigen over time of surveys overlaid with *P. vivax* microscopy infections captured by the current surveillance systems.



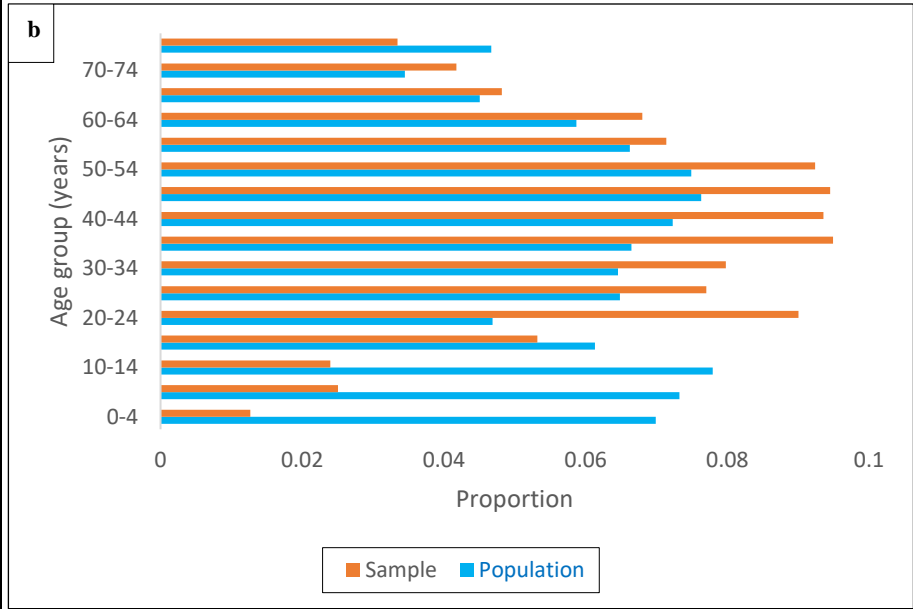
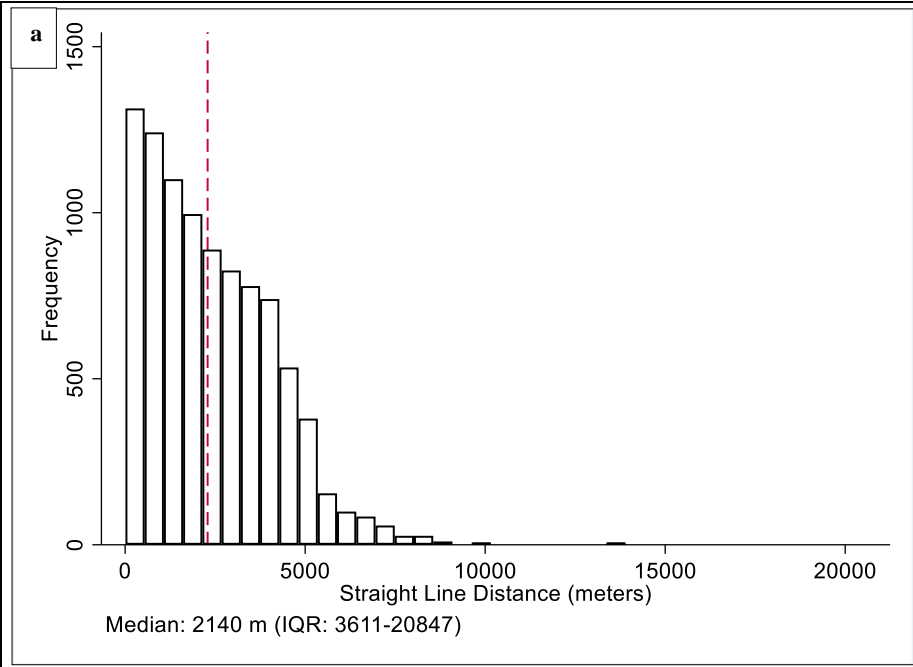
**Additional file 4.** Maps showing cluster of significantly higher than expected antibody responses to PvMSP-1-19 antigen over time of surveys overlaid with *P. vivax* microscopy infections captured by the current surveillance systems.



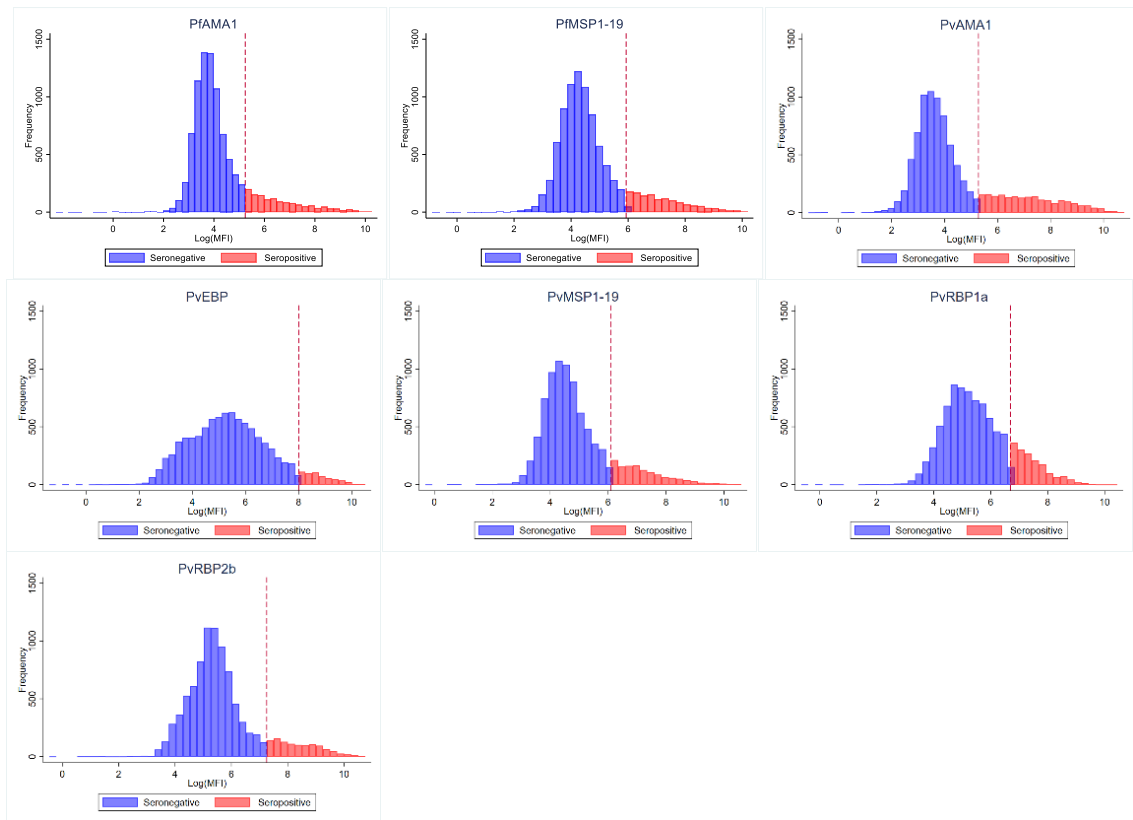
**Additional file 5.** Maps showing cluster of significantly higher than expected antibody responses to PfAMA1 antigen over time of surveys overlaid with *P. vivax* microscopy infections captured by the current surveillance systems.



**Additional file 6.** Maps showing cluster of significantly higher than expected antibody responses to PfMSP-1-19 antigen over time of surveys overlaid with *P. vivax* microscopy infections captured by the current surveillance systems



**Additional file 7.** Graphs showing summary of straight-line distance travelled by the health facility attendees (a) and population age distribution compared to the age distribution of those sampled in the health facility surveys (b). Data on age distribution of population of the studied areas i.e. Kokap, Samigaluh, Girimulyo, Kalibawang, and Pengasih districts were obtained from the 2016 population data published by the Central Agency on Statistics of Kulon Progo.



**Additional file 8.** Seropositive cut offs based on finite mixture model. Cut off was defined as the log MFI (median fluorescence intensity) plus three standard deviations of the seronegative component of each antigen. Broken red line represents the cut off.

### Additional file 9. Seroprevalence to each antigen per survey

Seroprevalence % (95% CI)	n	Q1 (May-July 2017)	n	Q2 (August-October 2017)	n	Q3 (November 2017-January 2018)	n	Q4 (February-April 2018)
<b>PfAMA-1</b>								
1-4 years old	0	0	0	0	0	0	0	0
5-14 years old	2	1.56 (0.39-6.03)	3	1.85 (0.60-5.59)	3	2.83 (0.92-8.41)	0	0
All ages	135	5.78 (4.91-6.81)	434	19.58 (17.98-21.28)	415	17.90 (16.39-19.51)	383	16.75 (15.28-18.34)
<b>PfMSP-1<sub>-19</sub></b>								
1-4 years old	3	10.71 (3.49-28.45)	1	2.50 (0.35-15.74)	0	0	0	0
5-14 years old	1	0.78 (0.11-5.34)	4	2.47 (0.93-6.40)	3	2.83 (0.92-8.41)	1	1.85 (0.26-12.02)
All ages	389	16.71 (15.25-18.28)	319	14.40 (13.00-15.93)	299	12.91 (11.60-14.34)	340	14.89 (13.48-16.41)
<b>PvAMA-1</b>								
1-4 years old	2	7.14 (1.79-24.49)	0	0	0	0	0	0
5-14 years old	1	0.79 (0.11-5.38)	0	0	4	3.77 (1.42-9.63)	4	7.55 (2.86-18.46)
All ages	532	22.90 (21.24-24.66)	538	24.27 (22.53-26.10)	542	23.37 (21.69-25.13)	506	22.21 (20.55-23.96)
<b>PvMSP-1<sub>-19</sub></b>								
1-4 years old	1	3.57 (0.50-21.44)	0	0	0	0	0	0
5-14 years old	1	0.78 (0.11-5.34)	0	0	2	1.89 (0.47-7.23)	3	5.77 (1.87-16.43)
All ages	429	18.38 (16.86-20.00)	271	14.68 (13.14-16.37)	280	12.12 (10.85-13.52)	345	15.61 (14.16-17.19)
<b>PvEBP</b>								
1-4 years old	2	7.14 (1.79-24.49)	1	2.50 (0.35-15.74)	0	0	0	0
5-14 years old	0	0	2	1.23 (0.31-4.80)	1	0.94 (0.13-6.39)	1	1.85 (0.26-12.02)
All ages	133	5.70	151	6.81	146	6.28	129	5.68
<b>PvRBP1a</b>								
1-4 years old	0	0	2	5.00 (1.25-17.92)	2	8.00 (2.01-26.96)	2	10.53 (2.64-33.76)
5-14 years old	11	8.59 (4.82-14.86)	17	10.49 (6.62-16.24)	16	15.09 (9.46-23.23)	0	0
All ages	373	15.98 (14.55-17.52)	516	23.26 (21.55-25.07)	378	16.29 (14.85-17.85)	372	16.85 (15.35-18.46)
<b>PvRBP2b</b>								
1-4 years old	0	0	1	2.50 (0.35-15.74)	0	0	0	0
5-14 years old	0	0	0	0	2	1.89 (0.47-7.23)	0	0
All ages	22	0.94 (0.62-1.43)	383	17.30 (15.78-18.93)	387	16.78(15.31-18.36)	326	14.37 (12.99-15.88)



### Additional file 10. Complete list of antigens tested in the study

No	Gene ID	Acronym	Description	Location	Reference
1	PF3D7_0304600	CSP	Most predominant and antigenic protein on sporozoite surface. Component of RTS, S vaccine	Sporozoite	(121)
2	PF3D7_1301600	EBA140 RIII-V	erythrocyte binding antigen 140; involved in invasion	Apical organelles, micronemes	(122)
3	PF3D7_0731500	EBA175RII_F2	erythrocyte binding antigen 175; RBC binding region via glycophorin A	Apical tip	(122)
4	PF3D7_0423700	Etramp 4 antigen 2	Early transcribed membrane antigen. Integral PVM protein. C-terminal	iRBC, PVM	(118)
5	PF3D7_0532100	Etramp 5 Ag 1	Early transcribed membrane antigen. Integral PVM protein.	iRBC, PVM	(123)
6	PF3D7_0402400	GEXP18	Gametocyte exported protein 18. Unknown function.	iRBC/Gametocyte	(118)
7	PF3D7_1035300	GLURP R2	Glutamate rich protein R2	Merozoite Surface	(124)
8	PF3D7_0501100.1	HSP40 Ag 1	Heat shock protein 40	iRBC	(118)
9	PF3D7_0501100.1	HSP40 Ag 3	Heat shock protein 40	iRBC	(118)
10	PF3D7_0206800	MSP2 CH150/9	CH150/9 allele of Merozoite surface protein (MSP) 2. Full-length.	Merozoite surface	(125)
11	PF3D7_0206800	MSP2 Dd2	Dd2 allele of MSP2. Full-length.	Merozoite surface	(126)
12	PF3D7_1133400	AMA1	Apical membrane antigen 1	micronemes	(127)
13	PF3D7_0930300	MSP1 <sub>-19</sub>	19kDa fragment of MSP1 molecule.	Merozoite surface	(128)
14	PF3D7_1021800	PfSEA-1	Schizont egress antigen.	iRBC	(129)
15	PF3D7_0424100	Rh5	Receptor for human protein Basigin.	Apical tip	(130)(131)
16	PF3D7_0501300	SBP1	skeleton-binding protein; essential for translocation of PfEMP1 to iRBS surface via Maurer's cleft.	iRBC	(132)
17	PF3D7_1002000	Hyp2	<i>Plasmodium</i> exported protein	iRBC / PVM	(118)
18	PF3D7_1036000	H103	Merozoite surface protein 11/H101/MSP3.7	Merozoite	(133)
19	PVX_092275	PvAMA1	Apical membrane antigen 1	micronemes	(134)
20	PVX_099980	PvMSP1 <sub>-19</sub>	19kDa fragment of MSP molecule	Merozoite	(135)
21	PVX_110810	PvDBP R2	Duffy binding protein region II	Merozoite	(136)
22	PVX_110835	PvEBP	Erythrocyte binding protein	Merozoite	(136,137)
23	PVX_098585	PvRBP1a	Reticulocyte binding protein amino acids 160–1170	Apical tip	(138)
24	PVX_094255	PvRBP2b	Reticulocyte binding protein amino acids 161–1454	Apical tip	(138)

## Unpublished data

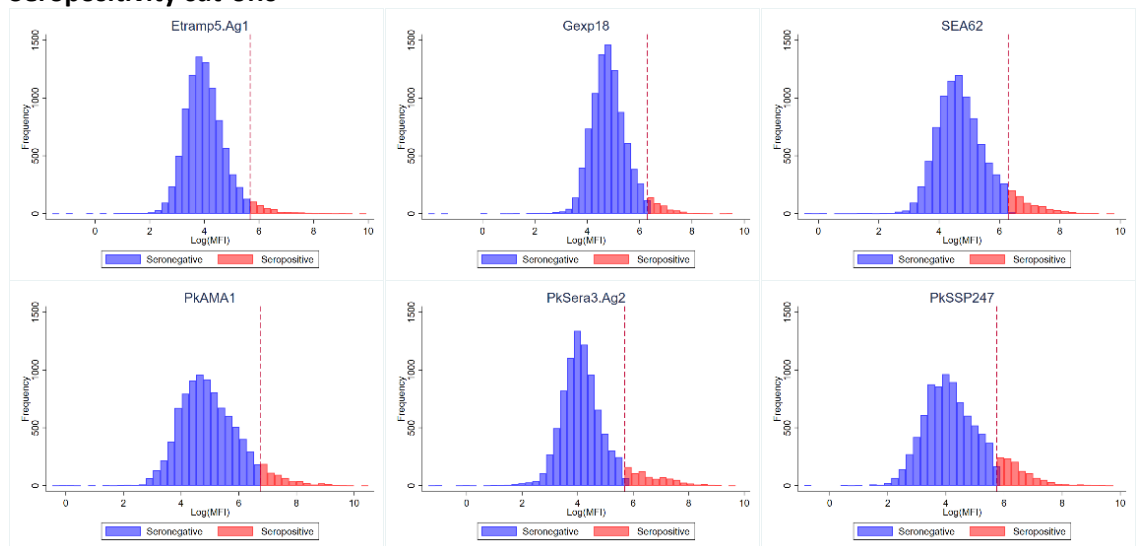
There were some unpublished data generated as part of the health facility-based serological surveillance study presented in this chapter. Some *P. falciparum* antigens associated with short-lived antibody responses (Etramp5.Ag1, GexP18 and PfSEA-1) were included on the panel of antigens tested against the samples, in addition to the other *P. falciparum* and *P. vivax* antigens reported in the paper. The measurement of the *P. falciparum* short-lived responses was strongly relevant and important as *P. falciparum* infection was historically more dominant than *P. vivax* in the studied region. However, recently, there was a significant change in transmission where *P. falciparum* seem to be disappeared, and *P. vivax* infections are increasing. Unfortunately, we do not have defined *P. vivax* antigens that elicit short-term responses that can be included in the panel at the time of the study was conducted. In addition, the number of human *P. knowlesi* infections are increasingly reported in other parts of Indonesia i.e. Kalimantan and Sumatera Islands, and the presence of macaques and efficient vector of *P. knowlesi* in the studied region support the rationale of including *P. knowlesi* antigens on the panel. Therefore, we aimed at evaluating the use of these antigens for investigating recent *P. falciparum* exposure as well as to describe the possibility of *P. knowlesi* exposure in the region.

Analysis of short-lived antibody responses to *P. falciparum* antigens illustrated some potential use for detecting malaria exposure in population. The seroprevalence data presented below showed there were individuals seropositive to each of these antigens. However, further analysis to assess the sensitivity and accuracy of the *P. falciparum* antigens could not be done due to the very low number of *P. falciparum* infection detected by microscopy in the study. Therefore, we could not make a clear interpretation of the seroprevalence to the *P. falciparum* short-lived antibody responses, although they have been reported as important serological markers to

detect recent exposure (i.e. can strongly predict infection in the last year). Based on this rationale, the results generated from these antigens were not included on the published paper and discussed separately. The sensitivity and accuracy of these antigens in predicting *P. falciparum* PCR positive infection was studied using a different dataset from a high transmission setting and is presented in the next chapter (Chapter 6).

The presence of *P. knowlesi* seropositive individuals showed the possibility of *P. knowlesi* exposure in the studied region. However, absence of evidence on *P. knowlesi* cases both in human and macaque populations suggested further study is needed to confirm the presence of *P. knowlesi* transmission in Kulon Progo. Potential future work is to perform molecular assays using blood spot samples collected in this study to validate initial results generated from the serological assay. Further investigation for risk factors and spatial distribution of *P. knowlesi* antibody responses will be conducted and discussed separately from this thesis.

### Seropositivity cut-offs



**Additional file 8.** Seropositive cut-offs for *P. falciparum* antigens (top row) and for *P. knowlesi* (bottom row) based on finite mixture model. Cut off was defined as the log MFI (median fluorescence intensity) plus three standard deviations of the seronegative component of each antigen. Broken red line represents the cut off.

## 6. Serological markers to assess malaria transmission intensity and infection in a high transmission setting, Indonesia

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#### SECTION A – Student Details

Student	Henry Surendra
Principal Supervisor	Chris Drakeley
Thesis Title	Operational applications of serology for malaria surveillance in different transmission settings in Indonesia

***If the Research Paper has previously been published please complete section B, if not please move to Section C***

#### SECTION B – Paper already published

Where was the work published?	
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#### SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published	Malaria Journal
Please list the paper's authors in the intended authorship order.	Henry Surendra, Jeanne R Poespoprodjo, Supargiyono, Elsa H Murhandarwati, Rizqiani A Kusumasari, Ajib Diptyanusa, Rumbiwati, Enny Kenagalem, Faustina H Burdam, Rintis Noviyanti, Kevin KA Tetteh, Jackie Cook, Zuleima Pava, Jutta Marfut, Ric N Price and Chris Drakeley
Stage of publication	In preparation

#### SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	First and corresponding author. I supervised the serological work, performed data analysis and wrote the manuscript.
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**Title: Serological markers to assess malaria transmission intensity and infection in a high transmission setting, Indonesia**

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## **6.1 Abstract**

### **Background**

Measurement of malaria species-specific antibody responses can potentially inform the assessment of transmission intensity, by quantifying recent exposure to infection. This study aimed to evaluate the use of multiple serological markers to estimate transmission levels and assess the association between serological markers with *Plasmodium* infections in a high transmission setting.

### **Methods**

Demographical data, peripheral blood smears and capillary blood samples were collected during a community-based cross-sectional survey conducted in Mimika District, southern Papua, Indonesia, in 2013. Slide microscopy and polymerase chain reaction (PCR) were performed to estimate parasite prevalence and identify sub-patent infections. Antibody responses to apical membrane antigen 1 (PfAMA1; PvAMA-1), merozoite surface protein 1 (PfMSP-1<sub>-19</sub>; PvMSP-1<sub>-19</sub>), merozoite surface protein 2 (MSP2.Ch150), duffy binding protein region 2 (PvDBPR2), erythrocyte binding protein (PvEBP), reticulocyte binding protein 1 [amino acids 160–1170] (PvRBP1a), early transcribed membrane antigen 5 Antigen 1

(Etramp5.Ag1), gametocyte exported protein 18 (GexP18), and schizont egress antigen 1 (PfSEA-1) were measured using a bead-based assay. Receiver operating characteristic (ROC) analysis was used to quantify the sensitivity and specificity of each *P. falciparum* and *P. vivax* antigens in predicting concurrent infection. Seroconversion rates (SCR) were estimated by fitting a simple reversible catalytic model to seroprevalence data.

## Results

A total of 2496 patients from 747 households provided samples. The overall parasite prevalence by microscopy and PCR was 17% (411/2496) for *P. falciparum*, 20% (489/2496) for *P. vivax*. The majority of infections were sub-patent with only 38% (154/411) of *P. falciparum* and 31% (149/489) of *P. vivax* parasitaemic individuals detected by microscopy. 65% of *P. falciparum* PCR positive children aged 1-5 years old were also seropositive to Etramp5.Ag1 antigen, with 76% prediction accuracy. The SCR to the most immunogenic antigen (PfMSP<sub>-1-19</sub> and PvEBP) was 0.156 person-year (95% Confidence Interval: 0.138-0.177) for *P. falciparum* and 0.141 person-year (95% Confidence Interval: 0.124-0.159) for *P. vivax*.

## Conclusion

This study highlights the potential of serological responses to multiple *P. falciparum* and *P. vivax* antigens to estimate transmission intensity and predict parasite prevalence in children in high transmission areas.

**Key words:** serology, surveillance, transmission, malaria, *P. falciparum*, *P. vivax*

## 6.2 Introduction

Microscopic examination of peripheral blood smears remains the gold standard diagnosis of malaria [1], with parasite prevalence used to estimate malaria transmission intensity in

endemic areas [2]. However, the derived estimates can be highly variable [3-5], dependent upon the skill of the microscopist or prevalence of malaria. Furthermore, microscopy can miss a high proportion of infections due to fluctuating parasite densities [6]. Sub-microscopic low-level parasitaemia, are not detected in clinical settings by standard diagnostic algorithms, and if left untreated these can result in subsequent clinical infection, severe malaria, and ongoing transmission of the parasite [7-9].

Although Rapid Diagnostic Tests (RDTs) are widely available and often used to diagnose malaria, the latest WHO malaria RDTs evaluation highlight that many RDTs result in poor specificity and sensitivity, especially when parasite densities are below 200 parasites/ $\mu$ l of blood [10]. RDTs are also significantly less accurate for diagnosing *P. vivax* infections compared to *P. falciparum*. Whilst molecular methods such as polymerase chain reaction (PCR) are highly sensitive [9,11] and can detect up to 50% more infections than microscopy [3,12], its application in the field is limited by high cost, time to process and the laboratory equipment and skills needed to perform the test [4].

Serology is an alternative approach to estimate malaria transmission. Utilising species-specific malaria antibodies as a proxy of infection can facilitate quantifying disease burden. The seroconversion rate (SCR) is used to define the annual rate at which individuals become seropositive [13-15]. Although the presence of long-lived antibodies such as *P. falciparum* and *P. vivax* apical membrane antigen 1 (PfAMA1 and PvAMA-1) and *P. falciparum* merozoite surface protein 1 (PfMSP-1<sub>-19</sub>) reflect cumulative exposure over time [13], seropositivity to any of these antigens in younger children are more likely to be an indicator of recent infection in the population [16,17]. Furthermore, several newly identified *P. falciparum* antigens such as Early transcribed membrane protein 5 (Etramp5) and gametocyte exported



protein 18 (GexP18) appear to elicit short-term antibodies and are therefore ideal for indicating recent infections [18].

Advances in the development of multiplex serological assays and novel informative antibodies have generated renewed interest in the use of serological to guide clinical practice and public health interventions. In this study we evaluated the use of *P. falciparum* and *P. vivax* serological markers in addition to microscopy and PCR data to estimate the level of transmission intensity and assessed the association between seropositivity and infection in a high transmission setting in Indonesia.

### **6.3 Methods**

#### **Study site**

This study was conducted in five sub-districts with ongoing malaria transmission in Mimika District (21,522 km<sup>2</sup>), the southern part of Papua Province, Eastern Indonesia (Fig. 1). The district had a population of approximately 196,401 in 2013, with the majority of the population (65%) living in Mimika Baru Sub-District [19]. The district is heavily forested [20], has a high humidity (average of 87%), with peak rainfall occurring between July and December [19]. There is significant economic migration due to the presence of a local mine resulting in the diverse ethnic origin of the population i.e. low lander Papuan, high lander migrant Papuan and Non-Papuan migrants living in the region [21]. Malaria transmission is restricted to the lowland areas, with three mosquito vectors: *Anopheles koliensis*, *An. farauti*, and *An. punctulatus* [22]. Despite extensive ongoing malaria control efforts, this region remains one of the highest malaria burdens in Indonesia, with an incidence rate of 249 per 1000 person-years for *P. falciparum* and 239 per 1000 person-years for *P. vivax* [23].

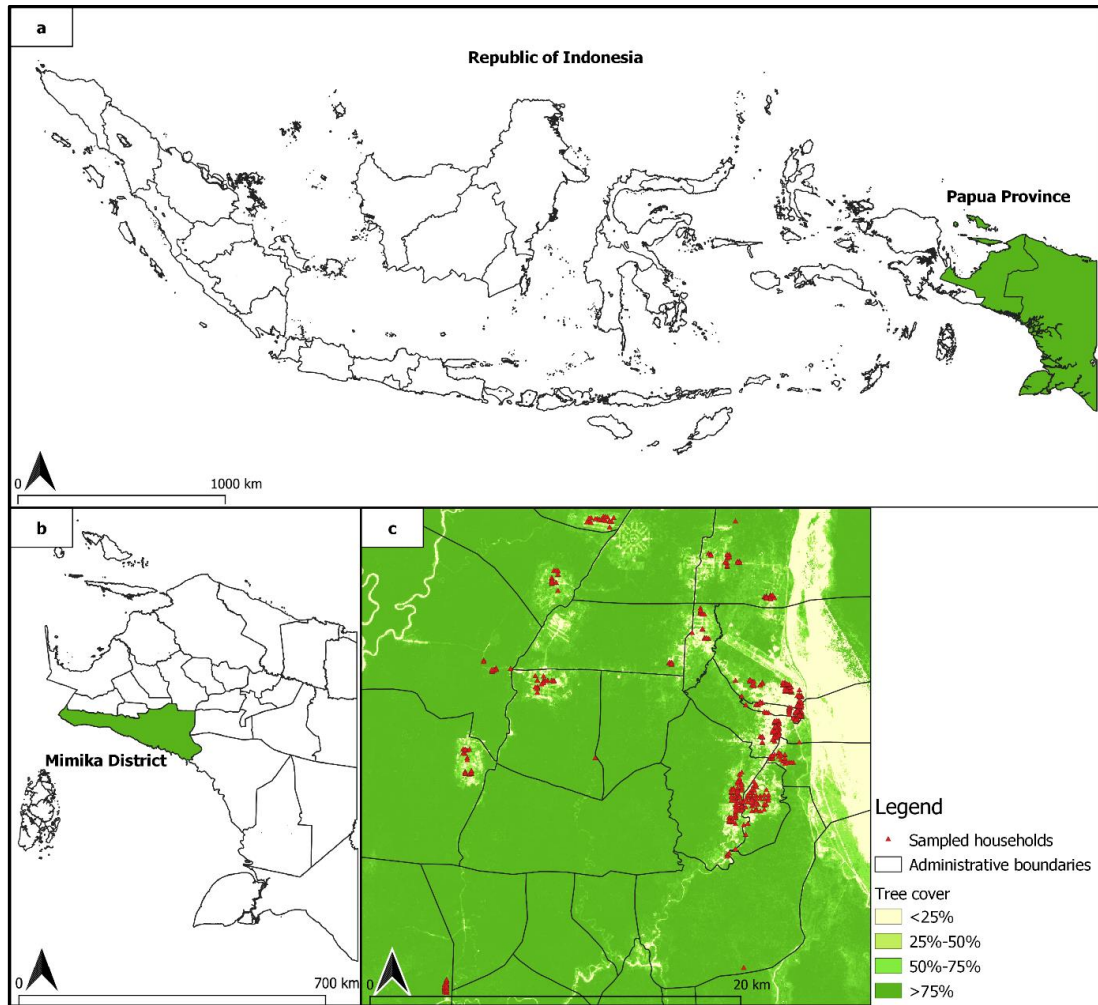


Figure 6.1 Maps showing a) location of Papua Province in Indonesia b) location of Mimika District in Papua Province, and c) geographical distribution of sampled households in Mimika District.

### Study design and data collection

Community-based cross-sectional surveys were conducted between April to July 2013. Household lists were obtained from local authorities and were arbitrarily assigned numbers according to their geographic location. Households were proportionally selected to represent each of the five sub-districts studied. Households were then randomly selected and invited to participate in the study. Households with no adult present were excluded from the survey and were replaced by neighbouring households. Individual written informed consent was obtained from all adults or guardians of the household members under 18 years

of age. Data on age, gender, ethnicity, history of fever in the last 30 days, bed net use, body mass index (BMI) to assess nutritional status, and pregnancy status among women aged over 14 years old were recorded using a short paper questionnaire. Samples were collected from all household members present if aged six months or older. Standard microscopy blood smears were collected as per routine national diagnostic standards. A 500 µl capillary blood sample was collected into a coded Microtainer® containing lithium heparin and fractionated then stored at -20°C at the laboratory at the Timika Research Unit, Papua. Household GPS coordinates were collected using handheld GPS, and shapefiles were obtained from the global administrative areas (GADM; <https://gadm.org/>), and the tree cover data derived from classified Landsat imagery at the 30-m resolution, were obtained from Hansen et al. [20].

### **Laboratory methods**

Parasite species was assessed from Giemsa-stained thick blood films. All positive films and 10% of the negative slides were cross-checked by a second microscopist at the Eijkman Institute for Molecular Biology (EIMB) in Jakarta and discrepancies reviewed by two expert microscopists for final assessment. PCR testing was performed by trained staff at EIMB, as described previously in Pava et al. [24]. Individuals were categorised as *P. falciparum* and *P. vivax* malaria infections if the malaria parasite was detected by either microscopy and or PCR. Serum samples were transported to the Parasitology Laboratory at the Department of Parasitology, UGM, Yogyakarta for serological testing using Luminex MAGPIX® (Luminex Corp, Austin, TX). A panel of *P. falciparum* and *P. vivax* antigens were assessed including apical membrane antigen 1 (PfAMA1; PvAMA-1), merozoite surface protein 1 (PfMSP-1-19; PvMSP-1-19), merozoite surface protein 2 (MSP2.Ch150) duffy binding protein region 2 (PvDBPR2), erythrocyte binding protein (PvEBP), reticulocyte binding protein 1 [amino acids

160–1170] (PvRBP1a), early transcribed membrane antigen 5 Antigen 1 (Etramp5.Ag1), gametocyte exported protein 18 (GexP18) and schizont egress antigen 1 (PfSEA-1) using a bead-based assay as described by Wu et al. [25] and read. To reduce confounding by the presence of maternally derived antibodies, results from infants under one year of age were excluded from the analysis [13]. MFI (median fluorescence intensity) values of samples were normalised against the MFI values of the positive control run on each plate, as described by Wu et al. [25].

### **Statistical analysis**

All statistical analyses were conducted in Stata IC 15 (Stata Corp, College Station, TX, USA). Parasite prevalence was defined as the proportion of infections detected by PCR in the sampled population. A cut off for seropositivity was defined as the mean MFI values plus three standard deviation of the seronegative population. Separate cut-off values were generated to determine seropositivity for each of the *P. falciparum* and *P. vivax* antigens tested [17]. Seroconversion rates (SCR) were estimated by fitting a reverse catalytic model to seroprevalence data for each antigen [13], except for three antigens associated with recent exposure (Etramp5.Ag1, GexP18, and PfSEA-1). Models allowing two forces of infection in SCR were fitted if deemed a better fit, using likelihood ratio methods. Receiver operating characteristic (ROC) analysis was performed to determine the sensitivity, specificity, and accuracy of serological markers in predicting PCR positive in comparison with microscopy.

## 6.4 Results

### Study population

**Table 6.1 Characteristics of sampled population and PCR positivity**

Variables (n=2496)	N	%	<i>P. falciparum</i>		<i>P. vivax</i>	
			n	% (95% CI)	n	% (95% CI)
<b>Age</b>						
< 5	426	17.1	34	8.0 (5.8-11.0)	68	16.0 (12.8-19.8)
5-15	638	25.6	123	19.3 (16.4-22.5)	160	25.1 (21.9-28.6)
> 15	1432	57.4	254	17.7 (15.8-19.8)	261	18.2 (16.3-20.3)
<b>Gender</b>						
Male	1011	40.5	178	17.6 (15.4-20.1)	198	19.6 (17.3-22.1)
Female	1485	59.5	233	15.7 (13.9-17.6)	291	19.6 (17.7-21.7)
<b>Ethnic</b>						
Non-Papuan	1434	57.5	141	9.8 (8.4-11.5)	244	17.0 (15.2-19.1)
Papuan	1062	42.5	270	25.4 (22.9-28.1)	245	23.1 (20.6-25.7)
<b>Sub districts</b>						
Mimika Baru	1345	53.9	154	11.4 (9.9-13.3)	270	20.1 (18.0-22.3)
Wania	487	19.5	84	17.2 (14.1-20.9)	99	20.3 (17.0-24.1)
Iwaka	426	17.1	107	25.1 (21.2-29.5)	85	20.0 (16.4-24.0)
Kuala Kencana	162	6.5	47	29.0 (22.5-36.5)	25	15.4 (10.6-21.8)
Kwamki Narama	76	3.0	19	25.0 (16.5-35.9)	10	13.2 (7.2-22.8)
<b>Bed net use</b>						
No	1625	65.1	258	15.9 (14.2-17.7)	329	20.2 (18.4-22.3)
Yes	871	34.9	153	17.6 (15.2-20.2)	160	18.4 (15.9-21.1)
<b>Pregnant</b>						
No	758	78.9	121	16.0 (13.5-18.7)	139	18.3 (15.7-21.3)
Yes	203	21.1	31	15.3 (10.9-20.9)	41	20.2 (15.2-26.3)
<b>Fever in last 1 month</b>						
No	2398	96.1	388	16.2 (14.8-17.7)	475	19.8 (18.3-21.5)
Yes	98	3.9	23	23.5 (16.1-32.9)	14	14.3 (8.6-22.7)
<b>Nutritional status</b>						
Normal	2363	94.7	397	16.8 (15.3-18.4)	466	19.7 (18.2-21.4)
Malnutrition	100	4.0	12	12.0 (6.9-20.0)	14	14.0 (8.5-22.3)
Severe malnutrition	33	1.3	2	6.1 (1.5-21.2)	9	27.3 (14.8-44.7)

In total, 2496 individuals from 747 households were included in the survey and provided blood samples. The general characteristics of these individuals are presented in Table 1. The mean number of people sampled per household was 3. The median age of participants was 21 years old (IQR: 7-35), and 60% (1485/2496) of individuals were female. The majority of the individuals came from Mimika Baru Sub-District (54%). The proportion of people

reporting having slept under a bed net the previous night was 35% (871/2496). Only 4% (98/2496) of the population reported experiencing fever in the previous month. Based on BMI assessment, 4% (100/2496) were malnourished, and 1% (33/2496) severely malnourished. There was a total of 203 pregnant women, representing 21% (203/961) of females over 14 years old.

### **Parasite prevalence by PCR**

The overall PCR-based parasite prevalence was 16.5% (411/2496) for *P. falciparum*, 20% (489/2496) for *P. vivax* and only 2% (60/2496) for mix infections. The *P. vivax* prevalence was higher than that for *P. falciparum*, and this was apparent across all age groups (Table 6.1). The prevalence was higher in children under 15 years old than adults for both *P. falciparum* and *P. vivax*. The prevalence varied between sub-district, with the highest *P. falciparum* prevalence detected in Kuala Kencana (29%), and *P. vivax* prevalence of 20% in three sub-districts (Mimika Baru, Wania and Iwaka).

### **Performance of microscopy and serological assay**

Overall, only 37.5% (154/411) of *P. falciparum* and 30.5% (149/489) of *P. vivax* PCR positive individuals were detected by microscopy. The sensitivity and accuracy of diagnosis significantly decreased by age, with consistent 100% specificity for both *P. falciparum* and *P. vivax* in all ages. For *P. falciparum*, the sensitivity of microscopy test was 59% (ROC area 80% (95% CI: 71-89)) in 1-5 years old, 45% (ROC area 72% (95% CI: 68-77)) in 5-15 years old and 31% (ROC area 66% (95% CI: 63-68)) in over 15 years old. For *P. vivax*, the sensitivity was 60% (ROC area 80% (95% CI:74-86) in 1-5 years old, 33% (ROC area 66% (95% CI:63-70)) in 5-15 years old and 22% (ROC area 61% (95% CI:58-63) in over 15 years old.

The sensitivity, specificity, and ROC area of seropositivity to each antigen for identifying *P. falciparum* and *P. vivax* PCR positive individuals are presented in Table 6.2. The age-adjusted ROC estimates revealed that seropositivity to Etramp5.Ag1 and PvEBP were the best predictor for *P. falciparum* and *P. vivax* PCR positivity, respectively. Whilst the sensitivity increased by age, the specificity and ROC area decreased by age for both *P. falciparum* and *P. vivax*, likely a reflection of cumulative exposure. The ROC area for Etramp5.Ag1 was 76% (sensitivity 65% and specificity 88%) in 1-5 years old, 72% (sensitivity 67% and specificity 77%) in 5-15 years old, and 64% (sensitivity 68% and specificity 60%) in over 15 years old population. The ROC area for PvEBP was 69% in 1-5 years old, 64% in 5-15 years old, and 51% in over 15 years old population.

**Table 6.2 Sensitivity (Sn), specificity (Sp) and receiver operating characteristics (ROC) area estimates of each antigen in predicting *P. falciparum* and *P. vivax* PCR positive**

	1-5 years old			5-15 years old			> 15 years old		
	Sn (95% CI)	Sp (95% CI)	ROC (95% CI)	Sn (95% CI)	Sp (95% CI)	ROC (95% CI)	Sn (95% CI)	Sp (95% CI)	ROC (95% CI)
<b><i>P. falciparum</i></b>									
<b><i>long-lived</i></b>									
<b>PfMSP-1<sub>-19</sub></b>	<b>79% (62-91)</b>	<b>69% (64-73)</b>	<b>74% (67-81)</b>	<b>91% (85-96)</b>	<b>43% (38-47)</b>	<b>67% (64-70)</b>	<b>97% (93-98)</b>	<b>19% (16-21)</b>	<b>58% (56-59)</b>
PfAMA1	65% (47-80)	79% (75-83)	72% (64-80)	88% (81-93)	56% (52-61)	72% (68-76)	93% (89-95)	24% (22-27)	58% (56-61)
MSP2.Ch150	47% (30-65)	86% (82-89)	66% (58-75)	59% (50-68)	81% (77-84)	70% (65-75)	70% (64-75)	49% (46-52)	59% (56-63)
<b><i>P. falciparum</i></b>									
<b><i>short-lived</i></b>									
<b>Etramp5.Ag1</b>	<b>65% (47-80)</b>	<b>88% (84-91)</b>	<b>76% (68-85)</b>	<b>67% (58-75)</b>	<b>77% (73-81)</b>	<b>72% (67-76)</b>	<b>68% (62-73)</b>	<b>60% (57-63)</b>	<b>64% (61-67)</b>
PfSEA-1	47% (30-65)	89% (86-92)	68% (60-77)	50% (41-59)	78% (75-82)	64% (59-69)	65% (59-71)	59% (56-62)	62% (59-65)
GexP18	59% (41-75)	75% (70-79)	67% (58-76)	62% (52-70)	77% (74-81)	70% (65-74)	58% (52-64)	67% (64-69)	62% (59-66)
<b><i>P. vivax</i></b>									
<b>PvEBP</b>	<b>68% (55-79)</b>	<b>69% (64-74)</b>	<b>69% (62-75)</b>	<b>82% (75-88)</b>	<b>46% (42-51)</b>	<b>64% (60-68)</b>	<b>86% (81-90)</b>	<b>15% (13-17)</b>	<b>51% (48-53)</b>
PvAMA1	60% (47-72)	74% (69-78)	67% (60-73)	62% (54-69)	55% (51-60)	58% (54-63)	80% (75-85)	23% (21-26)	52% (49-54)
PvMSP-1 <sub>-19</sub>	60% (48-72)	73% (68-78)	67% (60-73)	61% (53-68)	61% (56-65)	61% (56-65)	65% (59-71)	37% (35-40)	51% (48-54)
PvDBPR2	40% (28-52)	84% (80-88)	62% (56-68)	41% (34-49)	70% (66-74)	56% (51-60)	47% (41-53)	51% (48-54)	49% (46-52)
PvRBP1a	28% (17-40)	86% (81-89)	57% (51-62)	29% (22-37)	79% (75-83)	54% (50-58)	35% (29-41)	58% (55-61)	47% (43-50)



## Seroprevalence and seroconversion rate estimates

The median antibody responses to multiple *P. falciparum* and *P. vivax* antigens increased with age (Figure 6.2). The overall seroprevalence to *P. falciparum* long-lived antibody responses was 71% (1756/2485), 63% (1561/2489), and 41% (1019/2491) for PfMSP-1-19, PfAMA1, and MSP2.Ch150, respectively (Table 6.2). Seroprevalence to *P. falciparum* short-lived antibody responses was 37% (917/2496), 36% (876/2488), and 34% (851/2493) for Etramp5.Ag1, PfSEA-1, and GexP18, respectively. For *P. vivax*, the seroprevalence was 71% (1762/2496), 63% (2554/2486), 53% (1326/2495), 40% (983/2496), and 32% (797/2492) to PvEBP, PvAMA1, PvMSP-1-19, PvDBPR2, and PvRBP1a, respectively.

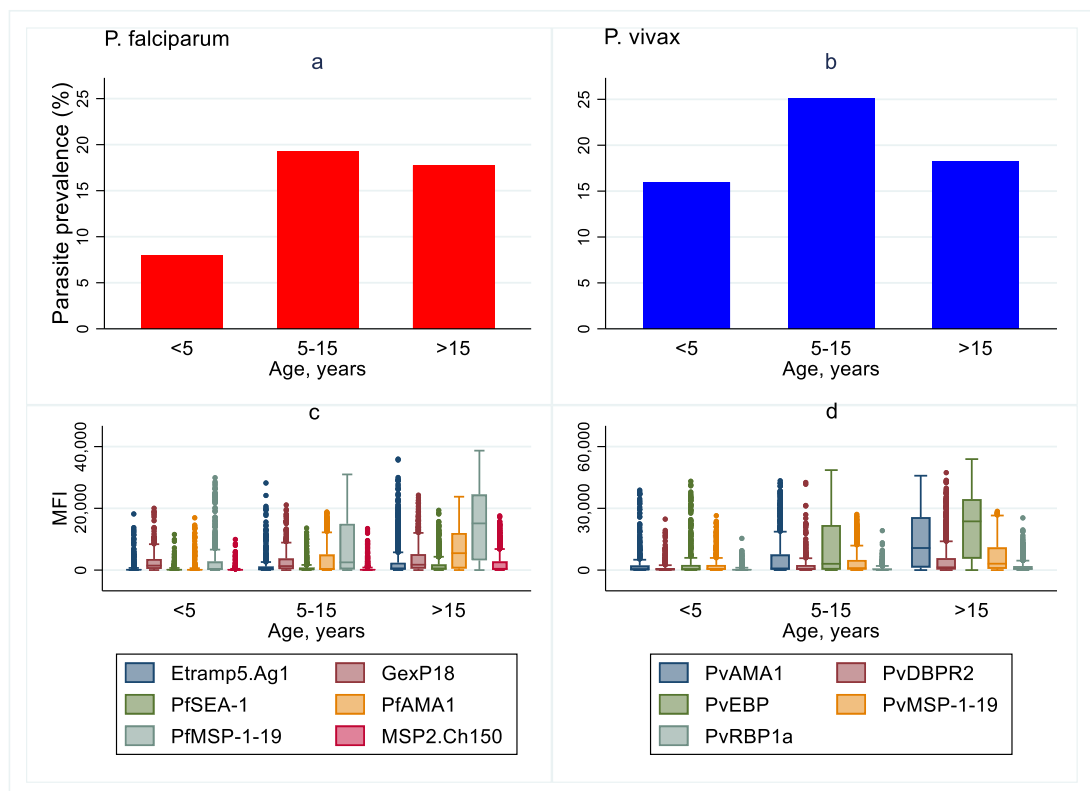


Figure 6.2 Parasite prevalence and antibody responses over age. **a-b)** *P. falciparum* and *P. vivax* parasite prevalence over age. **c-d)** Antibody responses to each of *P. falciparum* and *P. vivax* antigens over age. Etramp5.Ag1, GexP18 and PfSEA-1 are antigens associated with *P. falciparum* short-lived antibody responses.

The population-level SCR estimate for each of *P. falciparum* and *P. vivax* long-lived antibody responses suggested there was no evidence of historical changes in transmission intensity for either *P. falciparum* or *P. vivax* (Figure 6.3). The highest *P. falciparum* SCR was obtained from responses to PfMSP-1-19, with SCR 0.156 person-year (95% CI: 0.138-0.177). Whilst for *P. vivax*, the highest estimate was obtained from responses to PvEBP, with SCR 0.141 person-year (95% CI: 0.124-0.159).

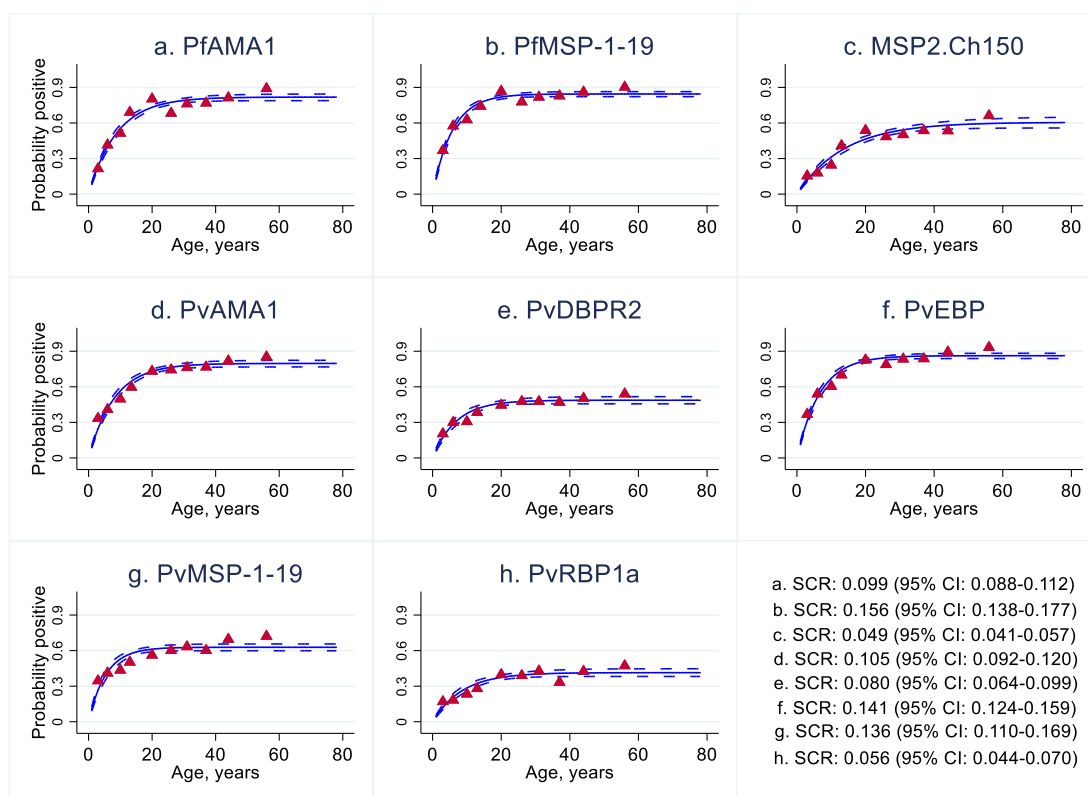


Figure 6.3 Age-seroprevalence plots for each of *P. falciparum* antigens (a-c) and *P. vivax* antigens (d-h) in Mimika, 2013. Solid lines represent the fitted probability for being seropositive to each of the antigens. Dashed lines represent the 95% confidence interval of these fits, and red triangles represent the observed proportion of seropositive per age decile. SCR value represents the average annual rate at which the population become seropositive to each of the *P. falciparum* or *P. vivax* antigen.

## 6.5 Discussion

This study presents an epidemiological analysis of parasitological and serological data from a community-based cross-sectional study in southern Papua in eastern Indonesia. The findings highlight the potential use of short-lived antibody responses to diagnose individuals with *P. falciparum* parasitaemia, including those with very low-density infections. The ROC estimates suggest that the prediction was better in children compared to adults over 15 years old as the specificity in adults decreased due to cumulative exposure to infection and the half-life of antibodies produced to previous infections. In addition, the analysis of *P. falciparum* and *P. vivax* long-lived antibody responses revealed that PfMSP-1<sub>-19</sub> and PvEBP were the most immunogenic antigens enabling the utility of these antigens to measure transmission intensity in this high transmission setting.

Seropositivity to each of the *P. falciparum* short-lived antibody responses (Etramp5.Ag1, GexP18 and PfSEA-1) had greater sensitivity for detecting any peripheral parasitaemia compared to microscopy alone. Whereas microscopy only detected 59% of PCR positive *P. falciparum* infection in children 1-5 years, the sensitivity of Etramp5.Ag1 in the same age group was 65%. Although the specificity of Etramp5.Ag1 was lower than microscopy (88% vs 100%), the ROC area was only slightly lower than microscopy (80% vs 76%). Whilst a fairly good performance also seen in children 5-15 years old (ROC area 72%), the seropositivity to Etramp5.Ag1 was less useful for predicting PCR infection in adults (ROC 64%). In a previous cohort study, *P. falciparum* antibody responses to short-lived antigens such as Etramp5.Ag1 and GexP18 predicted an individual's incidence in the preceding year in Ugandan children [18]. Our findings support a previous study in Kenya in which multiple *P. falciparum* antigens

were used as a proxy of recent transmission [26] and further highlighted the important application of serology for malaria surveillance [27].

Although seropositivity to the most immunogenic *P. vivax* antigen (PvEBP) had greater sensitivity for detecting any peripheral parasitaemia compared to microscopy alone, the specificity of this antigen was very low (69%, 46% and 15% in 1-5 years old, 5-15, and over 15 years old, respectively), thus is not suitable for predicting *P. vivax* recent exposure. Our study did not include antigens associated with *P. vivax* short-lived antibodies. However, suitable candidates were recently reported [28], and thus, future work should evaluate these in *P. vivax* endemic settings.

The SCR estimates generated from seropositivity to the most immunogenic *P. falciparum* and *P. vivax* long-lived antibody responses (PfMSP-1-<sub>19</sub> and PvEBP) revealed a higher transmission intensity for *P. falciparum* than *P. vivax*. Closer examination of the SCRs estimates suggested no step-change in transmission intensity, with population-level SCRs equating to approximately 156 per 1000 people seroconverting per year for *P. falciparum* and 141 per 1000 people for *P. vivax*. These serological estimates are consistent with previous findings reporting higher incidence rate for *P. falciparum* than *P. vivax* (249 per 1000 person-years for *P. falciparum* and 239 per 1000 person-years for *P. vivax*) [23], supporting a growing body of evidence that serological analysis can be used to determine population-level transmission intensity in a wide range of endemic settings [29-33].

In young children under five years old, the prevalence of *P. vivax* prevalence was twice as high as that of *P. falciparum* (16% vs 8%), as has been shown in previous cohort studies in

areas co-endemic to both species [34-36]. The SCR estimates revealed that seropositivity to both *P. falciparum* and *P. vivax* was higher in older children and adults compared to young children. These findings likely reflect the cumulative exposure in the adult population. However, this could also reflect a higher risk of malaria in the adult population, due to different behavioural activities such as forest-related jobs and night outdoor activities. These behavioural risks were previously reported in western Indonesia [31,37], Cambodia [38], and other places in Asia such as Malaysia, Bhutan, Philippines and Sri Lanka [39]. However, interpretation of these data is limited by the lack of information regarding the travel history and occupational risk that might explain the aged related differences. Whilst our study was conducted in the forested area, future studies will need to explore association behaviour and migration risks and how these relate to peripheral parasitaemia and seropositivity.

## 6.6 Conclusion

The serological analysis confirmed a high level of *P. falciparum* and *P. vivax* transmission intensity in Papua, Indonesia. Although seropositivity to Etramp5.Ag1 was a sensitive and specific predictor of concurrent *P. falciparum* PCR positivity in children, its utility was less useful in adults. Our study highlights the potential role of analysing multiple antigens to explore malaria epidemiology that can inform public health surveillance and programme evaluation in high transmission areas. However, analysis is ongoing to explore the best way to evaluate the use of serological data available in the present dataset. Future cohort studies evaluating *P. falciparum* and *P. vivax* antigens associated with short-lived antibodies to diagnose concurrent low-level parasitaemia are needed in different endemic settings so that

these hidden reservoirs of infection and ongoing transmission can be identified and eliminated.

### **Abbreviations**

CI: confidence interval; Etramp5.Ag1: early transcribe membrane protein 5 antigen 1; GexP18: gametocyte exported protein 18; PCR: polymerase chain reaction; PfAMA1: *P. falciparum* apical membrane antigen 1; PfMSP-1: *P. falciparum* merozoite surface protein 1; PfSEA-1: schizont egress antigen 1; PvAMA-1-19: *P. vivax* apical membrane antigen 1; PvEBP: *P. vivax* erythrocyte binding protein; PvMSP-1: *P. vivax* merozoite surface protein 1; PvRBP1a: *P. vivax* reticulocyte binding protein 1a [amino acids 160–1170]; SCR: seroconversion rates.

### **Ethics approval and consent to participate**

All biological samples and data were collected with written, informed consent from the participant or a parent or guardian. Ethical approval for this study and the process of informed consent, was obtained from the Eijkman Institute Research Ethics Commission, Eijkman Institute for Molecular Biology, Jakarta, Indonesia, (Ref: KE/FK/763/EC) and the Human Research Ethics Committee of the Northern Territory (NT) Department of Health & Families and Menzies School of Health Research, Darwin, Australia (Ref: HREC-2010-1434).

### **Consent for publications**

Not applicable

**Availability of data and materials**

The datasets used and analysed during this study are not publicly available due to the inclusion of identifying information on individuals but are available from the corresponding author on reasonable request.

**Competing interests**

The authors declare that they have no competing interest.

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**Authors' contributions**

HS performed the statistical analysis and wrote the manuscript. JRP and RNP designed the study and supervised the data collection. S and HS supervised the serological work. ZP performed PCR assay. EK performed the data collection. RAK and R performed the serological assay. KKAT produced the antigens and developed the serological assay. HS, RNP, JRP, JC, and CD interpreted findings and reviewed the manuscript. All authors read and approve the final manuscript.

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## 7. General discussion

### 7.1 Main research findings

This thesis brings together the application of serological methods in areas of differing endemicities and highlights the potential use of serological markers to identify receptive areas in low transmission settings (Chapter 3 and 5), the continued utility of seroprevalence and seroconversion rate as a metric to estimate transmission for both *P. falciparum* and *P. vivax* (Chapter 3, 5 and 6) and starts to evaluate the use of antigens that elicit short-term antibodies as a proxy for diagnosis (Chapter 6). This thesis also describes the potential application of a mobile technology-based participatory mapping as an alternative geolocation approach for research and public health surveillance purposes (Chapter 4) and use of health facility-based surveys recruiting both patients and accompanying people as an alternative sampling strategy to increase passive surveillance coverage (Chapter 5).

#### ***Potential markers for serological surveillance***

PfAMA1 and PvAMA-1 were identified as the most immunogenic antigens for *P. falciparum* and *P. vivax* in low transmission settings (Sabang and Kulon Progo). Importantly, although the spatial analysis of serological data did not add useful information in a high transmission setting due to uniform risk of transmission in the population (Chapter 6), the spatial analysis of age-adjusted antibody responses to PfAMA1, PfMSP-1<sub>-19</sub>, PvAMA-1 and PvMSP-1<sub>-19</sub> were sensitive and useful to identify areas at risk for malaria outbreak in very low transmission setting (Chapter 3 & 5). Interestingly, in the area where there was no active cases recorded in three consecutive years, the spatial analysis of age-adjusted antibody responses to PvMSP-1<sub>-19</sub> revealed the high-risk areas that subsequently became areas of *P. knowlesi* outbreak in the following year after the

area certified as malaria-free (12), most likely because of cross-reactivity between the PvMSP-1-<sub>19</sub> and several *P. knowlesi* antigens as recently found by Herman et al. in a study in Malaysia (194).

In the high transmission setting, Mimika, the seropositivity to the most immunogenic *P. falciparum* antigen that reflect short-term exposure (Etramp5.Ag1) was sensitive and specific in predicting *P. falciparum* PCR positive infections in children but was not useful in over 15 years old population. This finding suggests the potential use of Etramp5.Ag1 as a marker of recent exposure in children, which is important for surveillance and programme evaluation in elimination settings. For example, this antigen can be used to screen under 15 years old population as a proxy to better target area of intervention. Furthermore, PfMSP-1-<sub>19</sub> and PvEBP were identified as the most immunogenic antigens, enabling good estimate of SCRs for *P. falciparum* and *P. vivax* in this setting. This can mean that assessment of transmission intensity in a high transmission setting might be better done by testing samples against these two antigens using ELISA instead of multiplexing using the bead-based assay.

The relationship between serological and parasitological measures is presented in Figure 7.2. In a high transmission setting, Mimika, seroprevalence data show a poor relationship with the parasite prevalence data for both *P. falciparum* and *P. vivax*. This suggests that seroprevalence is less useful in high transmission settings, as parasite prevalence measures would be expected to remain sensitive. It has been known that serological measures are most useful in low transmission areas where conventional measures such as EIR and PR are insensitive. In low transmission settings where the parasite prevalence was very low (0.06% in Kulon Progo) and

zero (Sabang), serological measures were able to detect seropositive individuals, allowing identification of areas and population at risk of malaria exposure.

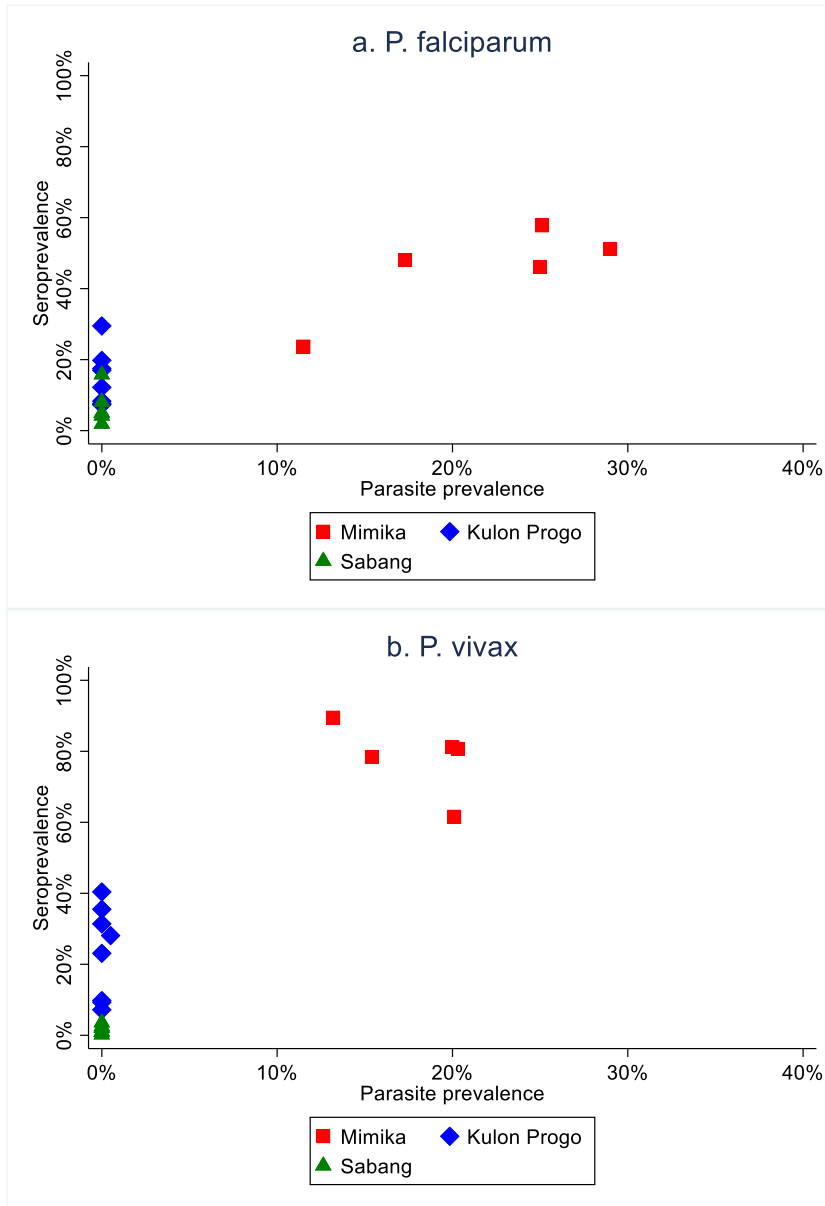


Figure 7.1 Relationship between a) *P. falciparum* parasite prevalence and seroprevalence, b) *P. vivax* parasite prevalence and seroprevalence.

For Mimika, seroprevalence was based on seropositivity to antigen associated with recent exposure (*Etramp5.Ag1*) for *P. falciparum* and to the most immunogenic long-lived antigen (*PvEBP*) for *P. vivax*. For Kulon Progo and Sabang, seroprevalence was based on seropositivity to the most immunogenic antigens i.e. *PfAMA1* for *P. falciparum* and *PvAMA-1* for *P. vivax*.

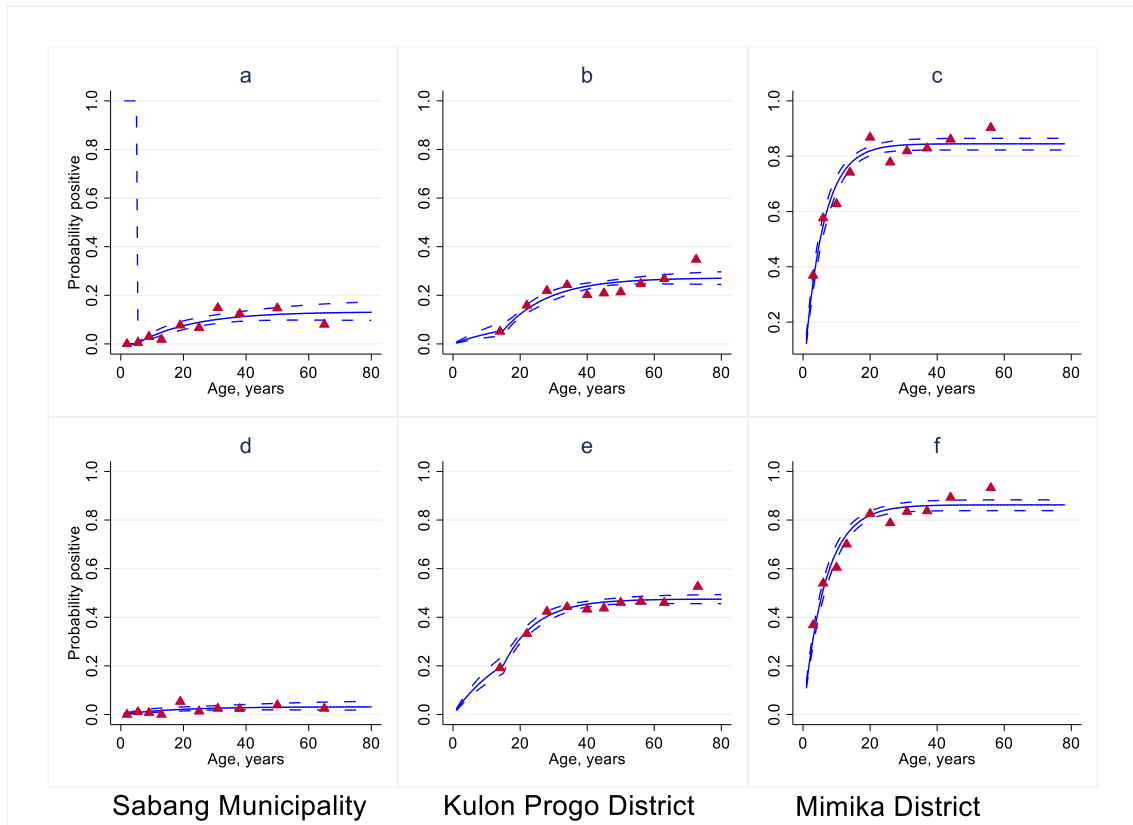


### ***Heterogeneity and risk factors of transmission***

This thesis provided evidence on the use of serological surveillance to better understand transmission dynamics in three different endemic settings where *P. falciparum* and *P. vivax* are co-endemic. Population-level SCRs estimates obtained from three different studies (Chapter 3, 5 and 6) highlights the heterogeneity of *P. falciparum* and *P. vivax* transmission in Indonesia (Figure 7.2). As expected, the SCRs estimates can clearly differentiate transmission level in the three study sites. The estimates also consistent with previous studies suggesting that the risk of exposure to both *P. falciparum* and *P. vivax* was higher in adults than children, especially in low transmission settings, most likely due to different behavioural activities such as forest-related jobs and night outdoor activities.

While the malaria risk is spatially homogeneous in the high transmission setting, the spatial analysis of antibody responses conducted in elimination settings (Chapter 3 and 5) suggest the spatial heterogeneity of malaria risk, with clusters of high exposure to *P. falciparum* and *P. vivax* identified in forested places. Moreover, the multivariable analysis also reveals that higher exposure to malaria was associated with bed net use in the districts nearing elimination but not in the district with high transmission. Although this finding can suggest a good bed net coverage in areas with high exposure to malaria, this can also mean that bed net use may no longer effective to prevent malaria transmission in low transmission setting where majority of the remaining cases are related to outdoor exposure such as when working at forest or sleeping in farm or plantation. This explanation is also supported by previous studies suggesting malaria vectors in Indonesia were more likely to rest and bite outdoor (166). These findings suggest the importance of targeting adults and those people who work and/or live at forested areas, for both surveillance and intervention programmes. Alternative interventions to prevent outdoor and

forest-related transmission are needed to support the country achieve malaria elimination by 2030.



*Figure 7.2 Population-level transmission intensity based on SCR estimates in Elimination (Sabang), Pre-elimination (Kulon Progo) and High transmission (Mimika) setting. Panel a, b and c represent *P. falciparum* SCR. Panel d, e and f represent *P. vivax* SCR. As described in Chapter 3 and 5, SCRs estimate for Sabang and Kulon Progo were based on seropositivity to any of *P. falciparum* and *P. vivax* antigens tested. SCRs estimate for Mimika was based on seropositivity to PfMSP-1-19 and PvEBP for *P. falciparum* and *P. vivax*, respectively.*

### **Alternative sampling and geolocation strategy**

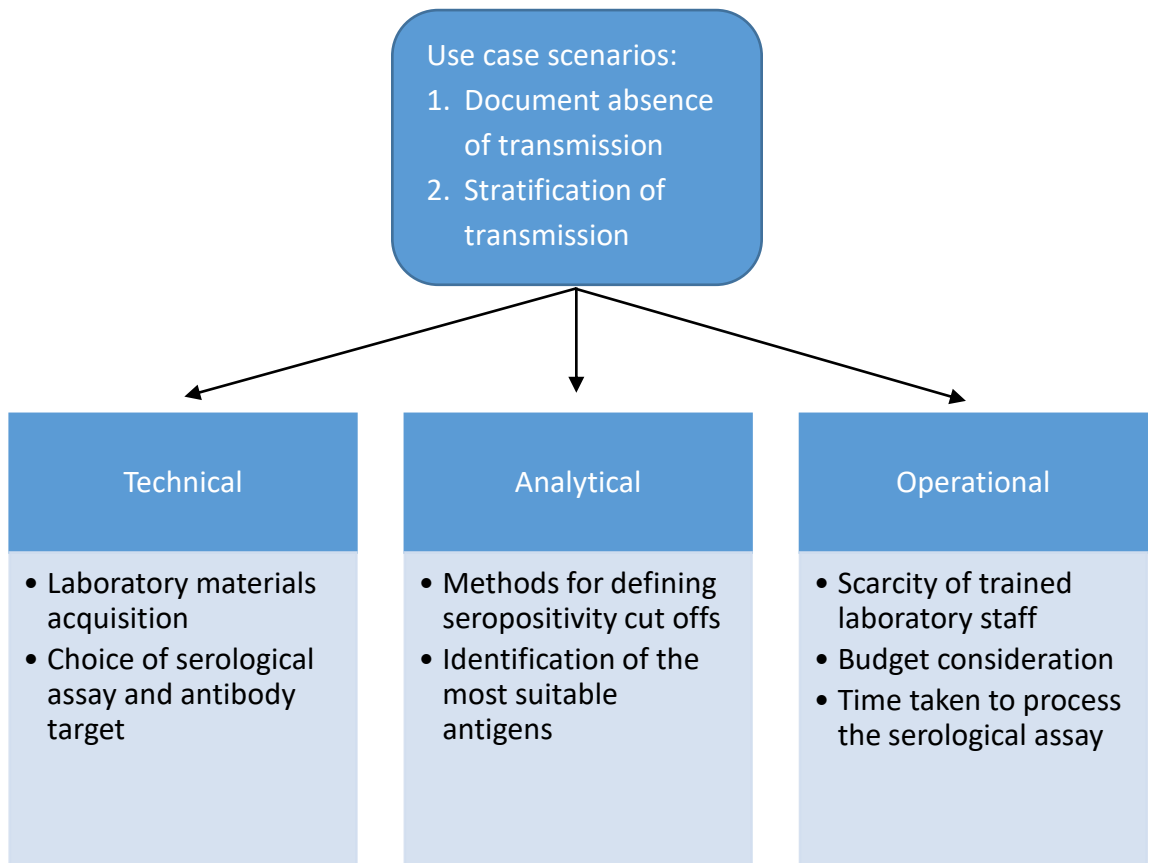
The choice of serological sampling strategy and the geolocation approach will be influenced by several factors such as characteristics of the population (i.e. health seeking behaviour, mobility and size of population) and the geographical area (i.e. catchment areas and geographical

accessibility), of the setting, and the transmission level. For example, the use of health facility-based sampling and participatory mapping approach might be suitable for low transmission areas with good access to health services and relatively large population or geographical catchment areas. The study in Chapter 5 has illustrated the feasibility of conducting serological surveillance utilising the existing health facility-based surveillance systems in a low transmission setting conducting elimination. The use of health facility surveys is appropriate for capturing malaria burden as shown by the high participation rates and consistency of demographical characteristics of people sampled over times. The inclusion of all health facility attendees (regardless of their symptoms) and their companions in the serological surveys was feasible and can improve sampling coverage of the existing health facility-based surveillance system. However, the use of different public facilities or different sampling approaches may be more appropriate in other settings. For example, active sampling may be more appropriate in areas where cases are associated with mobile populations and certain occupations such as mining or logging that make people unlikely to attend health facility to seek for treatment (167,195). The use of community-based collection of samples and household GPS coordinates might also be more suitable for moderate or high transmission areas where sample size required is small, and/or areas with poor access to health services due to factors such as treatment preferences (e.g. self-medication or traditional healing practices) or due to limited access to universal health coverage, and/or for hard to reach areas. Finally, the community-based method can also be useful for low transmission areas with relatively small population and geographical areas.

## 7.2 Future directions

### *Implementation of serological surveillance: challenges and opportunities*

Different applications of serological surveillance presented in this thesis could be used to better target surveillance and intervention, thus resources can be efficiently allocated in the future. However, potential challenges will need to be assessed to enable integration into the existing surveillance systems in different settings in Indonesia. These challenges can range from technical, analytical to operational aspects of the approaches. Several example of potential challenges at each aspect of serological surveillance evaluated in this thesis are summarised in Figure 7.3.



*Figure 7.3 Potential challenges in implementing serological surveillance to document absence of transmission and stratification of transmission.*

- **Technical**

At technical level, the potential implementation challenge is mainly related to the acquisition of laboratory materials and the choice of serological assay and antibody target. The majority of the malaria antigens used in this thesis were produced by our research group at the LSHTM, UK and other research collaborator at the Institut Pasteur, France, for research purposes. In addition, reagent and consumables used for the bead-based assays were purchased in the UK and transferred to Indonesia. Although majority of these materials are commercially available, cost will be much more expensive, and time needed for purchasing and shipping will be longer if order made locally. Although useful for research, the use of multiplex bead-based assay may be less suitable for programmatic use. ELISA is more available and commonly used for malaria and other diseases, thus can be a suitable option for programmatic use. However, as discussed above, the antibody target must be carefully chosen to best suit the use case scenarios for surveillance and programmes need.

- **Analytical**

At analytical level, issues are related to what is the best analytical methods in different scenarios. Firstly, there is an outstanding issue related to how is the best way to define seropositivity cut-offs for each antigen. Serological data analysis in this thesis was mainly based on reverse catalytic models estimating population level force of infection (SCR) which assume that seroprevalence will increase with age. A limitation of these approaches is that seroprevalence is potentially influenced by the seropositivity threshold. Seropositivity cut-offs in Chapter 3 was based on antibody responses of nonexposed individuals tested alongside with the studied samples. Individual was classified seropositive if the respective antibody levels exceed the mean plus 3 times the standard deviation of the seronegative population. Despite ensuring a high probability

of correctly classifying exposed individuals, this approach has the limitation of underestimating seroprevalence (142). On the other hand, seropositivity cut-offs in Chapter 5 and 6 were based on the two-component finite mixture model (FMM). This model was used on samples under analysis only (without additional data on nonexposed individuals). This approach relies on the basic assumption that the samples are a mixture of latent seronegative or seropositive populations (142). Similar to the cut-off used in Chapter 3, individuals were classified as seropositive if the respective antibody levels exceed the mean plus 3 times the standard deviation of the seronegative population identified by the model. Although the FMM model showed a good discrimination between seronegative and seropositive population in our dataset, this approach may not be ideal for identifying cut-off for several new antigens associated with more recent exposure in other low endemic settings.

Secondly, the choice of whether the analysis needs to be based on combined responses from multiple antigens or not can be influenced by the use case scenarios and level of transmission. For example, to document absence of transmission and stratify transmission, SCRs estimates in very low transmission settings (Chapter 3 and 5) were based on responses to multiple antigens. The rationale for this choice was based on idea that combining responses to multiple antigens will increase probability/analysis sensitivity in capturing exposure in the absence of active infections, as individuals may respond differently to each antigen. Whilst for estimating transmission intensity in a high transmission setting such as in Chapter 6, estimating SCRs from responses to a single most immunogenic antigen was the best option as majority of individuals responded to this antigen therefore provided representative estimate of the population.

- **Operational**

At the operational level, the potential challenges are there is a huge discrepancy in the existing malaria control program and surveillance capacity and budget allocation across districts in Indonesia. This is partly due to the decentralization of the health sector implemented in 2002 that put responsibility for planning and service delivery to local governments at the district level (196). The different capacity and budget allocation for malaria control and surveillance activities can limit the application of serology and any other new tools in some districts.

The serological assays used in this thesis (ELISA and bead-based assay) are relatively easy to perform and are available in several academic and government reference laboratories in Indonesia. However, while ELISA is a more commonly used platform for malaria and other diseases, the reagents and consumables required for the bead-based assays are relatively expensive for programmatic use. In addition, the time taken to order the reagents (3-6 months) may also affect the sustainability of using this assay for public health program.

Another potential challenge is the length of time taken from processing samples to generate the interpretable results for programmatic use. The WHO advised that as transmission declines, surveillance becomes more essential and requires more frequent data analysis and reporting to ensure adequate response can be taken in time (38). Diagnostic platforms such as serological lateral flow devices (sero-RDTs) that can perform more rapid combined antibodies test will be more useful for public health surveillance and control programme use, and research is ongoing to develop this tool (96).

The first alternative way to tackle these operational challenges is to integrate a multi-disease serological surveillance system that can include malaria, vaccine-preventable diseases (VPDs), neglected tropical diseases, and other notifiable diseases in population. Arnold et al. recently discussed about the potential of integrated serological surveillance to monitor infectious disease transmission and their interactions in population (197). The majority of currently available machines in Indonesia can test up to 50 different antigens in one run, offering a highly efficient way to monitor multi-diseases transmission and their interaction in the population. As the bead-based assay requires as little as 1  $\mu$ l of serum to determine up to 50 antibody responses simultaneously, measuring antibody responses to antigens from multiple diseases in one run will significantly reduce costs and time. The price of a Luminex MAGPIX machine used in this thesis was approximately £16,000 for order made in the United Kingdom. Our costing suggests that it costs approximately £1.00 per sample to test a minimum of 1000 samples against 25 different antigens. Although the machine is more expensive than ELISA reader, it was estimated that the cost of running multiplex assay using the Luminex MAGPIX can be up to four times less expensive than comparable ELISA assay (<https://www.luminexcorp.com/research-magpix/>). Furthermore, the integration of multi-disease surveillance can also greatly reduce cost and time needed to perform each surveillance components from data collection, analysis (both laboratory and statistical), reporting into formulating public health responses needed to control the diseases. Examples of this approach is given in Section 7.4.

Another option is to build collaboration between the ministry of health and universities and/or research institutes which are widely spread across Indonesia. One local example of this is the current ongoing collaboration between Indonesia Ministry of Health with UGM and Drakeley's group at LSHTM which aimed at implementing serological surveillance to evaluate changes in



malaria exposure upon implementation of intervention packages in high transmission settings in Indonesia. If successful, this collaborative serological surveillance exercise can be a model that can be widely implemented in Indonesia.

### ***Identifying and targeting hotspots of transmission***

This thesis has provided evidence for how serological surveillance can be used to identify hotspots of malaria transmission. Methodologies discussed in Chapter 3, 4 and 5, have a strong potential use in identifying hotspots of transmission (defined as clusters of higher than average anti body responses in this thesis) that potentially become source of malaria reintroduction in malaria elimination settings. It has been reported that hotspots can maintain malaria transmission in low transmission seasons and fuel transmission in the high seasons (43). However, this will depend on demographical and spatial representativeness of the sampling and mapping approaches employed. In Chapter 4, we have demonstrated that participatory mapping can generate a fine scale resolution that can accurately locate household into a correct hamlet unit. This indicates that a hamlet or village level intervention is likely a suitable choice for targeting hotspot identified using this approach.

Moving forward, the serological surveillance methods presented in this thesis could potentially be used by the malaria surveillance and control program to better target the intervention. Hotspot-targeted interventions has been reported as a highly efficient malaria control and elimination strategy that could rapidly reduce malaria burden at all levels of transmission intensity (43). This approach will supplement the current high-risk targeted approach such as providing LLINs and intermittent preventive treatment (IPT) to young children and pregnant women that aims to reduce severe morbidity and mortality. The hotspot-targeted interventions

offer a logistically attractive alternative to untargeted interventions that may need coverage levels nearing 100% to drive transmission lower, especially in moderate and low transmission settings where malaria transmission is highly heterogeneous (42,46,198). However, feasibility of implementing and integrating this approach into existing malaria surveillance and control programme needs to be assessed.

In future, serological measures could also play important role in helping the stratification of malaria risk as suggested by the WHO, especially in areas where EIR and PR are very hard to measure due to very low number of infected human and mosquitoes. As serological measures are increasingly recorded in many settings, these data could potentially be incorporated into a system such as in the Malaria Atlas Project (17,18) to allow risk stratification at minimum level.

### **7.3 Limitations**

First, while the use of health facility-based sampling is more efficient in a low transmission setting, this approach is likely to miss asymptomatic infections, as well as those occurring in people who choose not to use public facilities. However, the inclusion of all health facility attendees and their companions in the surveys has reduced this bias. In addition, people living further from facilities may be less likely to attend health facilities resulting in the methods being less likely to detect clusters of high exposure further from facilities. However, it is conceivable that iterative refinements of the maps over time with clinical and demographic data would improve this. Inclusion of mapping exercise in active surveillance performed by community health workers would be useful to capture heterogeneity in areas further from the facilities.

Second, although the mobile technology-based participatory mapping approach provides an alternative approach to remotely collect GPS coordinates of health facility attendees, the accuracy of this approach varied between health facilities and decreased in less populated areas with fewer prominent landmarks. Prior experience of using map may also influence the accuracy of this mapping approach. For example, younger people may have more experience in using digital maps compared to older. However, this factor was not evaluated in the study. Future studies need to improve the accuracy of this approach (i.e. adding as much as available landmarks), test the validity and feasibility of this approach in different settings to enable implementation in a broader operational context.

Finally, although our findings demonstrated the promising application of analysing multiple antibody responses data for surveillance use, the data generated will need to be translated into easily interpretable metrics of transmission. As previously discussed in Chapter 5, research is still ongoing to investigate the best analytical approaches to analyse the complex quantitative data generated by the multiplex assay. Methods applied in this thesis were based on standard seroprevalence, SCRs and regression models that have been widely used in previous malaria research. However, there is a more complex and robust methods such as Bayesian hierarchical models (199,200) that can be used to investigate the most epidemiological informative antigens but were not explored in this thesis. Although standard SCR and regression analysis in this thesis has generated important findings, future studies will need to explore any other approaches to combine these into more readily usable metrics.

#### 7.4 Implications for other infectious diseases

Methodologies used in this thesis can potentially be applied for multi-disease surveillance use. First, the multiplex bead-based assay used in this thesis provides an opportunity to collect data on a large number of diseases using a single sample. Studies have started to evaluate the use of this platform for the detection of malaria and other pathogens. For example, a study in Haiti was done to differentiate a newly introduced Chikungunya Virus to endemic dengue virus and other pathogens by measuring antibody responses to a recombinant chikungunya virus antigen, two dengue virus-like particles and three recombinant *P. falciparum* antigens in children. This study found that there had been a rapid and intense dissemination of chikungunya virus in Haiti and concluded that the multiplex bead assay is an appropriate serological platform to monitor the seroprevalence of multiple pathogens simultaneously (201). Another example is an integrated study measuring antibody responses to *P. falciparum*, *P. vivax*, *Wuchereria bancrofti*, *Toxoplasma gondii*, *Taenia solium*, and *Strongyloides stercoralis* recombinant antigens as part of a VPDs survey in Cambodia. This study concluded that the integrated serological surveys offer an opportunity to systematically assess the status of multiple public health programs and measure progress toward Millennium Development Goals (202).

Second, the mobile technology-based mapping approach validated in this thesis offers an attractive alternative approach to remotely collect household GPS coordinates of health facility attendees that can iteratively be improved and integrated with other environmental or disease outcomes data. This mapping approach has also been recently used to assess human mobility in Amazon (203). Combining this mapping approach with the multiplex bead-based assay will enable simultaneous investigation of malaria and other pathogens such as VPDs and NTDs

burden, monitor changes over time, as well as to investigate their spatial patterns and interactions in population. Employing this approach could generate information on the epidemiology and spatial pattern of multiple infectious diseases that can help Ministry of Health to better priorities and allocate resources for surveillance and control programme activities in national and sub-national level. Currently, there is an ongoing work carried out by Drakeley's group to evaluate the use of multiplex bead-based assay to investigate burden and spatial epidemiology of NTDs and VPDs.

## **7.5 Conclusion**

This thesis aimed to evaluate the operational application of serological surveillance for monitoring malaria transmission as an alternative additional approach to the existing case-based surveillance system in Indonesia. It has shown that the serological surveillance can provide additional important information that cannot be generated by the routine malaria surveillance which rely on standard diagnostics such as microscopy and RDT, particularly in low transmission settings. Firstly, analysis of community-based serological data can confirm malaria elimination and identify clusters with high exposure in area reporting zero cases in the last three consecutive years. Secondly, quarterly health facility-based serological surveillance and participatory mapping can predict receptive areas at risk for malaria outbreak and assess factors associated with exposure to malaria in a very low transmission area conducting elimination. Thirdly, whilst seropositivity to Etramp5.Ag1 in children is a potential marker of recent exposure, a single most immunogenic antigen associated to long-lived antibody responses is useful to assess transmission intensity in a high transmission setting. Additionally, mobile technology-based participatory mapping can be used to quickly obtain spatial residential information for

individuals presenting at health facilities in resource poor areas where formal addresses are typically not used, and internet connectivity is limited. Findings from this thesis could be used to better target disease surveillance and intervention in the region. Additionally, the collaborative work presented in this thesis has helped the establishment of local laboratory and capacity to perform a multiplex bead-based assay, participatory mapping and the associated analytical approaches that can be used for future malaria and other infectious diseases research in Indonesia.

Although this thesis shows potential operational applications of serological surveillance for malaria elimination, there are several challenges that need to be addressed before implementing the approaches in wider settings. Further operational and implementation research will be needed to identify and address bottlenecks of integrating serological surveillance into the routine surveillance systems.

There are ongoing serological studies of samples collected from three sites evaluated in this thesis. Work is ongoing for samples collected via community-based cross-sectional survey in 2014 in Sabang and a randomized controlled trial completed in 2018 in Mimika. Further testing for a panel of new malaria, NTDs and VPD antigens and further analysis and mapping for *P. knowlesi* exposure is planned for samples collected in 2018 in Kulon Progo. Catchment model analysis can be performed utilising data collected on travel distance, estimated time and mode of transportation used to travel from residence to the health facility. The future work arising from this thesis is summarised in Table 7.1.

Table 7.1 Summary of future work arising from this thesis

<b>Future work</b>	
Laboratory	<p>Process samples collected from Mimika in 2018 using a panel of new antigens for <i>P. falciparum</i>, <i>P. vivax</i> and soil transmitted helminths</p> <p>Process samples collected from Kulon Progo in 2018 using a panel of new antigens for <i>P. vivax</i>, NTDs and VPD antigens</p> <p>Identification of new antigens for measuring recent <i>P. vivax</i> exposure</p>
Analysis	<p>Sero-epidemiological analysis for <i>P. falciparum</i>, <i>P. vivax</i> and <i>P. knowlesi</i> data generated from study in Sabang 2014</p> <p>Sero-epidemiological analysis and mapping of <i>P. knowlesi</i> data generated from study in Kulon Progo</p> <p>Catchment model analysis using data collected on distance from residence to health facility, estimated time and mode of transportation used to travel to the health facility</p>
Operational	<p>Costing study to estimate budget needed for implementing various use cases scenario of malaria serological surveillance in different geographical settings</p> <p>Implementation research to identify and address bottlenecks of integrating the various use cases scenario into existing malaria surveillance systems</p>

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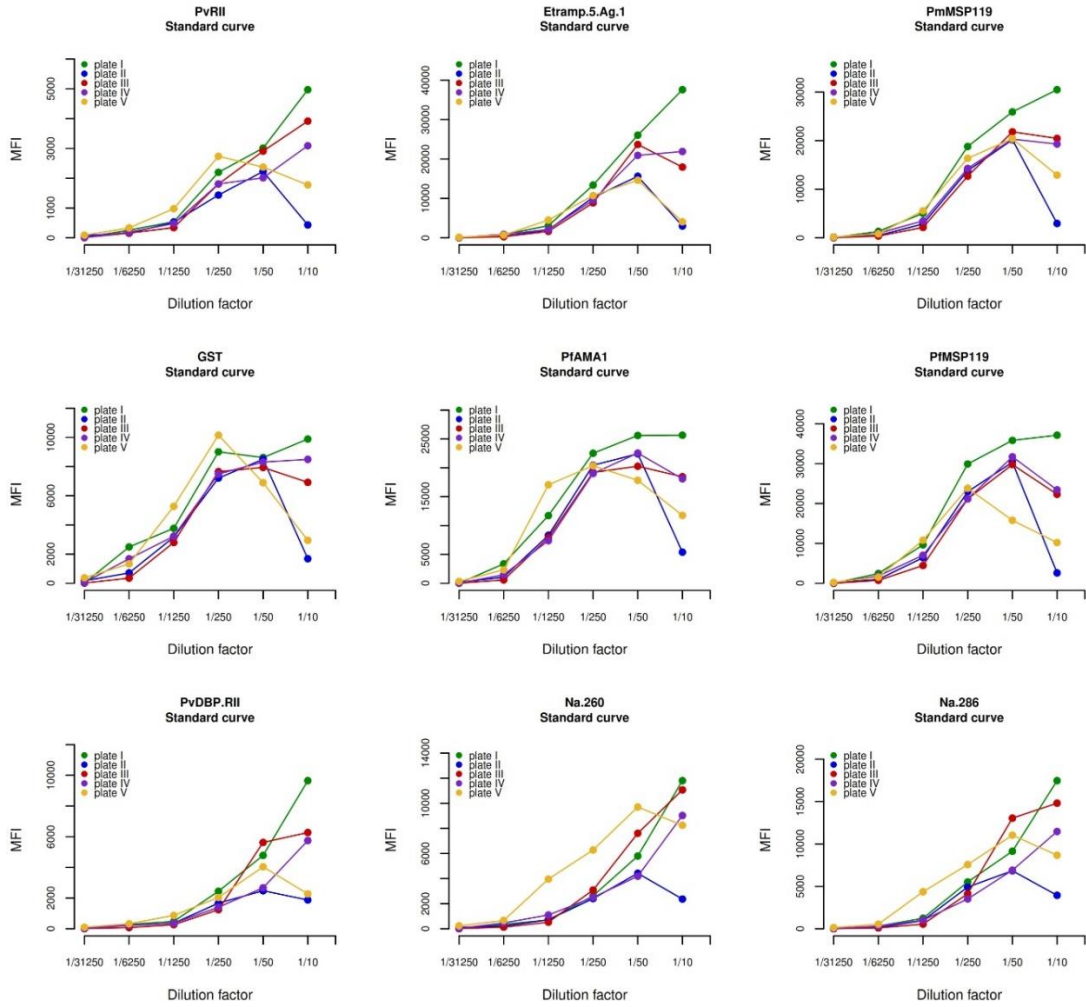
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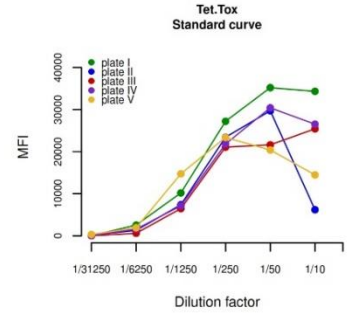
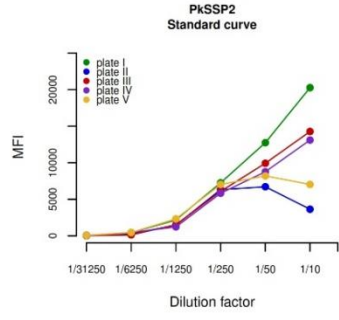
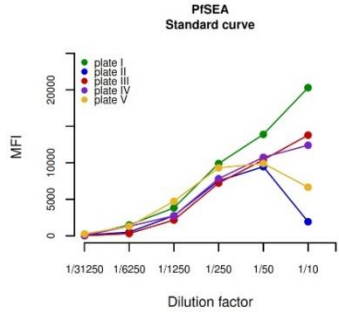
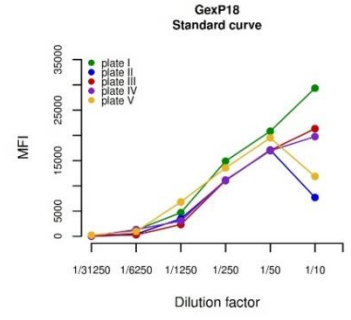
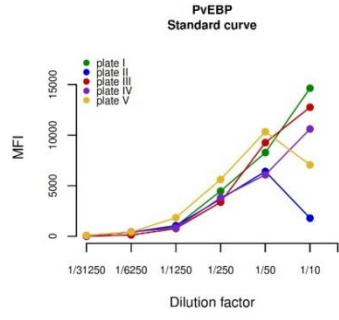
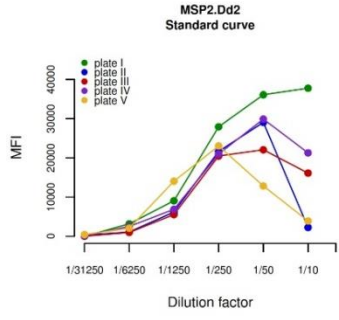
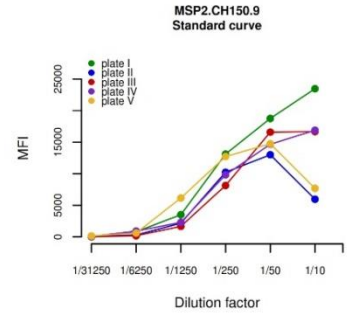
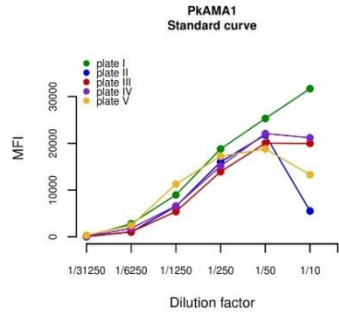
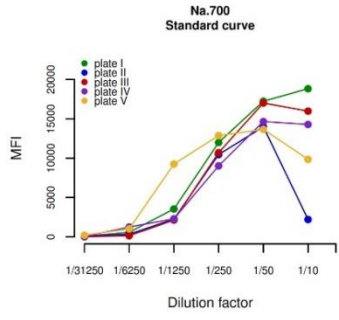


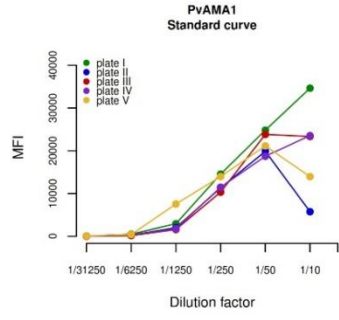
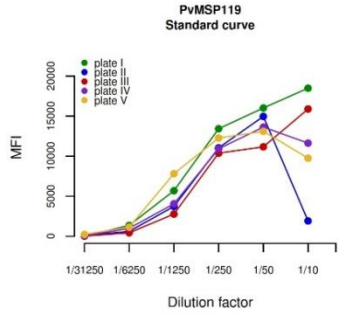
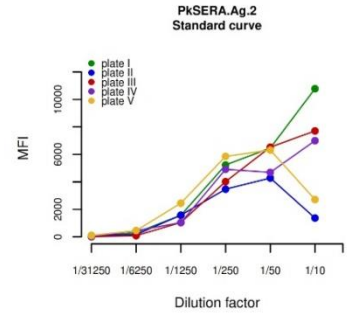
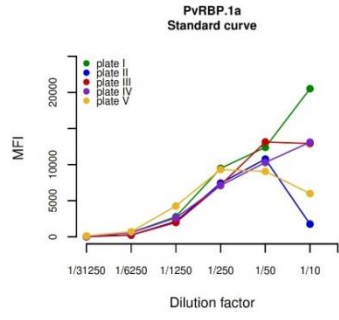
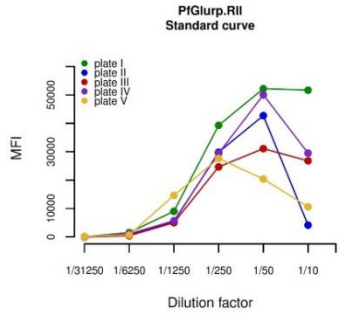
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# Appendices

## Annex 1: Example of Luminex assay quality control curves







## **Annex 2: Informed consent form**

INFORMED CONSENT FORM FOR PARTICIPANTS  
Version n° 1.0 of 01 Nov 2016

Optimising serological surveillance for malaria in Indonesia (OPSIN)  
(OPSIN protocol Version n° 1.0 of 01/11/2016)

**Study site:** Kulon Progo district, Indonesia

**Study centre:** Primary health centres in Kulon Progo district, Indonesia

**Indonesian PI:** Prof. dr. Supargiyono, DTM&H, PhD, SpParK (Universitas Gadjah Mada)

**UK PI:** Prof. Chris Drakeley, MI Biol, PhD (London School of Hygiene & Tropical Medicine, United Kingdom)

**This Informed Consent Form has two parts:**

- Information Sheet (to share information about the study with you).
- Certificate of Consent (for signatures if you agree to take part).

You will be given a copy of the full Informed Consent Form

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## **PART 1: INFORMATION SHEET**

### **Introduction**

Dear Sir/ Madam,

We are a group of researchers from Universitas Gadjah Mada, Yogyakarta and we are working with other researchers from London School of Hygiene & Tropical Medicine, United Kingdom with support from Indonesia Ministry of Health. We want to learn how to measure the risk of malaria in your community.

For this reason, we would like to know if you (or your child) might suffer from malaria exposure or infection and if you (or your child) have any clinical symptoms and risk factors of malaria. Therefore, we are asking you if you would like to participate in our study. Participation is voluntary, and you have the right to refuse. I will now give you some more information about the study. If my words are not clear, please ask me to stop and I will take time to explain. If you have questions later, you can ask me or the other study staff, at any time.

### **Purpose of the study**

Malaria is important public health problems in our region. Unfortunately, we do not always have good and appropriate methods to determine whether someone is infected or not. Some tests are expensive, or quite complicated to perform, other methods are not reliable. It is hard for malaria control programme to make sure whether some villages or sub districts are free from malaria infection and exposure. Another problem is that sometimes these infections are present in community but do not cause symptoms. It is then difficult for health provider to decide what controls are needed and for which areas these controls are targeted.

The aim of this study is to improve the capability of surveillance system to detect and measure malaria infection and exposure in our region. Also, we aim to improve our understanding of transmission dynamics and risk factors of malaria infection in the region. We do not know yet in which area is malaria infection still occur and which type of malaria is dominant in our region nowadays, but the study will help us to find out. We also want to determine the number of people in the community who have the infection but are not sick.

The full study will last for 12 months and we will collect samples from 20,000 participating members of your community, both adults and children.

### **Study procedures**

If you agree to participate in the study (or if you agree that your child participates), the study investigator will take your blood sample and ask you some questions about your health (or your child's health), your habits and your living environment. In addition, the investigator will show you a digital map and you will be asked to identify in which area you are living.

On the blood sample that you provide we will perform a standard malaria microscopic test that will be done in local health centre you attend. We also will perform additional tests to look for the presence of malarial antibody in your blood sample – the tests that will guide malaria control programme to identify presence or absence of malaria transmission in particular area in our region. If you agree to take part in the study, these tests will be conducted at Laboratory of Parasitology, Faculty of Medicine, Universitas Gadjah Mada in Yogyakarta.

The sample leftovers will be kept for several years in the Laboratory of Parasitology, Faculty of Medicine, Universitas Gadjah Mada in Yogyakarta for setting up further research on infectious diseases. These leftovers may also be shipped to laboratories in United Kingdom. If you do not agree that we store these leftovers, you (or your child) can still be part of the main study, just inform me or the study investigator.

### **Your participation is entirely voluntary**

It is your choice to decide whether you want to be in the study / whether you want your child to be in the study. Whatever your decision is, all the diagnostic tests done on your blood will be provided free of cost to you (or your child). Also, you can decide to join the study, and later change your mind. This decision will not affect the quality of your (your child's) care. Just tell me or the study investigator, you don't need to provide any justification for this.

### **What are the risks and benefits?**

You will likely benefit from taking part in this study. Everyone who participates will find out whether they have malaria. If we find malaria parasites in your (or your child) blood sample, you will receive the proper treatment. The study will be beneficial for your community. In

fact, we hope it will help the malaria control programme at the district and national level to better understand malaria transmission dynamics in our region.

There may be a small bruise or temporary mild pain on the finger or heel where the blood is taken. There is also a small chance of infection when blood is drawn. However, our careful procedures make this very unlikely.

### **Treatment**

This study is only about diagnostic tests and we will not test any new drug or other therapy. Any disease you may be diagnosed with will be treated following the current standard treatment in the local health center, in the district. If needed, you will be referred to another appropriate health facility and treated per standard of care available in Indonesia.

### **What are the costs?**

You will not be paid to take part in the study, however, we will make sure that you don't bear additional costs from your participation. All the diagnostic tests we do on your blood sample will be free of charge. The treatment of malaria infections that are detected during the study will also be free of charge. The study will not pay for the diagnostic tests and treatment related to any other health problems that you (or your child) may have.

### **What do we expect from you?**

If you accept to participate in this study, we will expect you to provide the blood sample and to undergo the physical examination. We also expect you to answer the questions of the study investigator to the best of your ability.

### **How will confidentiality be respected?**

We will not share any of your (or your child's) personal information outside of the Universitas Gadjah Mada study team. Your name (or your child's name) will not be mentioned on any sample, nor on the data collected during the study. You (or your child) will be given a unique number, which will be used to identify the samples and data collected. If the results of this study get published in a scientific journal, your name (or your child's name) will not appear on the publication. All members of the research team commit to protect the confidentiality of the information you provide. The members of the Ethics Committee, auditors, and Sponsor's representatives may access your (your child's) personal information, however, all



these people have to respect the confidentiality, and your (or your child's) personal information will not be revealed publicly.

**Whom to contact in case of problem or question?**

For any problem or question related to the study you may contact:

Coordinating investigator: **Henry Surendra, SKM, MPH** on +62 817 0741253 or Principal Investigator: **Prof. dr. Supargiyono, DTM&H, PhD, SpParK** on +62 812 2735246 in Universitas Gadjah Mada.

Otherwise, you may contact the **Centre for Tropical Medicine, Faculty of Medicine, Universitas Gadjah Mada**, Sekip Utara, Yogyakarta, 55281, Indonesia. Telephone +62 274 547147.

You may also contact the **Medical and Health Research Ethical Committee, Faculty of Medicine, Universitas Gadjah Mada**, telephone no. +62 274 7134 955 or by email: ***mhrec\_fmugm@ugm.ac.id***.

**PART 2: CONSENT FORM**

• **For adult, literate participants:**

I have read the participant information sheet, or it has been read to me, and I have understood the purpose of the study, the procedure to be conducted, and the risks and benefits related to my participation. I know that some of the samples that will be collected may also be sent abroad for analyses. I have had the opportunity to ask questions and all have been answered to my satisfaction. I consent voluntarily to participate in this study.

Print Name of Participant \_\_\_\_\_

Signature of Participant \_\_\_\_\_

Date \_\_\_\_\_

Day/month/year

- I agree that part of my samples get stored for future research on infectious diseases
- I don't agree that part of my samples get stored

• **For witnesses of adult, illiterate participants:**

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

*Print name of witness* \_\_\_\_\_

*AND*

*Thumb print of participant*

*Signature of witness* \_\_\_\_\_

Date \_\_\_\_\_

Day/month/year



- The participant agrees that part of his/her samples get stored for future research on infectious diseases
- The participant does not agree that part of his/her samples get stored

**Statement by the researcher/person taking consent**

I, the undersigned, have defined and explained to the participants in a language he/she understands, the procedures of this study, its aims and the risks and benefits associated with his/her participation. I have informed the participant that confidentiality will be preserved, that he/she is free to withdraw from the study without affecting the care he/she will receive at the hospital. I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

A copy of this Informed Consent Form has been provided to the participant.

Print Name of Researcher/person taking the consent\_\_\_\_\_

Signature of Researcher /person taking the consent\_\_\_\_\_

Date \_\_\_\_\_

Day/month/year

• **For literate parents or guardians of participants aged 0 to 18 years (minors)**

I have read the foregoing information, or it has been read to me, and I have understood the purpose of the study, the procedure to be conducted, and the risks and benefits related to my child's participation. I know that some of the samples that will be collected may also be sent abroad for analyses. I have had the opportunity to ask questions and all have been answered to my satisfaction. I consent voluntarily for my child to participate in this study.

Print Name of Participant\_\_\_\_\_

Print Name of Parent/Guardian\_\_\_\_\_

Signature of Parent/Guardian \_\_\_\_\_

Date \_\_\_\_\_

Day/month/year

I agree that part of my child's samples get stored for future research on infectious diseases

I don't agree that part of my child's samples get stored

- **For witnesses of illiterate parents or guardians of participants aged 0 to 18 years (minors)**

A literate witness must sign (if possible, this person should be selected by the participant and should have no connection to the research team). Participants who are illiterate should include their thumb print as well.

I have witnessed the accurate reading of the consent form to the parent of the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Print name of witness \_\_\_\_\_ AND Thumb print of parent/guardian

Signature of witness \_\_\_\_\_

Date \_\_\_\_\_

Day/month/year

The parent/guardian agrees that part of his/her child's samples get stored for future research on infectious diseases

The parent/guardian does not agree that part of his/her child's samples get stored

**Statement by the researcher/person taking consent**

I have accurately read out the information sheet to the parent of the potential participant, and to the best of my ability made sure that the person understands the procedures of this study, its aims and the risks and benefits associated with his/her child's participation. I confirm that the parent was given an opportunity to ask questions about the study, and all the questions asked by the parent have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

A copy of this ICF has been provided to the participant.

Print Name of Researcher/person taking the consent \_\_\_\_\_

Signature of Researcher /person taking the consent \_\_\_\_\_

Date \_\_\_\_\_

Day/month/year

• **Assent for literate participants aged 12 to 18 years:**

I have read the participant information sheet, or it has been read to me, and I have understood the purpose of the study, the procedure to be conducted, and the risks and benefits related to my participation. I know that some of the samples that will be collected may also be sent abroad for analyses. I have had the opportunity to ask questions and all have been answered to my satisfaction. I consent voluntarily to participate in this study.

Print Name of Participant \_\_\_\_\_

Signature of Participant \_\_\_\_\_

Date \_\_\_\_\_

Day/month/year

I agree that part of my samples get stored for future research on infectious diseases

I don't agree that part of my samples get stored

• **For witnesses of illiterate participants aged 12 to 18 years:**

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

*Print name of witness* \_\_\_\_\_

*AND*

*Thumb print of participant*

*Signature of witness* \_\_\_\_\_



Date \_\_\_\_\_

Day/month/year

The participant agrees that part of his/her samples get stored for future research on infectious diseases

The participant does not agree that part of his/her samples get stored

**Statement by the researcher/person taking consent**

I, the undersigned, have defined and explained to the participants in a language he/she understands, the procedures of this study, its aims and the risks and benefits associated with his/her participation. I have informed the participant that confidentiality will be preserved, that he/she is free to withdraw from the study without affecting the care he/she will receive at the hospital. I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

A copy of this Informed Consent Form has been provided to the participant.


Print Name of Researcher/person taking the consent\_\_\_\_\_

Signature of Researcher /person taking the consent\_\_\_\_\_

Date \_\_\_\_\_

Day/month/year

**Annex 3: Luminex SOP**

<b>SOP Title:</b>	<b>Luminex Assay</b>	<b>LONDON SCHOOL of HYGIENE &amp; TROPICAL MEDICINE</b> 
<b>SOP No:</b>	<b>Luminex Assay Indonesia</b>	
<b>Version:</b>	<b>001</b>	

Effective from:	05/06/2017
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Superseded Version Number:	
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<b>Author:</b>	
Name: Tom Hall	
Position:	Scientific Officer
Signature:	Date: /04/2015

<b>Revision History</b>
Comments
Reviewed by:
Date:
Next review due:
Signature(s):

## **1 Overview**

This SOP describes the method for the screening of serum samples by Luminex bead array

## **2 Equipment**

- a) Bio-rad 96 well plates
- b) Protective latex or nitrile gloves
- c) Pipettes 8 or 12-channel 30-300µl, single channel 100ul and tips
- d) Vortex
- e) Magnetic rack
- f) 1.5ml Eppendorf tubes

## **3 Reagents**

All reagents should be stored according to the instructions supplied with them and disposed of at the expiry date recorded on the product.

- a) Selected coupled bead regions
- b) PBS tablets
- c) Tween
- d) BSA
- e) Sodium Azide
- f) PVA
- g) PVP
- h) Casein
- i) Ecoli extract



#### 4 Preparation of Buffer Solutions

All buffer solutions should be clearly labelled with:

- \* Reagent name
- \* Expiry date
- \* Preparation date
- \* Name of person who prepared the buffer

Table 1- Preparation of buffer solutions

Buffer solution	Reagent/chemical	Amount/Volume
<b>1xPBS</b>	Oxoid PBS tablets	1
	Milliq water	100ml

Make up as needed daily, dispose of unused solution at the end of each day

<b>Wash buffer (1xPBS Tween)</b>	PBS 1X	1000ml
	0.05% Tween 20	0.5ml

Make up as needed daily, dispose of unused solution at the end of each day

<b>Buffer A (PBS-TBN)</b>	1x PBS	1000ml
	0.05% Tween	0.5ml
	0.5% BSA	5g
	0.02% Sodium Azide	0.2g

Store at room temperature

<b>Buffer B</b>	Buffer A	1000ml
	0.1% Casein	1g
	0.5% PVA	5g
	0.5% PVP	5g
	Ecoli Extract	1.4ml of 10.865ug/ml stock (15.25ug/ml)

Centrifuge at 3000 RMP for 30 minutes and Store at +4.

## **5 Buffer B Prep and sample dilution**

1. Prepare buffer B and leave mixing until large lumps are dissolved, may require mixing overnight.
2. Centrifuge buffer B at 3000 RPM for 30 minutes. Use a pipette gun and a 10ml glass pipette to remove most of the centrifuged buffer, aspirate slowly to avoid resuspending the particulate matter. Discard the last 10ml.
3. Prepare sample at least a day before testing.
4. Dilute samples at 1/200 (460ul of buffer B added to a 4.5mm blood spot) and leave overnight mixing at 300 RMP. Dilution in buffer B reduces the background noise caused by non specific antibody binding.
5. Leave samples mixing on a rotating platform for at least 8 hours to allow the full elution of the blood spot.

## **6 Multiplex magplex assay protocol**

1. Remove all the buffers, coupled beads, samples and controls from the fridge/freezer.
2. Measure out 5ml of buffer A per plate and add to a trough, 20ml will be required for 4 plates.
3. Calculate the required volume of beads; 8ul is required per plate (32ul for 4 plates).
4. Vortex the beads to resuspend and add the required volume to the buffer A in the trough. (protect beads from light at all times)
5. Once all bead sets are added to the buffer A mix well by tilting the trough up and down.
6. Mix again by aspirating and dispensing the liquid using as multichannel pipette set to 50ul.
7. Add 50ul of the bead mixture to all wells.
8. Place the plate on the magnetic separator for 2 minutes. Protect the plate from light between the incubations using aluminium foil or a plate lid.
9. With the microplate is still attached to the magnet remove the supernatant by rapid inversion with a sharp shake down the sink. Gently blot the plate on a paper towel to remove as much residual as possible.

10. Remove plate from the magnetic separator.
11. Wash the microplate once by adding 100ul of 1xPBS/T, place the microplate onto the magnetic separator and pause for 2 minute. Pour the liquid down the sink (rapidly invert plate while still attached to magnet) and blot on a paper towel.
12. Add 25ul of buffer B to wells A1 to H10. This is required to dilute samples to 1/400.
13. Using a multichannel pipette 25µl of prepared samples from a deep well plate onto the plate containing washed beads. Use the layout in figure 1.
14. Add 50ul of the required control (already diluted to 1/400) to the correct locations- see layout in fig 1. Add 50ul of buffer B to wells 11G/H and 12G/H
15. Cover plate with a lid, secure on a vibrating shaker. Mix at 200 rpm for 1.5 hrs at room temperature (RT).
16. Place the plate on the magnetic separator for 2 minutes. While the plate is still on the magnetic separator, pour off the supernatant by a rapid inversion with a sharp shake. Gently blot the plate on a paper towel to remove as much residual as possible.
17. Wash the plates three times with 100ul of 1xPBS/T as above, point 11.
18. Prepare secondary antibody at 1/200 in buffer A. For one plate add 25ul of the secondary antibody to 5ml Buffer A. For 4 plates 100ul of secondary will be added to 20ml of buffer A. (protect secondary from light).
19. Mix the buffer A and secondary mixture well (as in points 5 and 6) and add 50 µl/well of secondary antibody to each well.
20. Cover plate with a lid and secure on shaker. Mix at 200 rpm for 1.5 hrs at RT.
21. Wash plate X3 with 100ul of 1xPBS/T as above.
22. Add 50 µl of buffer A per well. Cover with lid, secure on shaker and shake at 200 rpm for 30 minutes at RT.
23. Wash plate 1X with 100ul of 1xPBS/T.
24. Add 100 µl of 1XPBS per well.
25. Leave plates over night in the fridge (+4°C) and read the follow day.

**Figure 1: Plate layout**

	1	2	3	4	5	6	7	8	9	10	11	12
<b>A</b>	<b>Test samples 1/400</b>  (Add 25ul of 1/200 samples to 25ul of buffer B)										<b>Pf pos</b>	<b>CP3</b>
<b>B</b>											<b>10/198</b>	
<b>C</b>											<b>1/400</b>	
<b>D</b>											<b>Pv/Pm</b>	
<b>E</b>											<b>pos</b>	
<b>F</b>											<b>72/96</b>	
<b>G</b>											<b>1/400</b>	
<b>H</b>											<b>Neg</b>	
											<b>Blanks</b>	<b>Blanks</b>
											<b>Buffer B</b>	<b>Buffer B</b>

**Annex 4: OPSIN questionnaire version 2.0 21 February 2017 (installed into GeoODK)**

Interviewer initial: \_\_\_\_\_

Health facility: \_\_\_\_\_

Participant ID: \_\_\_\_\_

Date of enrollment: \_\_\_\_\_(DD/MM/YYYY)

**Part I – DEMOGRAPHIC DATA**

***Participants < 6 months must not be included in the study.***

1. Date of birth: \_\_\_\_\_(DD/MM/YYYY) or Age: \_\_\_\_\_(Year) \_\_\_\_\_(Month)
2. Gender:
  - a. MALE
  - b. FEMALE
3. Participant status:
  - a. PATIENT
  - b. ACCOMPANYING PERSON

**Part II – GEOGRAPHIC INFORMATION**

1. Hamlet \_\_\_\_\_
2. Village Name \_\_\_\_\_
3. Head of Household \_\_\_\_\_
4. Nearest Market \_\_\_\_\_
5. Nearest Primary School \_\_\_\_\_
6. Nearest Health Facility \_\_\_\_\_
7. How did you travel here today:
  - a. WALK
  - b. MOTORBIKE
  - c. BICYCLE
  - d. CAR
  - e. OTHER (SPECIFY)
8. How many minutes did it take you to get here by [answer of Q7] \_\_\_\_\_
9. Locate residence on map using geolocation application (**coordinates populated by app**)
  - a. LAT \_\_\_\_\_
  - b. LONG \_\_\_\_\_
  - c. NOT ABLE TO LOCATE (**skip to part III**)
10. Are you willing to have your house labeled on the map as a point of interest to help other people find their house?
  - a. YES
  - b. NO

**Part III – CLINICAL DATA**

1. Current symptoms **(Check all that apply)**:
  - a. FEVER
  - b. HEADACHE
  - c. CHILLS
  - d. STOMACHACHE
  - e. VOMITING
  - f. NAUSEA
  - g. OTHER (SPECIFY)
  - h. NOT APPLICABLE
2. Temperature reading \_\_\_\_\_ °C
3. Has the participant been ill with a fever at any time in the last 2 weeks?
  - a. YES
  - b. NO **(if no skip to Q6)**
  - c. NOT SURE
4. Did the participant seek advice or treatment for the fever from any source?
  - a. YES
  - b. NO **(if no, skip to Q6)**
5. Where did the participant seek advice or treatment? **(Check all that apply)**
  - a. PUBLIC HOSPITAL
  - b. PRIMARY HEALTH CENTER
  - c. PUBLIC HEALTH POST
  - d. MOBILE CLINIC
  - e. FIELD WORKER
  - f. OTHER PUBLIC MEDICAL
  - g. PRIVATE HOSPITAL/CLINIC
  - h. PHARMACY
  - i. PRIVATE DOCTOR
  - j. OTHER PRIVATE MEDICAL (SPECIFY) \_\_\_\_\_
  - k. SHOP
  - l. TRADITIONAL PRACTITIONER
  - m. OTHER (SPECIFY) \_\_\_\_\_
6. Has the participant had a fever in the last 24 hours?
  - a. YES
  - b. NO
  - c. DON'T KNOW
7. Has the participant taken any drugs in the last 2 weeks? **(Check all that apply)**
  - a. SP/FANSIDAR
  - b. CHLOROQUINE (alone)
  - c. CHLOROQUINE + primaquine
  - d. AMODIAQUINE
  - e. QUININE
  - f. COARTEM
  - g. OTHER ANTIMALARIAL (SPECIFY) \_\_\_\_\_
  - h. ASPIRIN
  - i. ACETAMINOPHEN/PARACETAMOL

- j. IBUPROFEN
- k. OTHER (SPECIFY) \_\_\_\_\_
- l. DON'T KNOW

**Part IV – RISK FACTORS DATA**

1. What is the occupation of the participant?
  - a. FARMING
  - b. COCONUT/PALM TAPPING
  - c. MINING
  - d. FOREST RELATED JOBS
  - e. HOUSEWIFE
  - f. OTHER
  - g. JOBLESS
  - h. NOT APPLICABLE
  
2. Does the participant sleep where the farm is situated?
  - a. YES
  - b. NO
  - c. Not Applicable
3. Has the participant travelled outside (THIS HAMLET) in the last 4 weeks?
  - a. YES
  - b. NO (**Skip to Q7**)
  - c. DON'T KNOW (**Skip to question Q7**)
4. How many trips has the participant made outside (THIS HAMLET) in the last 4 weeks? \_\_\_\_\_
5. When did the participant come back from the participant's most recent trip?
  - a. <2 WEEKS AGO
  - b. 2-4 WEEKS AGO
  - c. >4 WEEKS AGO
6. Which Hamlet did the participant spend most time in during that trip? \_\_\_\_\_
7. Does the participant's household have any mosquito nets that can be used while sleeping?
  - a. YES
  - b. NO (**if no, skip to Q11**)
8. How many mosquito nets does the participant's household have? \_\_\_\_\_
9. Did the participant sleep under a bed net last night?
  - a. YES
  - b. NO
10. If NO, why not? (**Check all that apply**)
  - a. IT IS TOO HOT UNDER THE NET
  - b. THERE IS NOT ENOUGH SPACE UNDER THE NET/I FEEL TOO CLOSED IN
  - c. IT DOES NOT PROTECT AGAINST MOSQUITOES/INSECTS
  - d. NO MOSQUITOES AROUND
  - e. IT IS FOR ONLY CHILDREN/PREGNANT WOMEN
  - f. BEDNET USED BY PARENTS
  - g. BEDNET USED BY SIBLINGS
  - h. BEDNET BEING WASHED

- i. BEDNET OLD
  - j. BEDNET KEPT FOR VISITORS
  - k. IT IS TOO EXPENSIVE/CANNOT AFFORD ENOUGH NETS FOR EVERYONE
  - l. IT IS NOT THE RAINY/MALARIA SEASON
  - m. CANNOT HANG IT OVER MY SLEEPING PLACE/SLEEPING OUTSIDE
  - n. CHANGE MY SLEEPING PLACE TOO OFTEN
  - o. DO NOT KNOW
  - p. OTHER
11. What material is the flooring of the participant's house made of?
- a. DOESN'T HAVE FLOORING
  - b. BAMBOO OR OTHER WOOD
  - c. CEMENT
  - d. CERAMIC
12. What material is the exterior wall of the participant's house made of?
- a. NONE
  - b. WOVEN SPLIT BAMBOO
  - c. BAMBOO
  - d. CEMENT
13. What material is the roof of the participant's house made of?
- a. NONE
  - b. NIPA
  - c. BAMBOO
  - d. ROOF TILES
  - e. GALVANIZED IRON SHEETS
  - f. ASBESTOS

**Part V – LAB DATA**

1. Blood films prepared?
- a. YES
  - b. NO
2. Filter paper blood spots prepared?
- a. YES
  - b. NO
3. Microscopy Result
- a. POSITIVE
  - b. NEGATIVE
  - c. NOT DONE

Barcode Number: \_\_\_\_\_ (scan in)



## Annex 5: LSHTM Ethical Clearance

### London School of Hygiene & Tropical Medicine

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United Kingdom  
Switchboard: +44 (0)20 7636 8636

[www.lshtm.ac.uk](http://www.lshtm.ac.uk)



#### Observational / Interventions Research Ethics Committee

Mr Henry Saridah  
LSHTM

19 May 2017

Dear Henry

**Study Title:** OPSIN: Optimising serological surveillance for malaria in Indonesia

**LSHTM Ethics Ref:** 11944

Thank you for responding to the Observational Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

#### Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

#### Conditions of the favourable opinion

Approval is dependent on local ethical approval having been received, where relevant.

#### Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document Type	File Name	Date	Version
Investigator CV	CV - Henry-Surendra Saridah	11/07/2016	1
Investigator CV	CV - Jackie Cook	11/07/2016	1
Investigator CV	CV - Chris J Drakeley	11/07/2016	1
Investigator CV	CV - Supargiyono	15/02/2017	1.0
Protocol / Proposal	OPSIN_Research Protocol_V2_21.02.17	21/02/2017	2.0
Protocol / Proposal	OPSIN_Questionnaire_V2_21.02.2017	21/02/2017	2.0
Local Approval	EC OPSIN	03/03/2017	1
Information Sheet	ICF_V3_11.05.17	11/05/2017	3.0
Covering Letter	Clarification to LSHTM Ethics Committee	12/05/2017	1.0

#### After ethical review

The Chief Investigator (CI) or delegate is responsible for informing the ethics committee of any subsequent changes to the application. These must be submitted to the Committee for review using an Amendment form. Amendments must not be initiated before receipt of written favourable opinion from the committee.

The CI or delegate is also required to notify the ethics committee of any protocol violations and/or Suspected Unexpected Serious Adverse Reactions (SUSARs) which occur during the project by submitting a Serious Adverse Event form.


At the end of the study, the CI or delegate must notify the committee using an End of Study form.

All aforementioned forms are available on the ethics online applications website and can only be submitted to the committee via the website at: <http://leo.lshtm.ac.uk>

Additional information is available at: [www.lshtm.ac.uk/ethics](http://www.lshtm.ac.uk/ethics)

Yours sincerely,

A handwritten signature in black ink, appearing to be 'H. Saridah'.

  
**Professor John DH Porter**  
**Chair**

[ethics@lshtm.ac.uk](mailto:ethics@lshtm.ac.uk)  
<http://www.lshtm.ac.uk/ethics/>

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**Improving health worldwide**

## Annex 6: UGM Ethical Clearance



MEDICAL AND HEALTH RESEARCH ETHICS COMMITTEE (MHREC)  
FACULTY OF MEDICINE GADJAH MADA UNIVERSITY  
- DR. SARDJITO GENERAL HOSPITAL



### ETHICS COMMITTEE APPROVAL

Ref : KE/FK/0290/EC/2017

Title of the Research Protocol : OPSIN: Optimising serological surveillance for malaria in Indonesia

Documents Approved : 1. Study Protocol versi 02 2017  
2. Information for Subjects versi 02 2017  
3. Informed consent form versi 02 2017

Principle Investigator : Prof. dr. Supargiyono, DTM&H., Ph.D., Sp.ParK

Participating Investigator(s) : 1. Prof. Chris Drakeley, PhD  
2. Jackie Cook, PhD (LSHTM)  
3. Henry Surendra, MPH (LSHTM)  
4. Elsa Herdiana, PhD  
5. Riris Andono Ahmad, PhD  
6. Barandi Widartono, MSc  
7. Prof. Din Syafrudin, PhD

Date of Approval : **03 MAR 2017**  
(Valid for one year beginning from the date of approval)

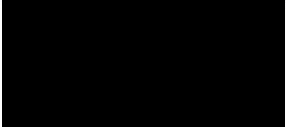
Institution(s)/place(s) of research : Kulon Progo, Purworejo and Magelang districts


The Medical and Health Research Ethics Committee (MHREC) states that the above protocol meets the ethical principle outlined in the Declaration of Helsinki 2008 and therefore can be carried out.

The Medical and Health Research Ethics Committee (MHREC) has the right to monitor the research activities at any time.

The investigator(s) is/are obliged to submit:

- Progress report as a continuing review : Annually
- Report of any serious adverse events (SAE)
- Final report upon the completion of the study

  
Prof. Dr. dr. Sri Sutarni, Sp.S(K)  
Chairperson

  
Dr. dr. Eti Nurwening Spolikhah, M.Kes., M.Med.Ed  
Secretary

Attachments:

- Continuing review submission form (AF 4.3.01-014.2013-03)
- Serious adverse events (SAE) report form (AF 6.1.01- 019.2013-03)

Recognized by Forum for Ethical Review Committees in Asia and the Western Pacific (FERCAP)  
1-Mar-17