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Evaluating and optimizing surveillance and response strategies for malaria elimination in the Asia Pacific region

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Abstract

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Malaria case investigation and reactive case detection (RACD) activities are widely implemented in low transmission settings to identify additional malaria infections and gather surveillance information, but with varying degrees of success. Challenges in conducting RACD include poor diagnostic sensitivity (particularly for low density and asymptomatic infections), knowledge gaps among those conducting RACD, financial and resource constraints, and operational and logistical difficulties. To improve infection detection and better target individuals at highest risk for infection, RACD strategies need to be evaluated and optimized to provide quality and nuanced surveillance information.

To support more effective surveillance and response strategies, this PhD project focused on evaluating RACD strategies to improve and optimize malaria surveillance in low transmission settings in the Asia Pacific region. Using a standardized monitoring and evaluation (M&E) tool, case investigation and RACD indicators were assessed, including the knowledge and practices of the staff conducting RACD. This PhD project explored the utility of molecular diagnostics and genotyping and targeted sociobehavorial RACD strategies for increasing infection detection and to understand the relatedness of infections identified during RACD. Also, the acceptability and feasibility of a presumptive treatment-based strategy to reduce malaria (referred to as reactive drug administration (RDA)) was evaluated.

Results revealed gaps in case investigation and RACD reporting completeness and timeliness and that staff were not always equipped with the appropriate documentation or have accurate knowledge on how to conduct RACD. Molecular diagnostics used in RACD in Thailand identified an additional 12 (0.6%) infections compared to no RACD-identified infections detected by microscopy. Of the four confirmed infections, only one (25%) was genetically related to the index case. In Indonesia, a sociobehavorial RACD strategy targeting high risk populations and work venues was able to identify 180 individuals for RACD yielding 8 infections compared to only one infection during household-based RACD. Shared risk factors between sociobehavorial RACD individuals and index patients include being male, 30-45 years of age, and occupation of logging or mining. In Thailand, an RDA strategy targeting within and around the household and forest-going co-workers was found to be acceptable by those that participated and feasible to be implemented by the malaria staff.

The quality of malaria case investigation and RACD activities and the knowledge of those implementing it can be improved using a standardized M&E tool. The molecular and genotyping findings may be useful for malaria programs in low transmission settings to increase infection detection in persistent malaria foci or among high-risk populations and to characterize local transmission patterns. When the highest risk individuals for malaria can be identified, a strategy like RDA may be useful to target and eliminate malaria to accelerate elimination efforts.

Keywords: malaria elimination, surveillance and response, reactive case detection, sociobehavorial, reactive drug administration, molecular diagnostics, microsatellite genotyping, *Plasmodium vivax*, low transmission

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To my life partner and love, Saida

List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I **Cotter C**, Sudathip P, Herdiana H, Cao Y, Liu Y, Luo A, Ranasinghe N, Bennett A, Cao J, Gosling RD. Piloting a programme tool to evaluate malaria case investigation and reactive case detection activities: results from 3 settings in the Asia Pacific. *Malaria*, 16(1) 347, 2017.

II Sudathip P*, **Cotter C***, Kitchakarn S, Zelman B, Sugaram R, Schwartz A, Murphy M, Vorster L, Greenhouse B, Roh ME, Mårtensson A, Imwong M, Gosling RD, Hsiang MS. Utility and costs of reactive case detection using molecular detection and genotyping in Thailand, a near-elimination setting with predominantly *Plasmodium vivax* transmission. *Submitted manuscript to PLOS Neglected Tropical Diseases*

***These authors contributed equally to this work**

III Bennett A*, **Cotter C***, Zarlinda I, Silaen G, Nora Lina R, Cueto C, Elyazar I, Jacobson J, Ekawati L, Hsiang MS, Noviyanti R, Smith JL, Coutrier FN. Sociobehavorial reactive case detection strategies targeting high-risk populations to increase the detection of malaria infections in Aceh Province, Indonesia. *Submitted manuscript to Malaria Journal*

***These authors contributed equally to this work**

IV Suwannarong K*, **Cotter C***, Ponlap T, Bubpa N, Thammasutti K, Chaiwan J, Finn TP, Kitchakarn S, Mårtensson A, Baltzell KA, Hsiang MS, Lertpiriyasuwat C, Sudathip P, Bennett A. Assessing the acceptability and feasibility of reactive drug administration for malaria elimination in a *Plasmodium vivax* predominant setting: a qualitative study in two provinces in Thailand. *Submitted manuscript to BMC Public Health*

***These authors contributed equally to this work**

These manuscripts are open access and therefore permission from the respective publishers is granted upon proper citation here within.

I have made primary contributions in the study design and field implementation during data/sample collection and analyses for studies included in this thesis. For **Paper I**, led the development of and training on the data collection tools, participated in field data collection and data analyses, prepared the figures and tables, and interpreted study findings. For **Papers II and III**, developed data collection materials, led field trainings, and observed data collection, supported the data analysis, prepared the figures and tables, and participated in the interpretation of study findings. For **Paper IV**, developed data collection materials, supported trainings and data collection, participated in the preparation of figures and tables, and supported interpretation of the study findings. For **Papers I – IV**, I wrote the first draft of the manuscript, incorporated co-author, and reviewer comments, and led editing of the final version for publication. For **Papers II, III, and IV**, I have shared first authorship with Dr Prayuth Sudathip, Dr Adam Bennett, and Dr Kanokwan Suwannarong due to my significant contributions to the research. The molecular work for **Paper II** was conducted at the Department of Molecular Tropical Medicine and Genetics at Mahidol University in Thailand. The molecular work for **Paper III** was conducted at the Malaria Pathogenesis Unit, Eijkman Institute for Molecular Biology, Jakarta, Indonesia.

Contents

Abbreviations

ACD	Active case detection
ACT	Artemisinin-based combination therapy
API	Annual parasite incidence
BMGF	Bill & Melinda Gates Foundation
CDC	Centers for Disease Control and Prevention
DBS	Dried blood spot
DDT	Dichloro-diphenyl-trichloroethane
DFAT	Department of Foreign Affairs and Trade
DFID	Department for International Development
DNA	Deoxyribonucleic acid
DOT	Directly observed therapy
DVS	Dominant vector species
FGD	Focus group discussion
GFATM	Global Fund to Fight AIDS, Tuberculosis and Malaria
GMEP	Global Malaria Eradication Programme
GMS	Greater Mekong Subregion
G6PD	Glucose-6-phosphate dehydrogenase
HH-RACD	Household-based RACD
HPH	Health promotion hospital
HRP2	Histidine-rich protein 2
IRS	Indoor residual spraying
ITC	Insecticide-treated clothing
ITN	Insecticide-treated net
IVCC	Innovative Vector Control Consortium
KII	Key informant interview
LAMP	Loop-mediated isothermal amplification
LLIN	Long-lasting insecticidal net
MDA	Mass drug administration
M&E	Monitoring and evaluation
MMP	Mobile and migrant populations
MMV	Medicines for Malaria Venture
MS	Microsatellite loci
MTaT	Mass testing and treatment
NMES	National Malaria Elimination Strategy

PATH MVI	PATH Malaria Vaccine Initiative
PCD	Passive case detection
PCR	Polymerase chain reaction
PMI	President's Malaria Initiative
PQ	Primaquine
PR-RACD	Peer referral reactive case detection
qPCR	Quantitative polymerase chain reaction
RACD	Reactive case detection
RBC	Red blood cells
RBM	Roll Back Malaria initiative
RCT	Randomized controlled trial
RDA	Reactive drug administration
RDT	Rapid diagnostic test
RNA	Ribonucleic acid
SB-RACD	Sociobehavorial reactive case detection
SOP	Standard operating procedures
STR	Short tandem repeat
SUFI	Scale up for impact
TTaT	Targeted testing and treatment
UCSF	University of California San Francisco
US	United States
USD	United States Dollars ($)
VBDU	Vector-borne disease unit
VB-RACD	Venue-based reactive case detection
VC	Vector control
VHV	Village health volunteer
WHO	World Health Organization

1 Introduction

1.1 Historical background on malaria control efforts

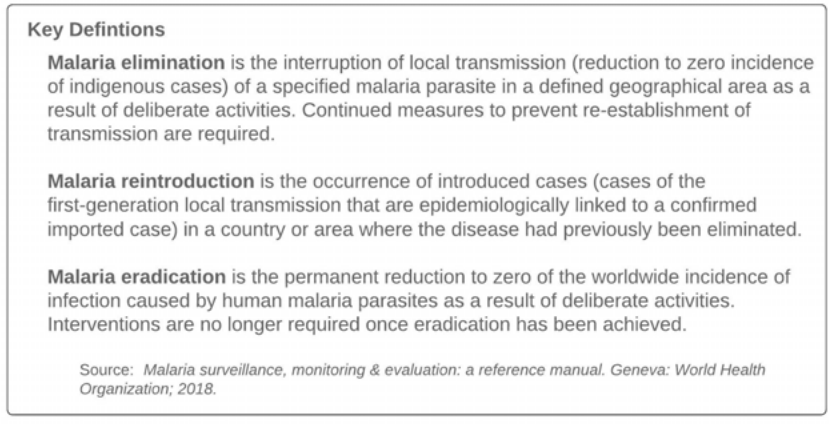
Malaria control and elimination efforts began in the late 19th century when researchers discovered the mechanism of malaria transmission: the *Plasmodium* parasite was transmitted by the anopheline mosquito.(1) This discovery laid a pathway for the development of tools to control malaria, namely personal protection, vector control targeting mosquito breeding sites and adult mosquitoes, as well as therapeutic and prophylactic drugs.(2) However, between 1900 and 1950, nearly 180 countries in the world still had endemic malaria.

Very little progress was made during this time due to two world wars, other global health pandemics (e.g., cholera, influenza)(3), and a lack of a coordinated global effort to aggressively control malaria. By the end of the 2nd world war, indoor residual spraying (IRS) and important drugs to treat and prevent malaria (e.g., chloroquine, amodiaquine, etc) were being used to limit the impact of malaria. In fact, in 1942 the Office of Malaria Control in War Areas was established to mitigate the impact of malaria and other vector-borne diseases during World War 2 around military training bases in the southern United States (US) and its' territories battling malaria.(4–6) This organized effort to control malaria established the Centers for Disease Control and Prevention (CDC) in the United States by 1946.

The National Malaria Eradication Program, an effort by state and local health agencies in the southern US, was started in 1947 by using IRS with dichlorodiphenyl-trichloroethane (DDT), but also by draining mosquito breeding areas and conducting aerial sprayings.(7) By 1950, nine countries in Europe had achieved malaria elimination with the US soon to follow when it ultimately eliminated malaria in 1952.(8) This achievement, as well as in other countries with temperate climates in Europe and the northern hemisphere applying similar malaria control interventions, increased the hope for eventual malaria eradication. (See Box 1 on definitions.)

In response to the positive achievements of eliminating malaria in several countries by the early 1950s, and armed with effective drugs and vector control tools at the time, the World Health Organization (WHO) launched the Global Malaria Eradication Programme (GMEP) at the eighth World Health Assembly in 1955(9) to "*...provide technical advice, and encourage research and coordination of resources in the implementation of a program having as its ultimate objective the worldwide eradication of malaria...*"(10) in all parts of the world,

except for Africa.(1) During the GMEP period, important lessons were learned on improving population coverage for interventions, the development of stronger community-based volunteer health workers, the use of local maps to guide malaria control activities, and improved quality of malaria surveillance systems.(11) Active case detection and treatment was another key intervention that helped to identify and treat malaria infections in the community.



Box 1 Key definitions

During the GMEP period, 68 countries successfully eliminated malaria, including Europe (37) and the Americas (22), compared to the rest of the world.(10) The GMEP effort brought about significant reductions in morbidity and mortality due to malaria; however, by 1969, fourteen years after announcing the launch of the GMEP program, the campaign was discontinued when WHO determined that malaria eradication was not achievable in many areas due to administrative, financial, and technical issues.(9,12) It was at this point that the WHO General Assembly re-examined the strategy and recommended a return to malaria control for countries still facing endemic malaria.

During the next three decades, most countries that had eliminated malaria were able to maintain their malaria-free status, and a few were able to continue on their path to elimination.(13) A total of 10 countries were able to eliminate malaria between 1979 and 2009, primarily in the Eastern Mediterranean (6), as well as small islands such as the Maldives and Seychelles. However, many of the countries that were not able to eliminate malaria experienced devastating disease resurgences (14) due to a lack of international financing and resources, demotivated and reduced number of malaria program staff, as well as drug and DDT resistance.(15,16) During the GMEP, many malaria elimination programs adopted a vertical organizational structure, outside of the routine public health system, and therefore were difficult to sustain.(9)

In the late 1990s the malaria control winds began to shift. Momentum was gaining for a new push for burden reduction through increased malaria control

efforts. An initiative by the WHO, the World Bank, and other United Nations agencies was launched in 1998 to re-invigorate malaria control efforts globally, called the Roll Back Malaria (RBM) initiative, with the aim to halve the suffering caused by malaria by 2010.(17) The RBM initiative was a social movement to stimulate international donors to invest money into achieving newfound gains in malaria control.(18) To highlight the political will supporting RBM and to discuss strategies to combat malaria in Africa, on April 25, 2000, African Heads of State and governments gathered in Abuja, Nigeria to attend the 'Summit on Malaria'.(19) In the early 2000's, international support for a renewed effort to control and eliminate malaria developed momentum and led to the creation of the Global Fund to Fight AIDS, Tuberculosis and Malaria (GFATM), in addition to other international organizations such as vaccine development (PATH Malaria Vaccine Initiative, PATH MVI), medicines to overcome drug resistance (Medicines for Malaria Venture, MMV), and new and improved insecticides to overcome mosquito resistance (Innovative Vector Control Consortium, IVCC). In 2006, the US President's Malaria Initiative (PMI) began investing in malaria-endemic countries to strengthen their malaria programs and respond to other public health threats by improving malaria and other disease surveillance.(20)

During the 2000s, when enormous sums of international funding was being raised for malaria control, malaria programs were deploying a package of proven malaria control interventions to attain high levels of population coverage with the goal of achieving maximum health benefits. Evidence showed that by scaling up multiple interventions simultaneously with high population coverage nationwide, optimal health effects can be achieved.(21) This strategy known as "Scale Up For Impact" (SUFI) was endorsed by the RBM initiative and was shown to reduce child mortality by more than 20% in some settings.

In 2007, the Bill and Melinda Gates Foundation (BMGF) put their weight and wallet behind a redefined effort to include eradication as an explicit goal.(22) Soon after the Gates' support for eradication was announced, momentum for malaria elimination and ultimately eradication was back on the table and up for debate as to whether it was feasible within a generation.(23–25) In 2015, three important documents advocated for malaria elimination and eradication and outlined key operational, technical, and financial strategies to achieve progress toward malaria eradication: *WHO's Global Technical Strategy for Malaria 2016-2030*, *RBM Partnership's Action and Investment to defeat Malaria 2016-2030*, and *From Aspiration to Action: What Will It Take to End Malaria?*. By 2019, a *Lancet* Commission on Malaria Eradication was convened to consider whether malaria eradication is feasible, affordable, and worthwhile.(26,27)

1.2 Global malaria burden

An estimated 150 million to 300 million lives have been lost to malaria during the 20th century alone. Although malaria control and elimination over the last

century has been uneven globally, an estimated 50% of the world's population live in malaria-free areas, compared with only 30% in 1950.(28,29) The 2022 WHO World Malaria Report estimates that in 2021 there were 247 million malaria cases in 84 malaria endemic countries and territories.(30) Malaria case incidence was an estimated 59 cases per 1000 population at risk. Globally, estimated malaria-related deaths reduced steadily between 2000 and 2021 from 897,000 to 619,000, respectively.

The highest burden of malaria remains to be in sub-Saharan Africa where an estimated 95% of global malaria cases occur, accounting for 234 million cases in the WHO African Region in 2021.(30) Although the true burden remains difficult to measure given the complexity of malaria,(31,32) estimates of malaria cases and deaths overall were declining prior to the COVID-19 pandemic, but progress slowed during the pandemic.(33) The majority of the malaria burden in Africa remains to be in the youngest children (< 5 years);(30) however, recent modeling shows the most impacted age group in Africa rising slightly to children older than 5 years.(34–36) This suggests that the increased protection for children under 5 is keeping the youngest alive until they are older, but also that older children are increasingly vulnerable to disease and maintaining control measures will be critical.

Malaria is commonly associated with poverty (37–40) and poverty exacerbates malaria given the increased healthcare costs (including travel to a health facility), lost ability to work due to illness, and increased malnutrition and disease susceptibility (especially in young children).(41,42) Malaria impacts countries economically, including with foreign investment and human productivity, therefore increasing the financial toll on the population affected.(43,44)

Every year more malaria-endemic countries make progress toward reducing their malaria burden and some achieving malaria elimination, further shrinking the malaria map. In 2000, there were 108 malaria-endemic countries compared with 84 in 2021. During that same period, countries reporting fewer than 100 indigenous cases increased from 6 to 27.(30) As a result of this continued progress, the epidemiology of malaria shifts – malaria is increasingly imported, caused by *Plasmodium vivax* in settings outside of sub-Saharan Africa, and clustered in small geographical areas or clustered demographically into subpopulations, which are often predominately adult men.(45)

This progress has been largely driven by international funding from wealthier countries financially supporting the GFATM,(46) as well as direct financial support provided to endemic countries from the US PMI,(20) the United Kingdom's Department for International Development (DFID),(47) and the Australian Department of Foreign Affairs and Trade (DFAT).(48) Private foundations and organizations also donate funding for malaria including research, such as the BMGF,(49) or provide in-country support for malaria endemic countries, such as the Carter Center.(50) Bolstered by *WHO's Global Technical Strategy for Malaria 2016-2030*, regional and country-level targets for malaria control and

elimination also provide political, financial, and technical support for countries.(51)

As malaria control interventions support a reduction in malaria morbidity and mortality and malaria transmission decreases, the goals of the malaria program and their interventions will need to adapt.(52) In areas of high transmission, monitoring and evaluation of control activities are based on aggregate numbers, and actions are designed to ensure that entire populations have access to malaria services. In areas of low and moderate transmission, the distribution of malaria is more heterogenous, and interventions need to be targeted toward populations that are most severely affected by the disease. In pre-elimination and elimination areas, ensuring efficient detection of and response to new malaria cases and foci is critical. Individual cases of infection or clusters of cases should be investigated to identify risk factors and eliminate foci of transmission.

Regional and cross-border initiatives to control and eliminate malaria are supporting reductions in the burden of malaria. These include the Elimination 8 in southern Africa,(53) the Asia Pacific Malaria Elimination Network,(54) and initiatives in Mesoamerica and Hispaniola.(55,56) In areas with contiguous borders and a shared challenge of combating malaria, cross-border collaborations have been initiated.(57–59) Progress in reducing the malaria burden have also been driven by lessons learned through program implementation and operational research.(23) An important component of progress in controlling malaria is through sharing of experiences and lessons learned among similar settings or adapting strategies for malaria from one setting to another.(60–63)

Challenges to eliminate malaria remain, and historically have led to significant malaria resurgences.(14) The reasons for these malaria resurgences are many, and not limited to: drug(6,64,65) and insecticide resistance,(6,64,66,67) international donor complacency,(64,68–70) human migration,(71,72) resource constraints,(64,70,73,74) war and conflict areas,(75–79) and accessing hard-to-reach areas and mobile populations,(64,65,80) among others.

1.3 The malaria parasite

Malaria is a disease caused by the *Plasmodium* parasite transmitted by the bite of an infected *Anopheles* mosquito. Malaria has existed alongside humans for over a millennia,(2,81) killing more humans than all wars and conflicts combined.(82) Over 200 species of *Plasmodium* exist, however only five infect humans. The *Plasmodium* species has two important stages: one lifecycle in a blood-feeding insect host (e.g., a mosquito) and a second lifecycle in a vertebrate host (e.g., mammals). (Figure 1) The malaria parasite is transmitted from an infectious mosquito to a human by inadvertently injecting sporozoites into the host during a blood meal. Once injected, the sporozoites search for soft tissue within the host, typically the liver, and invade hepatocytes. During the vertebrate host phase, the parasite emerges from the liver as merozoites and enter the blood

stream, infecting red blood cells (RBCs) and go through continuous asexual cycles of erythrocyte infection within the RBCs resulting in disease (known as malaria).

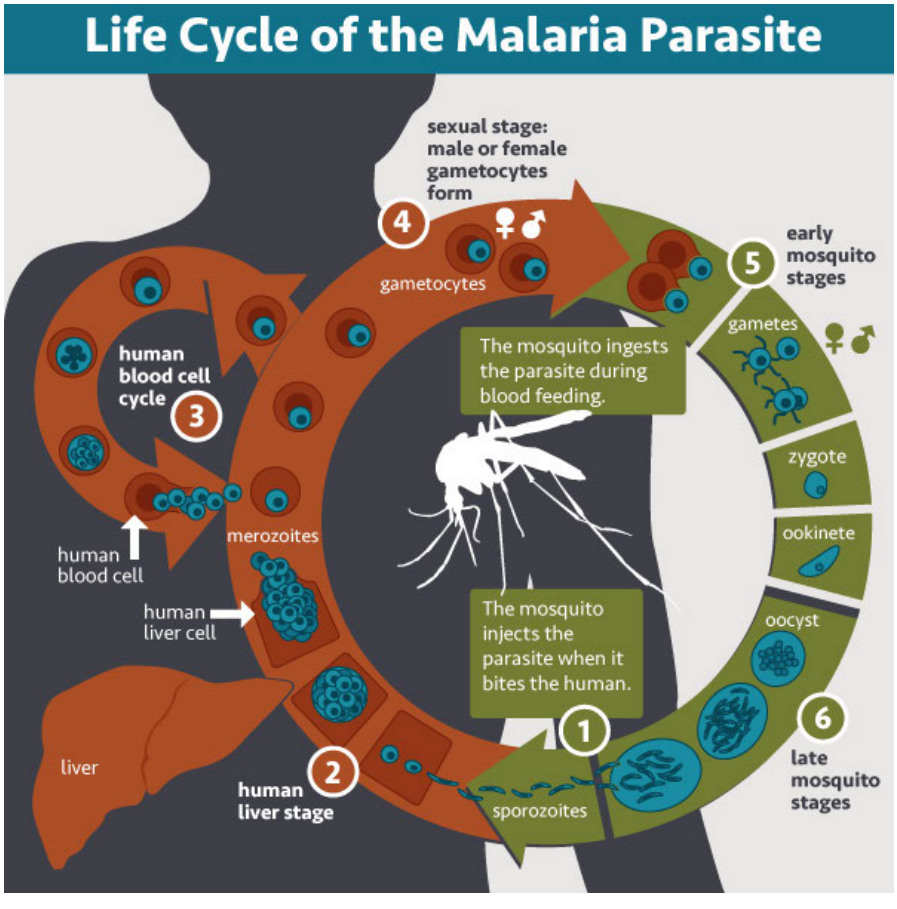


Figure 1 Malaria parasite lifecycle

Source: "Life Cycle of the Malaria Parasite" by NIAID is licensed under CC BY 2.0. To view a copy of this license, visit https://creative commons.org/licenses/by/2.0/?ref=openverse.

A small percentage of merozoites flowing through the host blood stream do not infect the RBCs, and instead differentiate into a sexual stage called gametocytes.(83,84) The gametocytes picked up by the mosquito host during a blood meal travel to the mosquito's midgut where they develop into male and female gametes, which fertilize each other, and form a zygote.(83,85) The zygotes develop into ookinetes, penetrating the wall of the midgut. The ookinete then embeds itself into the guts' exterior membrane and develops into an oocyst. These oocysts produce large numbers of sporozoites which migrate to the salivary

glands of the mosquito where they can be injected into the blood of the next mammal host, continuing the cycle of transmission.(85)

Plasmodium falciparum, most widespread in sub-Saharan Africa and southeast Asia causes the greatest number of deaths, especially in malaria immune-naive children under the age of five. *P. vivax* is more geographically widespread than *P. falciparum* and less severe.(31) *P. vivax* is typically a milder form of malaria, though far from benign. More recent evidence has shown severe *P. vivax* to be more frequent, including high prevalence of severe anaemia associated with the disease.(86,87) Additionally, evidence on the impacts on an individuals' health show that if *P. vivax* is not treated properly, it can sustain itself as a chronic infection contributing to poor health overall.(88) *P. vivax* is more common in Latin America and Asia, and not largely present in sub-Saharan Africa, particularly outside the Horn of Africa. This is due in part to the Duffy antigen and erythrocytic properties in the blood that prevent the *P. vivax* parasite from infecting the human RBCs.(89) RBCs that lack the receptor for the Duffy glycoprotein are relatively resistant to invasion by *P. vivax*. Furthermore, development of *P. vivax* within the mosquito gut can occur at lower temperatures than other *Plasmodium* species making it feasible to transmit in greater geographical climes and facilitate longer transmission seasons.(90) Other species such as *P. ovale* and *P. malariae* are also widespread in all areas with malaria transmission, but tend to be mild and not a dominant parasite species.(91) A newly-recognized fifth *Plasmodium* species prevalent in southeast Asia is *P. knowlesi*, which has also been identified in macaques as an additional host.(92)

1.4 Malaria transmission and epidemiology

1.4.1 Vector

Malaria transmission depends on the interaction between the host (typically humans) and the *Anopheles* mosquito at a time when malaria parasites are available to be transferred between hosts. There are around 40 *Anopheles* dominant vector species that commonly transmit malaria, despite more than 450 being formally recognized.(93) Male *Anopheles* mosquitoes do not transmit the malaria parasite and are primarily for mating. Adult female mosquitoes are directly responsible for transmitting malaria during blood meals. Both male and female *Anopheles* feed on sugar sources; however, females require a blood meal for the development of eggs.(84) After 2-3 days of resting following a blood meal, the female *Anopheles* will lay her eggs in water for further development as larva and pupae. It takes approximately 10-14 days for an egg to develop into an adult mosquito which can fly and continue the cycle of transmission by seeking out a host. Humans and animals have many similar odours and skin-derived compounds that attract mosquitoes.(94) Research has shown that female mosquitoes actively

carrying malaria parasites are significantly more attracted to human breath and odours than uninfected mosquitoes.(95)

The footprint of the *Anopheles* species spans nearly the entire planet from the northern parts of Russia and Europe in the northern hemisphere down to the northern tip of Australia and southern parts of the Amazon basin in the southern hemisphere. The *Anopheles* species vary geographically and depend on the local ecology to survive.(93) In Meso- and South America there are 8 dominant vector species (DVS) of which some include: *An. albimanus*, *An. albitarsis s.l.*, *An. darlingi*, and *An. pseudopunctipennis*. In Africa there are only 3 DVS in total, and include: *An. arabiensis*, *An. funestus*, and *An. gambiae*, with many geographical areas having significant overlap where the DVS co-exist. In the Asia Pacific region there are 16 DVS, highlighting the incredible diversity of *Anopheles* species and include, but are not limited to, *An. culcifacies s.l.*, *An. dirus s.l.*, *An. lesteri*, *An. minimus s.l.*, *An. sinensis*, and *An. stephensi*.(93)

Not only is there enormous diversity of the *Anopheles* species, the habitats in which the mosquitoes prefer for feeding, resting, and breeding are also diverse.(84) The presence of water is essential for mosquito breeding and larval development, and different water bodies attract different types of mosquitoes.(96) Mosquito feeding habits are endophagic (indoors) or exophagic (outdoors). After a blood meal, mosquito resting habits are either endophilic (indoors) or exophilic (outdoors). These factors, along with the time of day when the *Anopheles* mosquito is active (dusk, dawn, or at night) can influence the transmission of malaria between mosquitoes and humans, particularly when there is substantial overlap and exposure. In low transmission and prevention of re-establishment settings, these factors are particularly important where ecological conditions exist and *Anopheles* vectors are competent, especially in areas with highly mobile populations.(97)

Even with high levels of coverage of vector control interventions such as IRS or insecticide-treated nets (ITNs) meant for sleeping under at night, there are important gaps in protection that are often overlooked.(98) For example, ITNs have a gap in protection when individuals are outdoors without protection against potentially infective mosquito bites, a lack of use and suboptimal coverage of ITNs, and insecticide resistance by reducing the effectiveness of the intervention (e.g., the mosquito doesn't die when it comes into contact with insecticide or is repelled by it). In many malaria-endemic settings, ITNs are provided to households by the malaria program for use overnight when mosquitoes typically feed to protect the individuals sleeping in that residence. However, in settings with exophagic and daytime biting mosquitoes, ITNs are not being used but transmission is possible. This would also apply to mosquitoes that feed at dawn when an individual may be preparing to go to work or at dusk immediately after the sun sets when they are sitting outside unprotected but when a potentially-infected mosquito is seeking a human host. In forested settings where daytime biting *Anopheles* vectors exist, gaps in protection are an important challenge likely increasing the chance for infection. Furthermore, a second residence

at a farm or area within the forest are typically open-air structures with fewer walls or with no screening over the windows. Additionally, forest goers are not likely to have other vector control protection such as ITNs or have the structure be sprayed by IRS due to the remote location.

1.4.2 Human host

The human genome has evolved alongside the *Plasmodium* parasite for thousands of years unlike any other infectious disease, and in doing so both humans and parasites have evolved. When humans encounter a female *Anopheles* infected with malaria, a cascade of events occur within the human host. (see Figure 1 above) With at least one malaria infection some level of acquired immunity is induced; however, adequate protective immunity in a healthy host usually requires repeated infections over time.(99,100) Although the immune response to malaria is not fully understood, there is a clear age-structure of burden to clinical disease in endemic settings as older children and adults have resistance to severe morbidity and death due to acquiring natural immunity and surviving previous infection. Furthermore, children at the youngest age (birth to 6 months) are remarkably resistant to high parasitemia due to the protective immunity acquired with maternal antibodies.(99) It is well known that some humans have developed a survival advantage in the presence of malaria.(83) Human blood has evolved through developing protective strategies to prevent *P. falciparum* malaria from infecting RBCs, which has led to sickle cell anaemia.(101)

The presence of malaria symptoms may or may not be causing the disease.(102) Presence of 'malaria disease' is a tip of the 'malaria infection' iceberg regarding parasite densities in the human host and has implications for infection detection limits. In high burden areas, where individuals can be infected with malaria many times throughout the course of a year, large swaths of the population have high levels of immunity to the malaria parasite harbouring lower parasite densities compared with less-exposed individuals.(103) Adults are more likely than children to carry sub-microscopic infections due to repeated infections over time. In immune-naive populations, clinical symptoms are almost always observed with a *P. falciparum* infection, even at very low parasitemia levels. Parasite density is linked to disease with fewer parasite densities translating to less disease.(99)

Parasite densities may also fluctuate over time.(104–106) Low parasite density infections also have low gametocyte (sexual stage) densities, restricting the ability to transmit malaria given a mosquito must ingest both a male and female gametocyte to further the parasite life cycle.(107) Asymptomatic parasite reservoirs can persist and maintain an infectious reservoir of infection to sustain transmission. *P. falciparum* asymptomatic reservoirs have been shown to be more likely detectable through standard diagnostics compared to *P. vivax*.(108) The relative contribution of low-density infections to onward transmission varies widely in different endemic settings. Some cross-sectional studies have

shown that asymptomatic *P. vivax* carriers are estimated to contribute to a majority of human-to-mosquito infections; however, individuals with very low parasitemias are not likely to contribute significantly to transmission.(109,110)

To maintain parasite lifecycle endemicity, *P. vivax* and *P. ovale* have a unique latent form in the host, called hypnozoites, which hide in the liver and reactivate when appropriate. Hypnozoites can re-emerge from the liver months or years after initial infection, when the conditions are permissible for transmission, for example when mosquitoes are more abundant during the spring and summer months.(90) The majority of *P. vivax* relapses are likely asymptomatic and do not come to the attention of the health system and therefore maintain a potentially important infectious reservoir.(111,112)

1.5 Malaria infection detection and treatment in Asia Pacific

1.5.1 Malaria case presentation

Generally, individuals present to a health facility and undergo a malaria diagnostic test to determine the presence of malaria. If determined to be a positive malaria infection it is typically uncomplicated malaria which is an early-stage infection.(113) Symptoms of uncomplicated malaria generally include fever, chills, headaches, nausea and vomiting, among other symptoms.(114) If uncomplicated malaria goes untreated, the infection can develop into a medical emergency known as severe malaria.(115,116) This is particularly true in pregnant women,(117) those with spleen issues,(118) malnourished children,(119) and the elderly. In hyperendemic malaria settings, severe malaria is predominantly a disease of children aged 1 month to 5 years of age. Severe malaria is almost always caused by *P. falciparum* usually 3-7 days after the onset of fever. In countries that have little to no malaria, travellers returning from endemic areas can develop severe malaria because of a lack of acquired immunity against malaria.

1.5.2 Malaria diagnosis

Clinical or presumptive diagnosis of uncomplicated malaria, based off of whether the individual has or recently had fever, was once standard practice before widespread availability of diagnostic tests.(120,121) A prompt malaria diagnosis of a suspected case should be confirmed with a malaria-specific diagnostic test before treatment is provided. Clinical malaria diagnosis alone is not enough to rule out other bacterial or viral infections.(122) A correct diagnosis improves the overall management of patients with febrile illnesses and also helps reduce the emergence and spread of drug resistance.(123) Accurate measurement of a malaria infection is also important to define the burden of the

disease and understand the impact of control measures.(102) Malaria diagnostics vary in their difficulty of user operation, threshold of parasite detection, time for diagnostic result, and need for laboratory equipment and chemical reagents.(See Figure 2 on diagnostics.) There are important advantages and disadvantages to differing malaria diagnostics that should be considered depending on the setting in which they are being used.

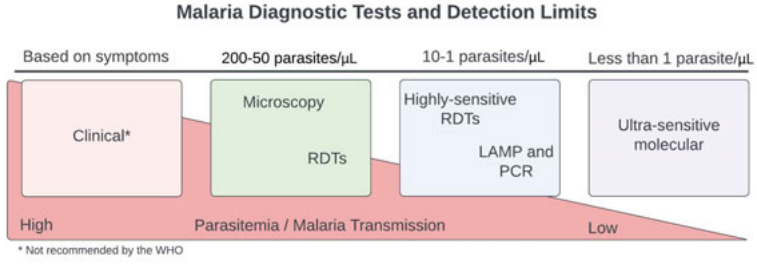


Figure 2 Malaria diagnostics and detection limits

1.5.2.1 Light microscopy

The gold standard to diagnose a malaria infection is optically using light microscopy,(124) and is the WHO recommended method to confirm a malaria infection.(125) The advantages of microscopy are that it allows for the detection of different malaria parasites for accurate treatment prescription, and to identify the *Plasmodium* lifecycle stages, including the presence of gametocytes. Microscopy is also able to quantify parasite density, and is particularly useful for patient follow-up to measure treatment outcome efficacy.(125) The limit of detection for microscopy is 50-200 parasites per µL of blood with parasite quantification of a blood smear in less than an hour.(126) This is important for low transmission and mixed *Plasmodium* species settings where low parasite density infections are more common.(127)

A small sample of blood is taken from a febrile individual presenting with suspected malaria and placed on a glass slide to make a thick and/or thin blood smear. Using a microscope, one hundred high powered fields are examined by a trained health worker who peers down the lens of a microscope to visually examine and identify whether parasites are present with a 3% solution Giemsa-stained blood slide. The Giemsa solution is the standard solution used to stain a blood slide for reading.(128,129) A thick blood smear, a more sensitive approach, is meant to determine whether a malaria infection is present or not.(126) If malaria positive, a thin blood smear is used to detect the morphology of the parasite species.

Microscopy is not as field friendly a point-of-care diagnostic, but can be operated in resource-limited settings that have limited or no electrical power source.(130,131) A disadvantage of microscopy is that it is dependent on the ability of the microscopist to read the slide accurately. Therefore, regular microscopy trainings are important for health staff, particularly as malaria declines and in mixed-species settings.(132) Additional skills are needed for microscopy including blood smear collection, fixing, staining and storage – all of which can alter the integrity of the blood slide sample and result.(133)

The cost of microscopy per test is fairly inexpensive ($0.12-$0.40 USD), depending on the setting and when excluding labor and the capital expense of the microscope. However, labor and human resources are important to consider to achieve an accurate diagnosis, but the cost can be shared across other disease diagnoses that require microscopy. Furthermore, maintenance of the microscopes is an important factor and potentially additional cost to consider.(134) In low transmission and prevention of re-establishment settings where microscopy is largely relied upon for malaria diagnosis, it is important to maintain malaria training on diagnosis and blood slide preparation, have adequate antimalarial stocks in facilities that see few patients, and raise malaria awareness among the public and targeted high-risk groups for malaria to encourage treatment seeking.(135,136)

1.5.2.2 Rapid diagnostic tests

In resource-limited settings where microscopy may not be feasible, point-of-care rapid diagnostic tests (RDTs) are a common method to identify malaria parasites. RDTs have been instrumental in malaria control and elimination by improving access to a confirmatory malaria test for improved differential diagnosis.(137) The main advantages of RDTs are that they are field-friendly, simple for community health workers to use without much training, have self-contained packaging useful for remote settings, and provide a rapid diagnostic result, typically within 15 minutes. RDTs are commercially available and typically indicate the presence of the parasite without speciation (PAN) or have some species-specificity to identify *P. falciparum* or *P. vivax* species.(138) The limit of detection of RDTs is comparable to that of microscopy at 50-100 parasites per μL of blood.

A small sample of blood is taken from a febrile individual presenting with suspected malaria and placed on the RDT. RDTs are designed to detect antigens by using an immunochromatographic strip where blood is placed on one end and the results are displayed by lines on a surface strip after placing reagent on the RDT.(138) Parasite antigens are detected when the blood sample and reagents are combined on the strip.

One disadvantage of RDTs, like microscopy, is their lack of sensitivity, particularly in low malaria transmission settings. This is true of infections that are low and very low density.(139) The lack of species identification in low-density infections limits the ability for an accurate diagnosis, and therefore appropriate

treatment regimen. Furthermore, RDTs are unable to be used for treatment follow up to determine whether an individual is still malaria positive, and may lead to false positives because the RDT detects malaria antigens which can remain in the blood for up to 30 days after treatment.(126) RDTs also require certain conditions to transfer and store the diagnostic as high temperatures and humidity can contribute to poor diagnostic performance.(140) The cost per unit is slightly more expensive than microscopy at around $0.85 USD per test when excluding labor. Ultra-sensitive RDTs are being evaluated in research settings and suggest that they may be more sensitive than standard RDTs and particularly in asymptomatic populations.(141)

1.5.2.3 Nucleic acid amplification

Malaria detection can be done through molecular methods, but are not implemented as part of routine diagnosis, largely due to the higher costs and resources associated compared to standard diagnostics.(142) However, molecular methods do provide a number of advantages over microscopy and RDTs in that these methods are more specific in identifying mixed infections and more sensitive to detect lower parasite densities.(143,144) The limit of detection of molecular methods can be as low as 1 parasite per µL of blood.(145) This is particularly important in low transmission settings where a majority of the infections are asymptomatic and sub-microscopic.(103,105) Similar to microscopy, some factors can affect the accuracy of detecting infections using molecular methods, such as methods for the collection and storage of blood samples. Furthermore, selecting appropriate primers and extraction methods can affect diagnostic performance.

There are several different types of molecular methods currently available, although the process for each is similar. For molecular detection, the parasite DNA or RNA must be extracted from the sample (whole blood or from a dried blood spot), then amplified using certain laboratory equipment, and finally detected and/or quantified depending on the method used. There are some differences between the types of molecular methods including related to the costs, infrastructure needed, field applicability, sample preparation and extraction, turnaround time for a result, and the detection limit.(146)

The disadvantages to using molecular methods compared to standard diagnostics includes the high cost of the equipment and laboratory facilities needed, consumables such as reagents and filter tips, as well additional training required for lab personnel. In addition, the turnaround time for a diagnostic result is longer for molecular methods, typically at least 12-24 hours.(146) Laboratory facilities are also likely to be far from rural and remote areas. The storage of blood samples for molecular detection is also important to prevent the degradation of the DNA sample collected, and therefore the loss of diagnostic sensitivity, particularly in hot and humid environments.(147)

1.5.2.3.1 Polymerase chain reaction

The most commonly used molecular detection method for malaria and other diseases is polymerase chain reaction (PCR), developed in the early 1980s.(148) PCR methods are very sensitive using DBS or whole blood, although the latter has been shown to reach a limit of detection of 0.022 parasites per µL when using higher blood volumes (1 mL).(149) In this thesis, the methods for analysis was nested PCR or real-time PCR targeting the 18S ribosomal(r)RNA gene that was developed by Snounou *et al* to identify the four human species of malaria.(143) Additional real-time quantitative PCR methods (qPCR) have been developed since.(150) Because study III is located in a mixed-species setting with known *P. knowlesi* transmission, a *P. knowlesi*-specific PCR test was used.(151) Having the appropriate set of primers can support the identification of all five *Plasmodium* species, particularly important for mixed-species settings.

PCR is frequently used to determine malaria prevalence at a point in time in a defined area when conducting community-based surveys to monitor disease burden and to measure the impact of malaria control interventions.(152) While not field-friendly for application in a routine setting, there are some ways in which PCR has been evaluated to improve its applicability in low transmission and mixed-species settings by increasing its throughput (numbers of samples able to be analyzed),(153) or using pooling strategies in low transmission settings where few infections are found overall to make PCR a more-efficient and cost-effective approach and still maintain diagnostic sensitivity.(152) Other efforts to improve the field applicability of PCR have resulted in the development of several different assays, all of which maintain low sensitivity thresholds, but remain limited in practice due to the need for a PCR amplification step.(146)

1.5.2.3.2 Loop-mediated isothermal amplification

A more recently developed molecular method to detect *Plasmodium* malaria and other diseases is loop-mediated isothermal amplification (LAMP).(154) Similar to PCR, LAMP has shown its ability to detect low-level malaria and asymptomatic infections with sensitivity thresholds down to around 1 parasite per µL depending on the blood volume being tested.(155) LAMP has been shown to identify all human *Plasmodium* species,(156) including *P. knowlesi*. (157,158) Loopamp kits are commercially available (Eiken Chemical co) and were used in this thesis for studies II and III, along with PCR methods for validation. Loopamp kits currently available have been validated for the detection of *P. falciparum*,(159) but can only indirectly detect *P. vivax* or other *Plasmodium* species using a combination of PAN-genus and *P. falciparum*-specific LAMP primers.(160) Currently, this lack of commercially available kits is a disadvantage compared to PCR in mixed *Plasmodium* species settings, but *P. vivax*-specific kits are being developed and evaluated.(161,162)

An advantage of LAMP compared to PCR is that less laboratory equipment is required to process samples. Loopamp kits use a cheaper and quicker boil and spin method for DNA extraction and requires a centrifuge. A simple heat block or water bath can be used for the LAMP reaction and does not require a PCR machine, which can be cost prohibitive.(163) Using a boil and spin method for DNA extraction speeds up the turnaround time for a LAMP result, making it potentially more field-friendly.(162,164) Furthermore, the Loopamp kits come pre-packed with a set of tubes that already contain reagent mixtures. However, like PCR, LAMP requires knowledgeable technicians to manage DNA contamination protocols, reliable electricity, a facility for preparing basic assays, ideally with separate work stations to avoid DNA cross-contamination.(162,165) Although cost of the commercially-available Loopamp kits remains a limiting factor in their uptake, compared to standard diagnostics,(166,167) there may be some appropriate settings, particularly in very low transmission areas that have high proportions of asymptomatic and low-density infections where LAMP may be useful.(146)

1.5.2.3.3 Microsatellite genotyping

Microsatellite genotyping is an important molecular identification tool that studies the genetic relatedness of *Plasmodium* infections. This technique increasingly is being used to investigate the population structure of malaria parasites, and to better understand the sources of transmission.(168–170) Purposes of genotyping for malaria include: 1) monitoring drug resistance;(171–173) 2) evaluating treatment outcomes; 3) characterizing parasite flow between regions;(174,175) and 4) distinguishing imported from locally transmitted infections. Microsatellite (short tandem repeat or STR) analysis was used in study II to compare allele repeats at specific loci in the DNA between samples. A STR is a microsatellite with repeat units that are 2 to 7 base pairs in length, highlighting the diversity of repeats among individuals.(176) Genotyping in study II was performed using fluorescently labelled PCR primers for a set of nine selected standardized microsatellite loci for *P. vivax*. Amplification of the microsatellite by PCR is used to determine how related the primers are to the *Plasmodium* DNA sample obtained. Genotyping is particularly important for Southeast Asia where *P. falciparum*-resistant malaria was discovered, sparking fears that malaria endemic countries would lose their first-line malaria treatment, artemisinin-based therapies, among others.(172,177)

As malaria transmission declines and the distribution of malaria infections becomes increasingly heterogenous within villages, and even among particular groups of individuals, hotspots of transmission are more easily detected and fuel transmission during the high malaria transmission seasons.(178) Additionally, in areas with high mobility of its population, passively detected malaria case data may not accurately characterize local transmission patterns. Importation of malaria cannot always be confirmed even when a travel history is accurate.(175) To characterize transmission, combining parasite genetic data with anonymized

mobile phone data, malaria epidemiological case data, and travel survey data has been shown to distinguish between imported and local transmission and estimate the direction and intensity of parasite flows.(174) By including a combination of human mobility and parasite genetic data it is feasible to show the connectivity of malaria parasite populations over great distances and between countries that have eliminated malaria and those with remaining endemic malaria.(179) However, there are inherent biases and limitations in epidemiological and genetic datasets, and these types of analyses can be prohibitive for malaria programs to perform.

Additional malaria infections detected near an index patient are assumed to be related by transmission to the primary index case infection. Research in *P. falciparum*-dominant areas have shown evidence of such infection clustering;(110,180–183) however, for *P. vivax*, some evidence has shown household infection clustering,(184) but in other studies that included genotyping, evidence of relatedness between index and additional infections identified was not clear.(185,186) Clustering of infections is an important factor when conducing any type of response to an index patient. In settings where infection clustering is evident (e.g., within the index patient household or nearby households) then conducting follow-up testing around the index patient could be an effective approach to identifying additional infections. However, in settings where infection clustering is not evident, follow-up of individuals nearby the index patient may not be an effective approach to identifying additional infections. Genotyping can help determine parasite relatedness by providing important information on whether parasites are from a common source (with shared risk factors such as behavior) or genetically different and the source is unrelated, possibly from either a new or relapse infection.(187)

PCR is required for genotyping, making it a cost-prohibitive method for most malaria endemic settings, particularly for routine use. However, many low transmission settings are conducting research trials in endemic areas within their borders, and therefore expertise in molecular genetics exists and could be leveraged. Including parasite genotyping into malaria program strategies for control and elimination face several barriers without a one-size-fits-all approach.(188) The challenges of integrating molecular technologies in Africa have been discussed;(189) however, in the Asia Pacific, the challenge of non-*falciparum* malaria is pervasive and is currently the epicentre of the emergence of resistance against frontline anti-malarials.(123) In the Asia Pacific region, there is consensus that data sharing and knowledge exchange across national borders is needed. Furthermore, establishing local laboratory expertise, collaborative research involving local and international research teams, and exploring the cost-effectiveness of genetic epidemiology for different use cases can support the expansion of genetics for more routine use in malaria programs. Importantly, engagement and data sharing between national malaria programs and local researchers working in this space will be needed.(188) Harnessing available expertise and using

it to characterize transmission dynamics in persistent or recurring malaria foci may help to accelerate elimination.(190,191)

1.5.3 Malaria treatment

Malaria cases are typically identified through the passive surveillance system at a health facility, malaria clinic, or hospital. Upon confirmation of a malaria diagnosis, antimalarial treatment should be initiated immediately based on the diagnostic result and in line with the recommended local malaria treatment guidelines. Treatment depends on five main factors: 1) infecting *Plasmodium* species; 2) clinical status of the patient (uncomplicated or severe); 3) drug susceptibility of the infecting parasite(s); 4) previous use of antimalarials; and 5) following local malaria treatment guidelines.(192) Antimalarial treatment dosage is different for adults and children, and is based on the weight of the patient. The objective of antimalarial treatment is to cure the infection (eliminate all parasites from the body) as rapidly as possible and to prevent progression to severe disease.(192) Appropriate treatment in an infected patient helps to prevent onward transmission of the infection to others and to prevent the emergence and spread of resistance to antimalarial drugs.(193)

1.5.3.1 Artemisinin-based combination therapies

Artemisinin-based antimalarial therapies are vitally important for malaria control and elimination. Using artemisinin treatment alone will compromise the drug effectiveness and increase the chance for drug resistance to emerge. Therefore, artemisinins are combined with partner medicines to prevent this selective pressure on the parasite. Artemisinins, and new quinoline derivates that are now used as partner molecules, were discovered by Chinese scientists in the 1970s through the *523 research programme*.(194) By the late 1990s, international research scientists were raising the alarm about drug resistant malaria and advocating strongly to combine malaria drugs to prevent mutations from arising, promoting greater parasite resistance to malaria drugs.(195) Artemisinin and its derivates had the most potent and rapidly acting properties of the antimalarial drugs available at that time.

The WHO recommends artemisinin-based combination therapies (ACTs) to achieve parasitological cure of a malaria infection and to prevent drug resistance.(196) There are at least half a dozen combinations of ACTs for the treatment of uncomplicated *P. falciparum* malaria. Duration of treatment is typically 3-days, covering two asexual cycles to ensure most parasites are eliminated, allowing for the partner drug to finish clearing any remaining parasites. In low transmission *P. falciparum* areas, a single low dose of primaquine (0.25 mg/kg) is given along with an ACT to reduce *P. falciparum* transmission by clearing any potential hypnozoites hiding in the liver.(197) In *P. vivax* areas, malaria treatment aims to cure the acute blood stage infection and to clear hypnozoites from the liver to prevent future relapses (also known as ‘radical cure’).(196)

ACTs are also highly effective at treating (but not curing) *P. vivax* malaria, even when the patient has a mixed species infection. For *P. vivax* and *P. ovale* infections, unless the patient is also treated with primaquine, relapses commonly follow.

1.5.3.2 8-Aminoquinolines

Quinine, the world's oldest treatment for malaria was discovered in the 17th century in Peru derived from the bark of the *Cinchona calisaya* tree, which grows high in the Andes mountains far from areas with endemic malaria.(198) Quinine is an active component in many other antimalarial drugs, including chloroquine, which was developed in the 1930s.(199) Following World War II, chloroquine became a major antimalarial drug. Chloroquine is an effective antimalarial therapy that is used to radically cure parasites from the body by clearing the dormant liver stage of *P. vivax* and *P. ovale* malaria, also known as hypnozoites.(200) By 1960, *P. falciparum* resistance to chloroquine began to emerge in Southeast Asia and South America and quickly spread across the world.(15) In areas that still have extensive resistance to chloroquine, primaquine (PQ) is used as an effective anti-relapse therapy.(201) A newer 8-aminoquinoline known as tafenoquine, has been recently introduced as another potential antimalarial drug for the treatment of relapsing malaria.(202)

To prevent relapses of *P. vivax* and *P. ovale* infections, a 14-day course of PQ (0.25 mg/kg) is recommended by the WHO in all transmission settings (except in the few populations with contraindications such as pregnant women, infants less than 6 months, people with G6PD deficiency, etc). Recent research has shown that a 7-day course of PQ with a larger dose per day (0.5 mg/kg) is feasible and well-tolerated to prevent relapse infections (except in the few populations with contraindications previously noted).(203) With the introduction of tafenoquine (300 mg) more recently, there is renewed hope that this anti-relapse therapy can improve treatment compliance because it is only a single dose treatment.(202) However, a major patient safety concern with any 8-aminoquinilines is the haemolytic toxicity in G6PD-deficient individuals as well as the general toxicity experienced in all patients.(204) G6PD-deficiency is an inherited X-linked genetic defect caused by mutations in the *G6PD* gene.(205) Where G6PD-deficiency is most common is also where *P. falciparum* malaria is endemic suggesting that G6PD-deficiency possibly provides a protective effect against malaria.(206)

Radical cure is important in low transmission settings where the goal is to interruption malaria transmission.(207) However, there are challenges for achieving safe and effective radical cure, including: a reliable point-of-care diagnostic that is inexpensive and easy to use, logistics of delivering diagnostics, ensuring appropriate training and quality control practice, and promotion of the need for G6PD testing, among others.(208) The effectiveness of radical cure can be limited because healthcare providers are reluctant to provide PQ without prior G6PD testing due to the risk of drug-induced haemolysis in individuals

with G6PD-deficiency.(209) The rates of G6PD-deficiency in the Asia Pacific region range from 0.1% (range of 0.0-0.4) to as high as 22.3% (range of 15.7-30.9).(210) In *P. vivax* and *P. ovale* dominant areas, G6PD testing is an important factor when deciding if PQ can be administered. Both qualitative and quantitative G6PD diagnostics are commercially-available for use in endemic settings.(211,212) Other barriers exist to wider implementation of G6PD diagnostics and their effectiveness, including: 1) perceived low risk of drug-induced haemolysis; 2) the perception that *P. vivax* malaria is benign and treatment with PQ was not a priority; and 3) the additional costs of introducing routine testing.(213)

1.5.3.3 Treatment follow-up and adherence

Antimalarial treatment follow up is determined by the parasite species infection, treatment provided, and patient accessibility. Adherence to the antimalarial medications depend on the side effects experienced while taking medicines and the patient tolerability of those side effects. There are ways to mitigate the side effects of antimalarial drug toxicity, including taking medications with food.(214) The duration of the antimalarial treatment course can also have an impact on adherence to the full course, an important consideration for longer duration treatment regimens such as 14-day PQ.(215) Even with a higher dose during a 7-day PQ course, the tolerability profile in a recent study was similar to the longer 14-day course, highlighting the potential to improve treatment adherence in G6PD-normal patients.(203)

Effectiveness of the malaria treatment, particularly with regard to relapsing malaria, is dependent upon adherence to the full treatment course,(216) typically when a large number of treatment courses are not directly supervised by health staff.(217) Without directly observed therapy (DOT), unsupervised antimalarial treatment regimens have little success at preventing recurrent infections.(218,219) Several factors can be the cause of poor medicine adherence, including: poor understanding of the treatment instructions, patient forgetting to take the medication, loss of the tablets or saving tablets for other family members in case of another infection once they are feeling better, and occurrence of side effects. DOT has been shown to be an effective way to support patients taking a full treatment course. However, this poses a challenge by requiring health staff to conduct DOT, which is not practical in most settings due to costs associated, including a lack of trained personnel and transport. In some settings, a community case management approach to malaria diagnosis and follow up has been shown effective.(220–222) Where resources exist, malaria cases are followed up by a health or malaria worker, sometimes a community-based worker or village volunteer, who can provide greater equity in accessing healthcare services, particularly in hard-to-reach areas.(223,224)

Acceptability is an important factor in taking malaria medicines, depending on whether the medicines are for malaria treatment or prophylaxis. Acceptability increases when known and trusted health care providers are involved in the

follow up process and recipients are given appropriate information about treatment adherence.(225,226) A study from Thailand showed that those who were adherent to antimalarial medication were significantly more knowledgeable of malaria and its aetiology than non-adherent individuals.(227)

1.6 Malaria control interventions

1.6.1 Vector control

Interventions that target the mosquito have been key factors in the control and elimination of malaria.(228,229) The purpose of vector control (VC) is to limit contact between mosquitoes and humans to prevent the transmission of the malaria parasite, thus interrupting the parasite life cycle.(230,231) Indoor residual spraying (IRS), or the application of insecticides on the interior housing surfaces (walls, ceiling) is a main VC intervention that has seen great reductions in malaria transmission. IRS was the main VC intervention during the GMEP era (1955-1969) and up until the introduction of more modern VC tools such as protective bednets while sleeping.

Bednets have been instrumental in reducing malaria globally.(232,233) Bednets are hung over a sleeping space and can accommodate several individuals overnight during peak mosquito biting times (between 10pm and 6am). Once insecticide was added to bednets, an additional protective effect enabled the killing of mosquitoes that landed on the nets, and further reduced the opportunity for transmission. Insecticide-treated nets (ITNs) and long-lasting insecticidal nets (LLINs) are a common form of bednets to protect those while sleeping or resting.(233) For those who work or travel in forested or forest-fringe areas and stay overnight in high-risk areas for malaria,(234–236) hammock ITNs are a convenient way to sleep off the ground and have additional VC protection from mosquito bites.(237) There is some evidence that insecticide-treated clothing (ITC) can reduce the incidence of malaria,(238) and has been shown to be highly acceptability among high-risk populations (rubber tappers) in Myanmar.(239)

Although VC using insecticides has been widely effective in reducing malaria morbidity and mortality, there are disadvantages to its use, including: insecticide resistance,(240) mosquito behavior change,(241,242) the need to reapply insecticides to walls and bednets,(243) human behaviors and the use of bednets,(244) and environmental considerations,(245), all of which may reduce the effectiveness of the VC intervention. In the United States, particularly during the GMEP era, the screening of porches, windows, and doors was another opportunity to provide human hosts additional protection from mosquitoes, especially during peak mosquito biting hours. Innovative housing designs for different settings have been evaluated to reduce the way in which mosquitoes and humans come in contact to prevent malaria transmission.(246–249) Even the

use of technology via lasers set up around the home are being evaluated for use.(250)

Water has a direct influence on aquatic habitats necessary for *Anopheles* larvae to survive and develop, as well as the impacts of temperature and humidity on their mortality rates.(251) Reducing the mosquito populations through larvaciding, thereby adding chemicals to common stagnant water areas (such as ponds or swamps) to kill the malaria parasite in its larval stages preventing its development, is another VC method used currently as well as during the GMEP period.(252) Larvaciding can be used in tandem with other VC methods such as IRS and ITNs to tackle persistent malaria foci. However, the disadvantage to this approach, as is similar to IRS, is the use of insecticides within the environment and risks posed in terms of resistance and ecological damage.(245)

Drainage of stagnant water is another method to reduce local mosquito populations to reduce malaria, but also for Dengue, Zika, and other vector borne illnesses. Finally, many consumer products are available that can be applied directly to the skin or sprayed on clothing,(96) as well as spatial repellents to deter mosquitoes.(253) Predicting larval habitats with remote sensing is another technique that has been developed to identify larval habitats for malaria control and elimination.(254)

1.6.2 Surveillance and response

Surveillance is the cornerstone of any successful disease reduction and public health program.(255) Surveillance contributes the data and information to characterize and assess the burden and distribution of adverse health events and identify emerging public health threats. The surveillance information gathered is then used to prioritize a public health response and monitor the impact of that response. Effective surveillance and response for malaria requires accurate monitoring of malaria cases and deaths, key entomological indicators, and the malaria control actions that were taken, relying on data and information from multiple sources.(30,256) To achieve a desired result, such as effective malaria burden reduction and elimination, a robust surveillance data collection system and a framework for using these data for action need to be in place and operationalized.(257)

Malaria surveillance and response systems are based on a number of factors such as the level at which details are recorded, promptness of reporting and investigations, and the frequency of analysis and response.(258) As transmission decreases, malaria becomes focal, and the intensity (level of detail) and frequency of reporting increases. Aggregate case data are reported on a monthly basis from large catchment areas in high transmission settings and as malaria transmission reduces to medium and low transmission, malaria reporting becomes near-real-time at the individual case level in small geographical areas (such as village or sub-village). Several factors impact the ability of a

surveillance response system to function, including: the level of transmission in a defined area, strength of the health system, and availability of resources.

As transmission decreases, heterogeneity in the malaria epidemiology becomes more evident.(45) The objectives of a malaria surveillance and response system and what is feasible to be conducted will need to evolve as the epidemiology changes. Surveillance and response becomes an integral part of the malaria program, much like ITNs, IRS, and malaria treatment have are main interventions during the high malaria transmission phase. The re-orientation of a malaria program from high levels of intervention coverage to prevent morbidity and mortality to a focus on completeness and timeliness of surveillance and response activities to seek out infections more effectively is a major challenge. Investments in a malaria surveillance system will be needed for immediate case notification (e.g., rapid reporting by mobile phone) and data analysis for decision-making.(259,260) For malaria surveillance and response systems to properly function, there needs to be a process whereby malaria data are being used for a response and incoming data are continually being analyzed to adapt to a changing epidemiological situation as transmission decreases. (See Figure 3 on the flow of health information.)

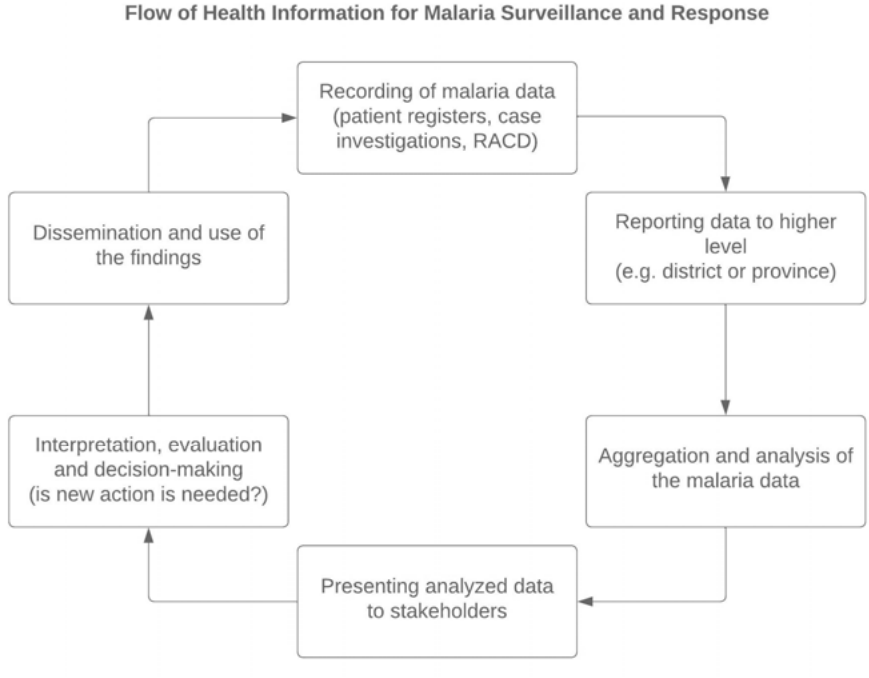


Figure 3 Flow of health information

Source: Adapted from *Malaria surveillance, monitoring & revaluation: a reference manual. Geneva: World Health Organization; 2018. Licence: CC BY-NC-SA 3.0 IGO.*

1.6.2.1 Passive case detection

In malaria endemic areas, a febrile individual visits a health facility or clinic that provides malaria diagnostic testing to determine if a malaria infection exists. Passive case detection (PCD) is the main way that index patients are identified for case confirmation and treatment. Upon visiting the health facility, blood is taken by fingerprick and either placed on a glass slide or a RDT to determine a diagnosis. If a positive result (typically uncomplicated malaria), the appropriate malaria treatment will be provided based on the *Plasmodium* parasite identified and the national malaria treatment guidelines. The patient is sent home and some basic information is typically obtained for treatment follow-up. If a febrile patient is determined to not have malaria based on the diagnostic test, a differential diagnosis should be made.(122,261) Differential diagnoses become more common in areas with declining malaria burdens, making it important to conduct regular malaria trainings for microscopists and health workers to maintain their diagnostic capacity to identify malaria and diagnose alternative causes of fever as well.(135)

PCD has several inherent challenges in the detection of all new infections(262) and underestimates the true magnitude of infections in a population.(263,264) One challenge of PCD is that it requires access to healthcare services, which may be difficult in remote and rural areas.(120) PCD also relies on treatment-seeking behaviors which can vary among malaria endemic settings.(265,266) In low transmission settings, where a large number of infections may be asymptomatic, infections may not always lead to clinical manifestations and no treatment is sought. In asymptomatic *P. vivax* carriers, there is some evidence that these individuals do contribute to a majority of human-to-mosquito infections.(109,110) Lastly, private sector health clinics and pharmacies are not typically included in the reporting structure for malaria surveillance; however, in countries where there is a strong private health sector, including their malaria-related diagnosis and treatment data would support malaria programs.(267)

1.6.2.1.1 Case investigation

As malaria transmission reduces, case investigations may be feasible to collect additional information on the index patient to bolster the malaria surveillance system for a potential response. The aim of case investigation is to determine whether an infection was acquired locally and the likely location of that infection. This information can help to determine whether local malaria transmission or other factors may lead to onward transmission. Some basic information is gathered from the index patient during the initial visit to a health facility, such as contact information, gender, age, diagnostic test result, date of fever onset and information on probable time and place of infection. A case investigation is carried out by a trained health worker and conducted typically within three days of initial index case presentation at the health facility.(60) Additional

information is collected during the case investigation including travel history and locations, occupation and other factors that may lead to onward transmission. This information, combined with the confirmatory diagnostic information (such as *Plasmodium* parasite species), and subsequent classification as either an imported or indigenous (or local) infection, is critical to identify an appropriate response.(262)

If the index patient lives in a non-malaria endemic area but reported recent travel history to an endemic area (within or outside of their country of residence), a health worker may conclude the index patient contracted malaria elsewhere, and that there is little risk of spreading that infection in the non-malaria area. However, even if there is no travel history but the conditions are favorable to transmit malaria (e.g., competent vectors exist) then the case investigation may have enough evidence to assume malaria was contracted locally (an indigenous infection). In lower transmission settings, where competent mosquito vectors exist, WHO recommends that case investigations be conducted.(262) There are challenges to completing a case investigation, particularly in areas with highly mobile and migrating populations.(268) Also, case reporting completeness to notify the surveillance staff that a case investigation is warranted, as well as the timeliness of case reporting, can make it difficult to complete case investigations.(269)

1.6.2.2 Active case detection

PCD identifies only a fraction of the malaria infections circulating in the population as it relies solely on a febrile patient visiting a health facility to determine a diagnosis.(262) To overcome the limitations of PCD, active case detection (ACD) strategies are important for detecting symptomatic individuals that are not detected by PCD and asymptomatic infections in the community. ACD is widely used to target the community- and household-level that are considered to be at high risk (e.g., occupational groups) by conducting large- and small-scale testing and treatment campaigns to identify additional malaria infections.(270) ACD complements PCD by targeting geographic areas of known malaria transmission and/or individuals who may be at high-risk of contracting malaria.(271) There are proactive and reactive strategies to detect additional infections in the population. Proactive and reactive strategies can be combined to tailor ACD strategies to high-risk populations and geographic areas.(272) ACD strategies can be more expensive than routine PCD because malaria staff will need to travel to the target areas, incurring greater human resource and travel costs, but the yield of infections detected may be higher than PCD.

1.6.2.2.1 Mass and targeted testing and treatment

One type of ACD strategy is mass testing and treatment (MTaT), whereby parasitological examination of all individuals, symptomatic and asymptomatic, in a population (typically a catchment area) is targeted. In this scenario, screening for malaria is done by microscopy or RDTs. The impact of conducting MTaT in

reducing malaria incidence and prevalence is marginal if any in most settings,(273–277) and typically conducted during national malaria indicator surveys to better understand community-wide malaria prevalence.(152) Some evidence has shown it can be beneficial to reduce asymptomatic malaria, particularly among older populations that may have some acquired immunity.(278) Furthermore, coverage of the MTaT population (the ability to reach every individual in a target catchment area) can have an impact on the results. This is particularly true if MTaT screening misses certain individuals in the target population who are more likely to carry an infection compared to the average individual in that population. MTaT can be costly although more research is needed on its cost-effectiveness compared to other screening strategies.(273)

Another type of ACD strategy is targeted testing and treatment (TTaT), whereby ACD can be proactively guided spatially by malaria prevalence risk maps, persistent malaria foci or may target certain high-risk groups, for example, individuals who may be exposed to infectious mosquitoes when working in the forest overnight.(271) This strategy also targets all individuals, symptomatic and asymptomatic, in these populations. Research from Cambodia showed that populations living in villages within the forest have significantly higher risk of infection compared to living outside of it, and that nearly all infections detected by PCR were asymptomatic and sub-patent.(279) Some evidence has shown that these high-risk populations can be targeted for testing and treatment;(234,280–283) however, the impact of such targeting is unclear. TTaT can also be done at border crossings to identify symptomatic and asymptomatic populations traveling and has been used to monitor *P. falciparum* artemisinin-resistance in the Greater Mekong Subregion (GMS).(284) Similar to MTaT, the cost-effectiveness for the TTaT strategy largely depends on the yield of infections detected compared to those tested and require more standardized costing methods for comparison.(285) Therefore, targeting in either space or time, such as areas of well-known seasonality, may have the greatest impact on transmission.

Missed individuals during ACD include refusals and may have an impact on the results. Reasons for not participating in ACD, include but are not limited to not being present at the time of testing or not having time/too busy to participate, being afraid of the needle, not wanting to provide blood with the fear that it will be tested for something other than a malaria infection (e.g., narcotics), not believing they are at risk of malaria or do not have an infection, or recently participated in ACD and provided a blood sample, but was not told the test result. It is important for any ACD campaign to be carried out by trusted health workers and staff and provide sufficient information about the reasons why ACD is being implemented to the target participants to reduce refusals and increase participation in the intervention.

1.6.2.2.2 Reactive case detection

In low transmission areas, where feasible and adequate resources are available, a follow-up visit is conducted to ascertain whether there is ongoing local

transmission in the index patient household or neighboring households to determine if additional vector control measures need to be undertaken to prevent potential onward transmission.(271) This strategy, known as reactive case detection (RACD), is conducted by health or malaria surveillance workers from the district- or subdistrict-level, and is an extension of the malaria case investigation. RACD is also an opportunity to check-up on the index patient treatment adherence and tolerability. RACD is not intended to impact malaria transmission, although there no evidence to date evaluating this. RACD is implemented in many low transmission settings with practices varying widely from country to country.(270)

RACD is conducted when suspected local malaria transmission has occurred and/or when competent vectors in that area are known to exist with the aim to detect additional infections and determine what vector control interventions need to be updated (e.g., providing new ITNs or updating household IRS).(52,271) RACD can also be used to investigate a malaria outbreak, such as when an above-normal number of index cases are passively-detected in a period of time.(52) RACD is typically conducted within a target range of 3 to 7 days after an index patient is identified through PCD, and depending on local resources available.(60,286) (See Figure 4 on RACD process.)

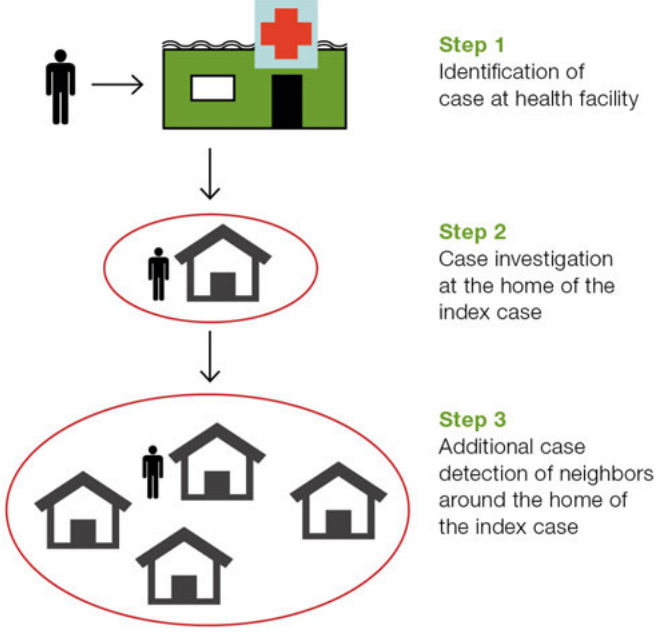


Figure 4 Overview of the RACD process

Source: Cotter C, *et al*. Piloting a programme tool to evaluate malaria case investigation and reactive case detection activities: results from 3 settings in the Asia Pacific. *Malar J*. 2017 Aug 22; 16(1):347

During RACD, a health or malaria surveillance worker conducts fever screening and diagnostic testing at the index patient household and surrounding households either with a RDT or by microscopy. Although the RACD screening radius should depend on local epidemiological and geospatial factors, the radius typically ranges from a few hundred meters up to 2 kilometers and depends on a number of factors including: household density, breeding sites, host availability for blood meals, and other ecological factors. RACD and the screening radius is also dependent on the financial resources available and the number of index cases that are required to have RACD follow-up conducted. If an RDT is used, a result can be identified at that time of the visit. If microscopy is used, the health worker typically must return to the health facility, process the slide for reading, and read the slide to determine a diagnosis. Anyone testing malaria positive is typically notified by phone or in-person so that an appropriate treatment course can be provided.

The RACD strategy takes advantage of the spatial and temporal aspects of malaria by focusing the screening activities within a specific time frame after an index patient is identified and at a particular location (typically the index patient household residence and surrounding households). A time-bound set of targets helps to guide the RACD activities and provides a clear set of indicators to monitor the implementation of RACD. Based off of China's '1-3-7' RACD strategy,(60) many malaria programs have adopted a similar framework for implementing RACD and has since been endorsed by the WHO.(258) In the '1-3-7' strategy, passively-detected cases are encouraged to be reported as suspected/confirmed malaria within one day of case presentation to the district-or higher-level depending on whether a rapid reporting system is available. A case investigation should be completed within three days and includes: 1) case confirmation, and 2) case classification (whether imported or local). Cases are typically 'confirmed' when a second diagnostic test (usually microscopy) is completed (quality assured PCR would be ideal if feasible to confirm species type). Lastly, a response (RACD) is conducted within seven days of initial index case presentation, whereby the index patient household and surrounding neighbors are visited to conduct malaria testing and determine what vector control interventions need to be updated.

Although the '1-3-7' timeframe is what most malaria programs intend to do, several factors and challenges influence the ability to achieve them. RACD is resource intensive mainly due to the human resources and travel costs required to visit the index patient household and surrounding neighbors, which may be in remote or rural areas.(287) Importantly for low transmission settings, poor diagnostic sensitivity to identify infections in low density and asymptomatic individuals is another challenge limiting the effectiveness of RACD.(127,139,180,185) A lack of complete and timely reporting and follow-up of index cases can lead to further decay of the effectiveness of RACD.(262) (See Figure 5.) Additionally, the utility of RACD can by influenced by *Plasmodium* species composition due to factors related to parasite development in the

mosquito gut and the local ecology (e.g., temperature) and affect the parasite lifecycle stages and their development making it more difficult to know exactly when RACD should be conducted.(288) In settings with *P. vivax*, the challenge of relapsing malaria when the infection has not been completely cured is a challenge and could affect how and when RACD should be conducted.

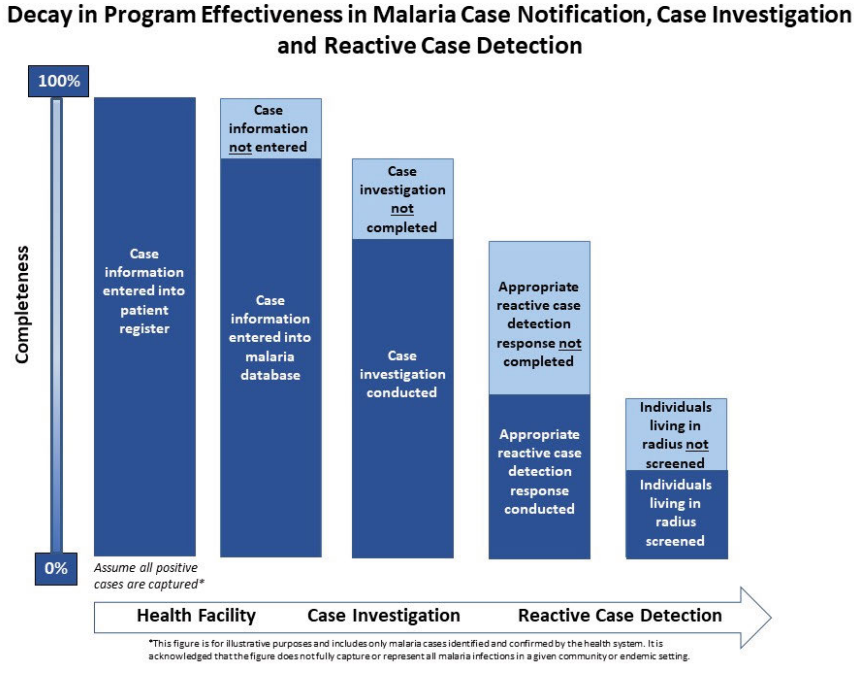


Figure 5 Decay in program effectiveness in RACD

Coverage of the intended target population during RACD is another major challenge that may impact the ability to detect additional infections and reduces the cost-effectiveness of the strategy.(286) RACD is typically conducted during working/business hours when government staff and vehicles are available, therefore likely missing some individuals who are away at work. By only screening those individuals at home during RACD increases the screening bias to mostly women and children. The missed individuals may likely be working elsewhere and be conducting activities which put them at higher risk for malaria (e.g., forest work). Ensuring full screening coverage of those individuals who reside nearby an index patient may require government staff to work after normal business hours and on weekends to ensure full coverage for maximum impact. Refusals for RACD can also have an impact on the target population screened. Reasons for refusing to participate in RACD, include but are not limited to: not having time/too busy to participate, being afraid of the needle, not wanting to provide blood with the fear that it will be tested for something other

than malaria infection (e.g., narcotics), not thinking they are at risk of malaria or do not have an infection, or recently participated in RACD due to a separate nearby index case and provided a blood sample, but was not told of the testing result. RACD that is carried out by trusted health workers and staff may help to reduce refusals and increase uptake of RACD. Given that RACD is resource and time intensive, it is important that the RACD reaches everyone in the intended target population.

Despite widespread implementation of RACD in low transmission settings, there were no standardized metrics or tools available to monitor and evaluate (M&E) malaria case investigation and RACD activities until recently. As with any intervention it is important to monitor and evaluate RACD-related activities to focus resources where necessary. To fill this gap a RACD M&E tool was developed and piloted in three settings in the Asia Pacific (related to study I),(289). As more malaria programs are implementing RACD, evaluations are being increasingly carried out on the reporting and timeliness of their RACD activities, as well as improving the knowledge and practices of malaria and health worker staff conducting RACD.(287,290–295)

RACD is typically initiated in a pre-elimination setting (medium to low transmission areas) when some follow-up is feasible with the aim to find additional infections around an index patient. However, without a critical evaluation of whether and when RACD should be incorporated (if at all) and if a timely and complete case investigation and RACD follow-up is feasible, RACD will not likely provide the surveillance intel that programs can benefit from. Given the financial and human resources required for RACD to be conducted, RACD can initially be a mechanism for additional surveillance intel if that information is used to identify a risk profile of those with the highest risk of infection and therefore be targeted with interventions. Conducting RACD prematurely in areas where the burden is too high for a timely and complete response may lead to a waste of resources and not support the aim of additional infection detection and gathering surveillance. Furthermore, as malaria transmission decreases, the use of molecular diagnostics in RACD may be necessary as there will likely be a larger proportion of low density and asymptomatic individuals missed using standard diagnostics.

1.6.2.2.2.1 Household-based RACD

RACD is typically conducted at the household-level, including the neighboring households of the passively-detected index case.(52) Procedures for household-based RACD (HH-RACD) include the screening of all eligible household members residing or visiting the index case household as well as the neighboring households within a particular geographic radius (determined by local policies). HH-RACD is the standard form of RACD in low transmission settings. A diagnostic test is conducted and basic demographic information is collected on

standardized reporting forms then input into a malaria surveillance system database, whether online or electronically.(260)

HH-RACD is conducted around an index case residence due to spatial and temporal aspects of malaria infections and clustering.(271,178) Research in *P. falciparum*-dominant areas have shown evidence of such infection clustering; (180–182,186) however, for *P. vivax* dominant areas some evidence has shown household-level infection clustering,(184) but in other studies that included genotyping, evidence of relatedness in *P. vivax* settings was not clear.(185,186) The lack of evidence of parasite relatedness may be because the RACD-identified infections are unrelated to the index case infection or either a new or relapse from a previous infection.(187) The unrelated infection could be an individual who shares infection risk with the index patient (e.g., forest goers), but did not contract malaria at the same time or place. To better understand whether RACD-identified infections are related, genotyping of the infections detections could provide additional epidemiologic surveillance.(296)

One challenge of HH-RACD in low transmission settings is that the risk of infection is often not related to where an individual lives, but what that individual does, linked to occupation or other behavioral factors. Contacting individuals at high-risk for malaria is difficult because these individuals may be working or traveling to/from occupational or forested areas as RACD is typically conducted during daytime working hours when government health staff are available. Further, the individuals being screened during RACD are those at the residence at the time of the visit, typically women and children, and not adult men who are at work, the latter of which may be the population at risk of infection.(297) Additionally, RACD is expensive due to travel and human resource costs, so applying HH-RACD to a low-risk population may not be cost-effective, and therefore improved targeting of RACD may be a better approach in terms of infection yield and costs per infection identified. In elimination settings the cost per infection identified will increase – fewer infections are available to be found as the total number of individuals being screened increases. However, if the goal is elimination, adequate resources need to be available to support it.

In addition to the challenges of RACD being costly, and having limitations using standard diagnostics,(286) some evidence has shown that in low transmission settings the use of molecular diagnostics (such as PCR and LAMP) in RACD may have the added benefit of identifying subpatent and asymptomatic infections. Although these molecular methods can be more costly,(166,298) infections below the threshold of standard diagnostic detection may be responsible for up to 50% of malaria transmission.(109,110) Therefore, the added cost by using molecular methods and the increased yield of subpatent infections detected (and treated) could potentially make RACD using molecular methods a more cost-effective approach in the final stages of elimination.(166)

The value of conducting HH-RACD depends on the local epidemiological situation, particularly where exposure to malaria occurs away from the residence or community (e.g., in forested areas).(299) Research in the GMS suggests that

given the low yield of infections, high demand for resources, and limited evidence for impact on transmission, HH-RACD may not be worth the cost.(300) However, in Latin America, also a dominant *P. vivax* setting, HH-RACD was found to be more effective for identifying infections where malaria transmission is variable and unstable.(185) In medium transmission settings, HH-RACD can yield anywhere from 2-32% RACD-identified positives by RDT or microscopy among those screened. In low and very low transmission settings, HH-RACD can yield anywhere from 0-13% and 0-3.4% RACD-identified positives by RDT or microscopy among those screened, respectively. (Personal communications with Michelle S. Hsiang)

1.6.2.2.2.2 Sociobehavorial RACD

In addition to the spatial and temporal dynamics of malaria transmission, targeting other risk factors, including behaviors, has been shown to yield more RACD-identified infections compared to HH-RACD alone.(301) Research in both *P. falciparum* and *P. vivax*/mixed species settings has shown the importance of other risk factors for malaria to consider, particularly in forested settings.(281,282,297,302–304) As malaria transmission declines and becomes increasingly clustered in specific populations due to various social, occupational, and behavioral risk factors, targeting interventions to effectively identify and track these populations is important.(283,305) Furthermore, understanding the complexity of mobile and migrant populations (MMPs) and different social and occupational groups in any endemic setting is important to enable more effective targeting of interventions.(234,280,281,306,307)

Conducting RACD focused on targeting high-risk groups and including social and occupational groups, including in areas like the GMS with forest-related malaria transmission and forest goers, is referred to as sociobehavorial RACD (SB-RACD). A SB-RACD strategy originates from other diseases such as HIV and tuberculosis that rely on contact tracing-related infection strategies.(305) Although the disease is different, there are overlapping factors in their dynamics which include: focalized in high-risk populations, barriers to proper testing and treatment, long infectious and asymptomatic periods, and may be missed by traditional surveillance measures. Among the SB-RACD strategy there can be a peer-referral focus (PR-RACD), such as co-travellers or co-workers, and/or a venue-based focus (VB-RACD), whereby RACD, and its associated activities including vector control, is conducted at a known venue in the forest, forest-fringe, or high-risk location.

A SB-RACD strategy helps to overcome the challenges faced with conducting standard HH-RACD. A SB-RACD strategy is important for several reasons: 1) HH-RACD will likely miss these MMPs and forest goers because they are frequently away from their households; 2) individuals with the greatest exposure to potentially-infective mosquitoes may likely develop partial immunity resulting in asymptomatic or sub-clinical infections and may be less likely to seek

care;(308,309) 3) high-risk individuals may be conducting illicit forest work or are undergoing undocumented travel;(234) and 4) most passive malaria surveillance systems capture only limited data necessary to identify behavioral risk factors and risk groups.(280) China developed a novel MMP-targeting strategy along the China-Myanmar border area and evaluated a grid-based surveillance strategy to increase the effectiveness of community-based malaria case management and identify and target high-risk populations for malaria screening.(310) The grid-based strategy is a grassroots governance approach that shifts administrative resources at the community level to areas with the most need and provides necessary training to community members on controlling diseases of importance. This strategy was developed during the 2003 SARS crisis and is maintained by the Chinese government. The grid-based strategy, adapted for low malaria transmission settings, is a useful community-based and surveillance strategy to increase the local stakeholder ownership in delivering malaria elimination activities and maximize resources from all sectors involved in malaria delivery strategies.

As with HH-RACD, there are inherent challenges to conducting SB-RACD. Firstly, coverage of the intended target group is a challenge, particularly if illicit forest work or undocumented travel exists and the individuals do not want their identities, or their work location discovered. Second, and similar to HH-RACD, human resources and travel costs make up a large share of the total RACD cost,(166) but likely even more so with SB-RACD due to longer distances and more difficult travel conditions. Third, standard diagnostics, and their limitations in diagnostic sensitivity to identify infections in low density and asymptomatic individuals, is another challenge. Fourth, it may be difficult to return to the forest location and provide treatment if an infection is identified through SB-RACD because microscopy requires a basic laboratory, and the individual may have moved on from that work location or communication with that individual is hampered by poor mobile connectivity due to the remote location.

1.6.2.2.3 Treatment-based strategies and prophylaxis

1.6.2.2.3.1 Mass drug administration

Mass drug administration (MDA) for populations in malaria-endemic areas is another malaria control and elimination strategy that has been implemented since the early 1900s.(311) MDA is the administration of a full therapeutic course of antimalarial medicine to a defined population residing in a defined geographical area, irrespective of the presence of malaria symptoms or infection (excluding individuals whom the medicine could be harmful).(312) Several other tropical diseases regularly use MDA as one of their main control and elimination strategies, including lymphatic filariasis,(313) onchocerciasis,(314) and schistosomiasis.(315) The purpose of implementing MDA for malaria is to reduce or interrupt transmission, reduce morbidity and mortality, or prevent reoccurrence and resulting malaria transmission.(312) MDA is designed to clear the

human parasite reservoir by administering a curative antimalarial dose, providing a treatment effect to clear any malaria parasitemia as well as a temporary prophylactic effect to prevent new blood stage infections.

The impact on parasite prevalence when MDA is implemented is significant and shows large decreases in malaria initially before reverting back to pre-MDA levels within 1-3 months (depending on incidence levels and seasonality) once MDA is stopped.(312,316) There has been one exception to this observation on Aneityum Island, Vanuatu, when eight rounds of MDA with chloroquine, sulfadoxine-pyrimethamine, and primaquine, in conjunction with ITNs, interrupted malaria transmission.(317) In China, large-scale MDA campaigns have been implemented since the GMEP era for the control of *P. falciparum* and *P. vivax* malaria treating tens of millions of people.(318) More recently, China implemented what is referred to as 'Spring Treatment' in high-risk individuals to eliminate any potential *P. vivax* liver stage parasites just prior to the transmission season.(319,320) In the GMS, where multidrug resistance exists, 3-monthly rounds of MDA reduced malaria incidence and prevalence over a 1-year period, but *P. falciparum* infections quickly returned once the intervention ceased, largely because malaria infections were reintroduced from surrounding areas.(321)

The implementation of MDA is complex and requires significant investment of resources and careful planning. Conducting MDA on a large scale is operationally difficult, largely because high levels of population coverage are required.(312) Due to antimalarial contraindications a proportion of the population will be excluded. And like RACD, individuals may be absent during the MDA campaign, and there will be refusals to participate. The use of MDA is currently recommended by the WHO for *P. falciparum* elimination in areas of multidrug resistance in the GMS, in areas approaching elimination that still have *P. falciparum* transmission, and during epidemics and complex emergencies.(316) However, for MDA to be have an impact on transmission it must be repeated regularly for sustained effect and be combined with other vector control interventions such as IRS.(320,322,323) Additionally, the efficacy of the drug regimen is important for MDA to be successful. Furthermore, community acceptability of MDA is critical and supports achieving higher intervention coverage of the population.(324–326)

Another important challenge with MDA is the administration of antimalarial medications to individuals without having symptomatic malaria.(326) Rigorous pharmacovigilance should be implemented during a MDA campaign to monitor the safety of those participating. This is particularly true in *P. vivax* endemic settings where G6PD-deficient populations are prevalent (327) and longer 8-aminoquinoline drug regimens are used for radical cure of *P. vivax*.(216,326,328) Concerns around treating G6PD-deficient individuals can be mitigated with point-of-care G6PD diagnostics that have a reported accuracy of around 90%,(212) although barriers to routine rollout of G6PD testing exist.(213) Another challenge to sustaining post-MDA gains once reducing

malaria is human and mosquito migration into areas that have successfully interrupted transmission, as was experienced in Costa Rica after their successful MDA campaign.(329) This is certainly a challenge for any post-elimination areas that are trying to prevent the re-establishment of malaria. Having the necessary prevention of re-establishment approaches in place, including entomological surveillance and a sensitive malaria surveillance system that is quick to respond to any infections identified (imported or local) is critical to sustain the MDA gains achieved.

1.6.2.2.3.2 Reactive drug administration

To overcome the challenges described previously for RACD, including importantly the detection limits of standard diagnostics in asymptomatic and subclinical infections, a reactive drug administration (RDA) strategy is a novel approach that has been shown to reduce malaria transmission in a *P. falciparum* dominant setting,(323) and was recently evaluated in a *P. vivax* dominant setting.(330) Much like RACD, RDA targets the spatial and temporal aspects of malaria by providing presumptive malaria treatment to household and nearby community members of a passively-identified index patient. The eligible target population for RDA is offered an ACT (plus an 8-aminoquinoline) based on the location and following guidance by the national malaria medicine committee. In *P. vivax* settings, the target population should also be tested for G6PD function by taking a small blood sample and using a quantitative handheld testing device.(211) If G6PD-normal (males ≥ 4 IU/g Hb, females ≥ 6 IU/g/ Hb), health or malaria surveillance staff should provide a 14-day course of PQ per national drug policy. For patient safety purposes, individuals testing G6PD-intermediate or G6PD-deficient should only be provided the recommended ACT, per national drug policy.

Concerns over haemolysis and, until recently, the lack of availability of point-of-care G6PD diagnostic tests have contributed to low rates of PQ uptake for the cure of *P. vivax* malaria in endemic settings.(209) G6PD testing is critical when implementing an RDA strategy to increase the safety of those individuals targeted. A pharmacovigilance tool initially developed in a *P. falciparum* setting for the rollout of single low dose PQ was found to be a feasible strategy to promote safe medicine administration using 8-aminoquinolines.(331) In the Asia Pacific region, there is evidence that single low dose PQ is tolerable among patients infected with *P. falciparum*.(332) Patient safety is important,(333) particularly in *P. vivax* settings, where the malaria medicine regimen is prolonged due to the use of 14-day PQ.(216)

As with a MDA campaign, the acceptability of a RDA strategy among the community is critically important to support population coverage, and therefore potentially greater intervention impact.(227,326,334,335) In areas of declining malaria transmission with low-density (127,336) and asymptomatic infections,(180,185) a low perceived threat of malaria may outweigh the risk of

taking medicines presumptively.(337) Evidence has shown that understanding the target populations and providing appropriate and tailored information may support the uptake of the intervention.(226)

RDA may have some additional logistical benefits to its implementation compared to RACD, including the need for reduced human resources and travel costs associated with follow up if only a single visit is required by malaria surveillance staff. This assumes that the necessary features for drug adherence and monitoring of adverse events is in place and functional, likely by community or village-based volunteers for malaria treatment follow-up and management.(220) Rigorous pharmacovigilance should be implemented during RDA to monitor the safety of those participating.

Like HH-RACD, RDA implemented at the household level introduces bias in who is being provided the intervention relative to who is most at risk of infection. Individuals typically available when RDA is being implemented at home during the day, women and children, may not be the population at highest risk of infection. Instead, adult men who are at work or elsewhere may be a better target for RDA. RDA can be targeted to high-risk populations and known groups of individuals with exposure to malaria (i.e., forest goers) when an index patient with similar risk factors is identified, similar to the targeting of SB-RACD. Implementing RDA among high-risk populations, particularly forest-goers, may help eliminate asymptomatic and sub-clinical reservoirs of infection, and provide antimalarial prophylactic effects when staying in the forest for longer periods. RDA should be appropriate for the epidemiology of the local malaria transmission context to target the groups that are at highest risk for malaria transmission. Should RDA be implemented, it must be carefully thought through to ensure maximum resources are available for its implementation to be done well with the highest coverage possible among the population targeted. Strengthening the capacity of local village and health workers would be needed, including ensuring acceptability of the RDA intervention among high-risk populations.(338)

As with any mass malaria drug campaign there is potential risk for increased selective pressure on the *Plasmodium* parasite and therefore increased risk of parasite resistance.(224,324) Given that RDA would be implemented within a select group of individuals at higher risk of having an infection and geographically or behaviourally linked with the index patient, development of parasite resistance is possible. Therefore, it is important that if RDA is conducted among higher risk groups of malaria infection, it must be done well to reduce the chance for drug resistance development by ensuring complete cure of the infection. Additionally, complete drug adherence to the antimalarial drug regimen could be supported by DOT,(339) mHealth platforms to improve adherence and support health workers, and/or quality improvement strategies embedded within malaria program activities. (340,341)

2 Rationale and aims of the thesis

2.1 Rationale

Malaria case investigation and reactive case detection (RACD) activities are widely implemented in low transmission settings with varying degrees of success. This is due to the challenges faced in conducting a quality response to identify additional malaria infections around a passively-identified index patient to gather additional surveillance information. Challenges include poor diagnostic sensitivity (particularly for low density and asymptomatic infections), knowledge gaps among health workers conducting RACD, financial and resource constraints, and operational and logistical difficulties, among others. To improve infection detection and better target individuals at highest risk for infection, RACD strategies need to be evaluated and optimized to provide quality and nuanced surveillance information. To support more effective surveillance and response strategies, this PhD project focused on evaluating RACD strategies to improve and optimize malaria surveillance in low transmission settings in the Asia Pacific region. We generated new data evaluating malaria case investigation and RACD indicators and RACD-related activities using a standardized monitoring and evaluation (M&E) tool, including assessing the knowledge and practices of the staff conducting RACD. This PhD project explored the utility of molecular diagnostics and genotyping and targeted sociobehavorial RACD strategies for increasing infection detection and to understand the relatedness of infections identified during RACD. Also, the acceptability and feasibility of a presumptive treatment-based strategy to reduce malaria (referred to as reactive drug administration (RDA)) was evaluated. The research conducted during this PhD project provides important tools and evidence for low malaria transmission settings to consider and explore as they scale-up and implement more targeted malaria surveillance and response strategies to accelerate elimination efforts.

2.2 Aims

- To evaluate malaria case investigation and RACD-related activities in three settings using a novel standardized monitoring and evaluation program tool to determine its feasibility and reliability to identify key gaps in reporting of malaria indicators, and knowledge and practices among health and malaria staff. (study I)

- To determine the utility of using molecular testing and genotyping in RACD in Thailand: 1) to assess the yield of using LAMP versus microscopy to detect *Plasmodium* infections; 2) and to assess the use of microsatellite genotyping to determine the relatedness of index and additional infections detected by RACD. (study II)

- To evaluate sociobehavorial RACD strategies (venue-based and peer-referral) that target high-risk populations in Aceh Province, Indonesia: 1) to determine whether a sociobehavorial RACD (SB-RACD) strategy can effectively identify individuals who have recently had contact with an index case and identify elevated risk of malaria infection compared to household-based RACD (HH-RACD); and 2) to compare parasite prevalence of RACD-identified infections through SB-RACD to HH-RACD. (study III)

- To evaluate the acceptability and feasibility of RDA among key public health staff, village health volunteers, and community members in Thailand, and to identify the necessary improvements in RDA activities and operational considerations required for future scale-up. (study IV)

3 Materials and methods

3.1 Study sites and population

This thesis includes manuscripts that are a result of two prospective surveillance studies, and one feasibility and reliability and one acceptability and feasibility evaluations. A feasibility and reliability evaluation was conducted across three study provinces in China, Indonesia, and Thailand (one province in each country) between 2013 and 2015 and provided data for study I. Two prospective surveillance studies were conducted and provided data for study II in Thailand in 2016 (two provinces included) and for study III in Indonesia between 2017-2018 (two districts included). A qualitative acceptability and feasibility evaluation was conducted in Thailand (two provinces included) between 2021-2022 and provided data for study IV.

3.1.1 Study sites

3.1.1.1 Study sites in Thailand (study I, II, IV)

Thailand is a low malaria transmission country in the GMS bordering Cambodia and Laos to its east, Myanmar to its west, and Malaysia to its south. The total population in Thailand is estimated to be 71 million with just over 13 million population (18%) at risk for malaria, predominantly along its borders.(30) Thailand has 77 provinces in total, 35 (45%) of which have ongoing local malaria transmission. Malaria transmission is low overall and occurs seasonally with peaks from April to June and September to November with some variation of those periods between provinces. *P. vivax* is the dominant species responsible for 90% of local malaria cases in 2016. The dominant vector species are *An. dirus s.l.* and *An. minimus s.l.*.(93)

Malaria incidence in Thailand reduced from 3.57 per 1000 population in 2012 to 0.58 per 1000 population in 2016, although some areas along its borders remain persistently high.(30,293) This steep overall reduction in malaria was due to a number of factors starting with The Artemisinin Resistance Containment Project from 2008 – 2012, a precursor to real-time electronic capture of malaria-related data at the national level.(342) With substantial national government support, as well as from donors such as the GFATM and US PMI, Thailand launched their *National Malaria Elimination Strategy (NMES)* in 2017 and adopted China's '1-3-7' strategy for malaria case reporting, investigation, and

response to focus on the rapid identification of infections and using timely and active surveillance and response to prevent infections from spreading.(343)

Study site selection in Thailand was based on whether the malaria program was currently conducting case investigation and RACD activities, the study timing and duration of the study activity, availability of program staff and data to conduct the studies, and recent malaria caseload to ensure an adequate number of malaria cases. Participants that provided secondary data for the feasibility evaluation (study I) were district- and provincial-level (Ranong Province) malaria program staff involved in surveillance and response activities. Study site selection for the prospective surveillance study (study II) was based on recent malaria case data in the provinces and districts with the highest malaria incidence (Chanthaburi Province: Pong Nam Ron, Kang Hang Maew and Soi Dao districts; Kanchanaburi Province: Zaiyok district). The annual parasite incidence (API) of malaria in 2015 just prior to study start was 0.21 and 1.38 per 1000 population for Chanthaburi and Kanchanaburi Provinces, respectively. Participants for study II included confirmed malaria index cases reported from four district-level malaria clinics (vector borne disease units or VBDUs) and their household members, and neighbors residing within a one-kilometer radius of the index case (up to a maximum of 75 individuals or minimum of the five nearest households). Figure 6 identifies the study provinces and districts included in their respective studies. (I, II, IV)

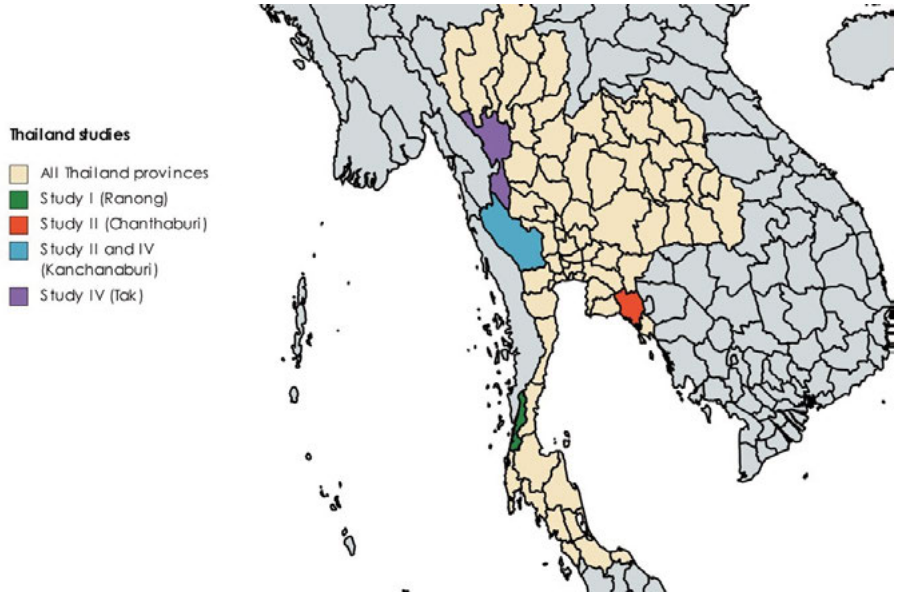


Figure 6 Thailand

Study sites for the qualitative study (study IV) were selected from two of the four study provinces (Kanchanaburi and Tak) that had the highest number of

response events in the parent RCT (registered at clinicaltrials.gov: ID NCT05052502). Sub-districts with at least three malaria cases between October 2018 and September 2019 were eligible for inclusion in the parent RCT and stratified based upon API (high/low), total population (high/low), and geography (east/west). Study participants for the qualitative interviews were individuals in the parent trial that were key malaria and health staff or community members and high-risk populations that received the RDA intervention during the RCT.

3.1.1.2 Aceh Province, Indonesia (study I, III)

Indonesia is a highly diverse archipelago in Southeast Asia and Oceania consisting of over 5,000 inhabited islands.(72) The total population in Indonesia is estimated to be 273 million with nearly 17 million (6%) of the population at high risk for malaria, predominately in eastern Indonesia bordering Papua New Guinea and in Borneo to the north.(30) In 2010, 450 districts reported suspected malaria, and was reduced to 266 districts by 2018.(72) Malaria transmission is medium to low overall. Malaria incidence in Indonesia reduced from 2.89 per 1000 population in 2007 to 0.9 per 1000 population in 2017, a 3-fold reduction. All five *Plasmodium* species exist in Indonesia, and particularly in Aceh Province located on Sumatra Island in the far most western part of the country.(344–346) Indonesia has up to 20 dominant and secondary *Anopheles* vector species, including *An. balabacensis*, *An. latens*, and *An. leucosphyrus*, among others.(347)

Reductions in malaria have been due to strong subnational action supported by evidence-based policy and advocacy. This spurred the national government to pass a *National Ministerial Decree on Malaria Elimination* in 2009 and provided decentralized authority to the provinces and districts to have locally tailored approaches to malaria control and elimination.(72) Similar to China, Indonesia adopted a surveillance and response strategy of '1-2-5' for malaria case reporting, investigation, and response.

Study sites in Aceh Province were chosen based on the malaria program currently conducting case investigation and RACD activities and recent malaria caseload to ensure an adequate number of malaria cases. The study district of Aceh Besar was chosen for the feasibility evaluation (study I) and included five sub-districts: Aceh Besar, Aceh Timur, Banda Aceh, Bireun, and Sabang. Participants selected for study I were provincial-, district-, and sub-district-level health staff involved in malaria surveillance and response activities. Figure 7 identifies the study provinces and districts included in their respective studies (I, III).

Due to declining levels of malaria in Aceh Besar sub-districts, study III areas were expanded to include the highest burden sub-districts, including the neighboring district of Aceh Jaya bordering the south. Sub-districts for study III included: three sub-districts in Aceh Besar district (Kuta Cot Gli, Lembah Seulawah (Saree), and Lhoong) and two sub-districts in Aceh Jaya district (Krueng

Sabe, Sampoiniet). The API of malaria in 2016 just prior to the study starting ranged from 0.26 in Lembah Seulawah sub-district to 4.1 in Krueng Save sub-district.

Figure 7 Indonesia

Participants for study III were confirmed malaria index cases reported from five sub-district-level health facilities (puskesmas) with a recent history of forest work (previous 60 days), household members and neighbors residing within a one-kilometer radius of the index case (up to a maximum of 25 individuals or minimum of the five nearest households), and referrals of those index cases who worked together with the index case in or near the forest.

3.1.1.3 Jiangsu Province, China (study I)

The Republic of China, a country with 1.4 billion population, was previously a high malaria transmission country averaging 60,000 malaria cases per year in 2006.(318) Malaria case incidence reduced from 0.46 per 10,000 population to 0.2 per 10,000 between 2006 and 2008. An important component of this incredible decrease in malaria cases was a focus on improving surveillance and response for malaria.(61) Starting in 2010, China launched its *National Action Plan for Malaria Elimination* and set a goal for countrywide elimination by 2020.(63) Between 2010 – 2016, while malaria incidence overall greatly reduced, the proportion of *P. falciparum* malaria increased relative to the total, particularly imported malaria cases from exported labour groups returning from overseas.(348) By 2017, no indigenous cases were reported, although imported malaria remains a concern.(349) Despite no local transmission occurring, China does have competent vector species for malaria including, *An. lesteri* and *An. sinensis*.(93)

Figure 8 China

Study sites in China were chosen based on the malaria program having recently conducted case investigation and RACD activities. Jiangsu Province, just west of Shanghai, was chosen for the feasibility study (study I). The participants selected that provided secondary data were Jiangsu Provincial-level and county CDC staff who conducted malaria surveillance and response activities. At the time of the feasibility evaluation, indigenous malaria case counts were zero as well as malaria incidence; however, imported infections did occur. This provided an opportunity to conduct the evaluation in an area that was in the malaria prevention of re-establishment phase in a setting with only imported malaria. Figure 8 identifies the study province included in study I.

3.2 General methodologies

Description of the general and specific study methodologies are detailed in the methods section of the respective study papers. Below, some brief methodologies are included.

3.2.1 Feasibility and reliability evaluation

Study I assessed key malaria case investigation and RACD indicators, and knowledge and activities using a standardized M&E program tool. Between 2013 and 2015, the M&E tool was implemented by provincial- and district-level health and malaria staff in two low transmission settings (Aceh Province, Indonesia and Ranong Province, Thailand) and one prevention of re-establishment setting (Jiangsu Province, China) using the most recent 12 months of program data (Indonesia evaluated 3 months of program data). Table 1 provides a summary of pilot study areas.

Study I included secondary data collected through health facility patient registers and malaria information systems on malaria case reporting, case investigation and RACD activities. Specific outcomes include: availability of key malaria documents such as notification forms, SOPs, or documentation to support implementation activities, malaria case reporting completeness and timelines, case investigation and RACD completeness and timeliness, and RACD positivity. Standardized questionnaires were used to assess the knowledge and practices of health facility and malaria program staff on case reporting, case investigation and RACD activities. Summary statistics and proportions were analyzed to determine results.

Table 1 Summary of study I areas

Location	Data collection period	Tool implementation period	Program phase	Pilot scale	No. of facilities reporting	No. of staff that provided responses
Aceh, Indonesia	June-September 2013	June-September 2013	Elimination	5 Districts[1]	34	34
Jiangsu, China	January-December 2012	June-August 2013	Prevention of reintroduction	3 Counties[2]	6	10
Ranong, Thailand	January-December 2014	January-March 2015	Elimination	5 Districts[3]	10	15

[1]Aceh Districts: Aceh Besar, Aceh Timur, Banda Aceh, Bireun, Sabang

[2]Jiangsu Counties: Baoying, Gulou (Nanjing City), Haimen

[3]Ranong Districts: Kapoe, Kraburi, Laun, Meaung, Suksamran

3.2.2 Prospective surveillance studies

Studies II and III were prospective surveillance studies performed through the passive and RACD malaria program activities in Thailand (2016) and Indonesia (2017-2018). Individuals presenting to malaria clinics, malaria posts, or border malaria posts (study II) or health facilities (study III) with suspected malaria were asked to provide a fingerprick blood sample, and thick and thin smears for microscopy were prepared. Microscopy-confirmed malaria index cases were invited to participate in the studies and per national treatment guidelines appropriate treatment was provided. Per local surveillance guidelines, a team of health or malaria staff conducted RACD within 7 days of index case diagnosis. Household members of the index case and neighboring households were targeted for RACD (study II and III).

In study III, in addition to household-based RACD screening, index cases with a recent history of forest work (previous 60 days) and referrals of those cases who worked together with the index case in or near the forest were also invited to participate in the study population. Enrolment of a malaria index case in sociobehavorial-RACD (SB-RACD) triggered one of two reactive recruitment responses to enroll individuals who work, or who have recently worked, at the index case's recent work sites. A peer referral-based RACD (PR-RACD) response targeted the specific co-workers or co-travellers who were with the index case at forest or forest fringe work sites and spent the night there in the previous 60 days. A venue-based RACD (VB-RACD) response targeted individuals directly from the work sites identified by the index case (the work venues), as accessible, so long as they spent at least one night there in the previous 60 days.

In consenting individuals, blood was collected by finger prick and used to prepare thick and thin smears for microscopy as well as dried blood spots (DBS) on filter paper (3MM; Whatman). For all malaria cases identified, whether index case or during RACD, up to an additional 250 µL of blood by fingerprick was captured in a microtainer (study II) or up to 3mL (based on participant age) of venous blood in a heparinized tub (or EDTA) (study III) and stored for genotyping. Household- and individual-level questionnaires were developed in English, translated into local language (Thai or Bahasa), and administered by health facility malaria surveillance staff using Google Nexus tablet computers for the index case as well as household, community members, and forest-goers.

3.2.3 Qualitative study

Study IV was a qualitative study performed as part of a prospective two-arm cluster randomized controlled trial (RCT) to evaluate the effectiveness of reactive drug administration (RDA), targeting high-risk villages and forest workers, compared to standard RACD for reducing sub-district incidence and

prevalence of *P. falciparum* and *P. vivax* malaria in Thailand.(330) The RCT was carried out between November 2020 and November 2021 in four of the remaining provinces with active foci in Thailand (Kanchanaburi, Mae Hong Son, Tak, Ubon Ratchathani).

Target participants for study IV were individuals in the two provinces with the highest number of RDA events conducted during the RCT period (Kanchanaburi and Tak). Study participants were key malaria and health staff or individuals who received the intervention during the parent RCT and were invited to participate in focus group discussions (FGDs) and key informant interviews (KIIs). FGDs were conducted among Health Promotion Hospital (HPH) staff and Village Health Volunteers (VHVs) with a target of six individuals per FGD.

For study IV, FGDs and KIIs were conducted from December 2021 – February 2022 in the intervention sub-districts. Interview guides were developed in English and translated to Thai, and were tailored to each respondent group, which explored: 1) their roles and responsibilities in RDA; 2) perceptions and knowledge of G6PD testing and referral; 3) drug adherence and tolerability; 4) acceptability of and attitudes toward RDA; and 5) feasibility and barriers to implementing RDA.

3.3 Study specific methodologies

3.3.1 Blood sampling and storage

During RACD for studies II and III, microscopists collected blood smears and transported them in closed slide boxes for further examination. Slides were fixed and stained with 3% Giemsa and examined in triplicate at three levels: sub-district or district, provincial, and national. Thick smears were examined to determine the presence of *Plasmodium* parasites in 100 high-powered fields and if no parasites were seen then that smear would be considered negative. If *Plasmodium* parasites were identified in a thick smear, an additional 100 high-powered fields on the thin smears were examined to determine species. Quality assurance for microscopy was performed by an expert level-certified microscopist at the provincial level (study III) and the national level (study II) for all positive cases and 10% of randomly selected negatives, per national microscopy guidelines.

In study II, all DBS samples collected were dried overnight at the malaria clinic then stored in sealed plastic bags with desiccant before being transported to the national laboratory in Nonthaburi, Thailand for DNA extraction and LAMP and PCR analysis. DBS were stored at 4°C within one week of collection and microtainers 4°C starting the same day of collection. All DBS and microtainers were then stored at -20°C within one month of collection.(147)

In study III, all DBS samples collected were dried overnight at the sub-district health facilities then stored in sealed plastic bags with desiccant before being transported to the Provincial Hospital in Banda Aceh for DNA extraction and LAMP analysis. DBS were stored at 4°C within one week of collection and then stored at -20°C within one month of collection. Venous blood samples were stored at 4°C for up to one week after collection, then were transported to Eijkman Institute in Jakarta for DNA extraction and then stored at -20°C.

3.3.2 DNA extraction

In study II, DNA extraction from the DBS was performed using the Saponin/Chelex method.(350) Using 15 µL of Chelex-extracted DNA, Pan-LAMP testing followed by *P. falciparum*-LAMP-specific testing for Pan-LAMP positive samples was performed using a commercial Loopamp detection kit in accordance with the manufacturer's instructions (Eiken Chemical, Co., Ltd., Japan). Low parasitemia controls (down to 1 p/µL) were used and Pan-LAMP was duplicate tested.(351,352) Using 5 µL of Chelex-extracted DNA, all Pan-LAMP positive samples and 10% of randomly selected LAMP negative individuals from RACD were tested by the Mahidol University laboratory using real-time PCR targeting the 18S rRNA gene.(150) For PCR, low parasitemia positive controls were also used (down to 0.4 p/µL).

In study III, DNA extraction from the DBS was performed using the Saponin/Chelex method. Using 15 µL of Chelex-extracted DNA, Pan-LAMP testing followed by *P. falciparum*-LAMP-specific testing for Pan-LAMP positive samples was performed using a commercial Loopamp detection kit in accordance with the manufacturer's instructions (Eiken Chemical, Co., Ltd., Japan). Low parasitemia controls (down to 1 p/µL) were used and Pan-LAMP was duplicate tested. Using 5 µL of Chelex-extracted DNA, all Pan-LAMP positive samples and 10% of randomly selected LAMP negative samples were tested by the Eijkman Institute malaria laboratory using nested PCR targeting the 18S rRNA gene with species-specific identification.(151)

3.3.3 Genotyping to determine interrelatedness

Microsatellite (short tandem repeat or STR) analysis was performed in study II to compare allele repeats at specific loci in the DNA between samples. Genotyping was performed using fluorescently labelled PCR primers for a set of nine selected standardized microsatellite loci for *P. vivax* (1.501, 3.27, 3.502, MS1, MS5, MS6, MS7, MS8, MS16) using published protocols.(353,354) *P. vivax* microsatellite genotyping markers were included in a cluster analysis using Network 10 software (http://www.fluxus-engineering.com/sharenet.htm), which is based on median joining algorithms.

3.3.4 Qualitative analysis

In study IV, FGDs and KIIs were led by experienced facilitators supported by trained notetakers. Audio recordings of the FGDs and KIIs were transcribed into Thai, then translated into English. The transcriptions were reviewed, coded by two separate researchers, and gathered into key themes using an inductive approach. A codebook was developed with both a priori codes from the interview guides and themes that emerged from reviews of the interview summaries and notes. Dedoose qualitative data analysis software (version 9.0.62) was used to perform content analysis to obtain study results.

4 Ethical considerations

Study I was approved by the malaria programs of the three pilot study areas. The Committee on Human Research at the University of California San Francisco (UCSF), USA determined this study to be a program improvement activity (14-13399) because only secondary data analysis was being used.

Study II was reviewed and approved by the Committee on Human Research at UCSF (13-10988), the Institutional Review Board at the University of Texas Southwestern Medical Center (STU 052016-041), and the Ethics Committee for Research in Human Subjects at the Department of Disease Control in Thailand (41/2558). Written informed consent was obtained from all subjects. For individuals under the age of 18, written informed consent and risk factor questionnaires were administered to a parent or guardian.

Study III was reviewed and approved by the Committee on Health Research Ethics of the National Institute of Health Research and Development in Indonesia (LB.02.01/2/KE.083/2017), and the UCSF Committee on Human Research (16-20220). Written informed consent was obtained from all study participants. For individuals under the age of 18, written informed consent and risk factor questionnaire were administered to a parent or guardian. For participants between the ages of 12-17, written informed assent was also obtained.

Study IV was reviewed and approved by the Ethics Committees for Research in Human Subjects at the Office of the Permanent Secretary Ministry of Public Health in Kanchanaburi Province (Kor Chor 0032.002/2185), Maehongson Province (MHS REC 030.2563), Tak Province (009/63), and Ubon Ratchathani Province (SSJ.UB 090), and the UCSF Committee of Human Research (19-28060). Written informed consent was obtained from all participants. The parent trial for study IV was registered at clinicaltrials.gov (NCT05052502).

5 Results and discussion

5.1 Study I: Evaluating malaria case investigation and RACD activities

This study was an evaluation of malaria case investigation and RACD activities, which are widely implemented in low transmission settings, yet no standardized metrics or tools exist to monitor and evaluate these activities. Through the use of a standardized M&E program tool, this study: 1) assessed whether key documents and personnel involved in the RACD process were available and in use; 2) assessed a minimum set of indicators on index case notification/reporting, case investigation, and RACD timeliness and completeness at health facility and district levels to identify gaps in reporting; and 3) evaluated the knowledge and practices of health facility and malaria program staff on case reporting, case investigation, and RACD activities.

This study obtained secondary data from 50 health facilities in the study areas. Program data were entered manually into Microsoft Excel to determine summary statistics and proportions. 71.8% (97/135) of the proper notification and reporting forms and 20% (27/135) of the standard operating procedures (SOPs) for case investigation and RACD were available. Gaps in reporting key malaria data on the completeness for case reporting/notification (98.8%; min 93.3-max 100%), case investigations (65.6%; min 61.8-max 78.4%), and RACD follow-up that was required (70%; min 64.7-max 100%) were reported. In addition to reporting gaps identified, the timeliness of malaria case reporting/notification highlighted the time bounds set for many study areas were unable to be met. All study areas have a policy for RACD follow-up of 7 days from index case notification, and 84.7% of required RACD events were followed up within that time frame. Of note, the study area in China (Jiangsu Province), a prevention of malaria re-establishment setting, had 100% reporting for both completeness and timeliness of the malaria indicators evaluated.

A total of 59 health and malaria staff provided responses to the standardized questionnaire on the knowledge and practices of malaria case reporting, case investigation, and RACD activities. Questions focused on '*who should be screened when conducting RACD?*' and '*what is the radius to screen around an index case household?*', as examples. Results highlighted that RACD practices varied widely, even among the health or malaria staff within the same study area. A notable difference was identified related to the minimum

geographic screening radius for RACD: no study area evaluated had a majority correct response with 40% (4/10) in China, 44% (15/34) in Indonesia, and 18% in Thailand identifying the correct policy for RACD screening radius.

Key takeaways from this study

- Prior to this study there were no standardized M&E tools or approaches to help malaria programs identify gaps in reporting completeness and timeliness. Through the use of a novel standardized RACD M&E tool during this study, programs can now evaluate the performance of, and aspects related to, malaria case reporting, case investigation and RACD follow-up. Here we've demonstrated that it is feasible and reliable to apply this M&E program tool in a variety of low transmission and prevention of re-establishment settings and can be used as a template to introduce basic malaria case reporting, investigation and RACD principles and related M&E activities. Gaps in malaria reporting completeness and timeliness identified through use of the RACD M&E tool can be appropriately addressed.

- Health and malaria staff did not always have the reporting forms and materials they need at the health facility to ensure proper and timely malaria-related reporting to the district level. This includes SOPs and/or guidelines to support their activities for reporting, case investigation and RACD follow-up. In the prevention of re-establishment study site (Jiangsu Province, China), all key documents and forms were available. This audit of key documents highlights the uneven distribution of proper forms and SOPs, particularly in areas with remaining malaria transmission. Having the reporting tools and materials available, providing regular trainings on their use, and conducting regular documentation audits may help to improve reporting practices for malaria notification, particularly in areas of underreporting.

- Gaps in malaria case reporting/notification, case investigations and RACD follow-up were identified in study areas with ongoing local malaria transmission. The key case investigation and RACD indicators evaluated in this study did show gaps in reporting completeness in Indonesia and Thailand study areas (low transmission sites) compared to China (prevention of re-establishment of malaria site), which reported 100% of all cases, investigations, and RACD. This is likely due to the China study setting (Jiangsu Province) not having ongoing local malaria transmission (imported cases only) at the time of data collection, and overall having a much smaller case burden compared to other study areas evaluated. Importantly, China has mandatory malaria case reporting

laws in place which require any malaria case to be reported (local or imported) within 24 hours of presentation to a health facility.(60) Timely reporting can also be facilitated by having an online reporting system, which both China and Thailand are using.

- A lack of timeliness for health or malaria staff to conduct case investigations and RACD follow-up was identified in study areas with ongoing local malaria transmission. All study areas have a policy for case investigation and RACD follow-up from index case notification to completion of RACD screening of individuals around the index patient. In this study, 84.7% of RACD required events were followed up within 7 days of case notification. China study areas conducted RACD follow-up within 7 days 100% of the time. This difference is again likely associated with a higher case burden in low transmission study sites in Indonesia and Thailand. Given the temporal dynamics of malaria transmission, a timely RACD response may support the detection of additional infections, some infections that may possibly be related to the primary index case or others that can be identified by chance. As the malaria case burden reduces in some settings, the timeliness of the RACD response should improve. And ensuring adequate resources are available for malaria staff to conduct follow-up may bolster a quicker response.

- Standardized questionnaires of key activities related to malaria case investigations and RACD showed that both the knowledge and practices varied widely, even among the health or malaria staff within the same study area. Standard questions on the target individuals to screen during RACD, how often to screen household members of the index case, triggers for conducting RACD, geographical radius to screen around an index case household, and the minimum number of individuals to screen during RACD, as examples, highlighted the differences between staff knowledge and practices. Regular use of a standardized questionnaire to evaluate the knowledge of health and malaria staff may be useful to identify where gaps in knowledge can be improved, as they relate to the standard surveillance guidelines. This data can be used to document progress in knowledge attainment over time and after malaria surveillance trainings.

5.2 Study II: RACD using molecular detection and genotyping in Thailand

This was a prospective surveillance study performed through the passive and RACD program in a low transmission setting with predominantly *P. vivax.*

This study aimed to: 1) assess the yield of using LAMP versus microscopy to detect *Plasmodium* infections during RACD; and 2) assess the use of microsatellite genotyping to determine the relatedness of primary and additional infections detected by RACD. The study was conducted over a 9-month period and included three districts in Chanthaburi (Pong Nam Ron and Kang Hang Maew) and Kanchanaburi Provinces (Zaiyok), the highest malaria burden districts in each respective province.

A total of 27 malaria index patients reported through the passive surveillance system in study areas triggered 27 RACD events. Twenty (74%) of the microscopy-positive index patients were due to *P. vivax*, five (19%) due to *P. falciparum*, and the remaining two (7%) mixed *P. falciparum/ P. vivax* infections (all confirmed by PCR). A total of 1,973 household members and neighbors who resided in the target screening area were interviewed for household and other malaria risk factors and provided blood samples on filter paper for malaria testing by microscopy and LAMP. Whole blood for any positively-identified individuals (index and RACD-identified) was collected in a microtainer for molecular genotyping and stored at -20°C within one month of collection until further analysis.

Key takeaways from this study

- No positive infections were detected in RACD by microscopy after testing a total of 1,973 household members and neighbors residing around the index patient, likely due to low parasite densities undetectable by standard diagnostics. However, 12 (0.6%) confirmed or probable infections were detected in RACD by molecular testing using LAMP. Of the 27 RACD events, 9 (33%) had at least one additional confirmed or probable infection suggesting: a) the possibility of local transmission; and b) RACD using molecular detection methods can increase the detection of confirmed and probable infections making it a more effective strategy to identify infections in the community compared to microscopy alone.

- Four of the 12 LAMP-identified infections in RACD were confirmed by PCR, all four of which were *P. vivax*. Among these four, microsatellite genotyping showed that one (25%) was genetically related (7/7 markers matched) to the index case (and not living in/around the same residence) suggesting that most transmissions in this study may have occurred away from the household or residential area. The remaining three RACD-identified infections were unrelated and either new or a relapse from a previous *P. vivax* infection. In low transmission and *P. vivax* dominant settings, the evidence for clustering of infections is limited. RACD using genotyping can provide important surveillance information for malaria programs by determining if infections found in a

particular area have similar or different genotypes. If parasites are similar, a focused response on the active foci could be deployed. If the parasites are different, a response may be better targeted by providing interventions among individuals with shared risk factors (e.g., behaviors).

5.3 Study III: Sociobehavorial RACD targeting high risk populations in Indonesia

This was a prospective surveillance study performed through the passive and RACD program in a low transmission, mixed-species setting with predominantly *P. vivax* and *P. knowlesi*. This study aimed to: 1) determine whether a sociobehavorial RACD (SB-RACD) strategy can effectively identify individuals who have recently had contact with an index case and identify elevated risk of malaria infection compared to household-based RACD (HH-RACD); and 2) compare parasite prevalence of RACD-identified infections through SB-RACD to HH-RACD. The study was conducted over an 18-month period from March 2017 to August 2018. The highest malaria burden sub-districts in each respective district were included in the study: three sub-districts (Kuta Cot Gli, Lembah Seulawah (Saree), and Lhoong) in Aceh Besar district and two sub-districts (Krueng Sabe, Sampoiniet) in Aceh Jaya district.

A total of 34 malaria index patients reported through the passive surveillance system in study areas triggered 34 HH-RACD events. Twenty-one (62%) and 10 (29%) of the microscopy-positive index patients were due to *P. knowlesi* and *P. vivax*, respectively, with the remaining three (9%) undetermined (all confirmed by PCR). Of the 34 index patients, 33 SB-RACD events were conducted resulting in 21 peer referral-RACD (PR-RACD) (64%) and 12 venue based-RACD (VB-RACD) (36%) events. A total of 847 household members and neighbors who resided in the target screening area were enrolled in HH-RACD and provided a blood sample on filter paper. In SB-RACD, a total of 180 individuals enrolled and provided a blood sample on filter paper (59 in PR-RACD and 121 in VB-RACD). A total of 8 (4.4%) confirmed or probable infections were identified through SB-RACD using LAMP compared to 1 (0.1%) confirmed or probable infection through HH-RACD. Laboratory testing on blood samples was conducted by microscopy, LAMP, and PCR.

Key takeaways from this study

- A SB-RACD strategy identified individuals who have recently had contact with an index case and identify elevated risk of malaria infection compared to HH-RACD. Of the 34 HH-RACD events, 33 had a recent history of forest or forest-fringe work, and therefore SB-RACD was also

conducted either at the worksite of the index case (n=12 VB-RACD events) or among the co-workers/travellers (n=21 PR-RACD events) of the index case. SB-RACD yielded an additional 180 individuals to screen during RACD, many of whom had an increased risk of malaria infection due to shared behaviors such as working in the forest and staying overnight.

- Targeting the sociobehavorial contacts of malaria index cases when conducting RACD was able to detect more RACD-identified LAMP-positive infections compared to HH-RACD alone. A total of 8 (4.4%) confirmed or probable infections were identified through SB-RACD (5 from PR-RACD and 3 from VB-RACD) using LAMP compared to 1 (0.1%) confirmed or probable infection through HH-RACD (*P*-value= <0.001). PCR-corrected LAMP positive infections identified during SB-RACD yielded a total of 3 VB-RACD (2.5%) infections compared to 1 from HH-RACD (0.1%) (*P*-value= 0.018). These data highlight that although the overall yield of infections is very low in this setting, SB-RACD yielded more PCR-corrected LAMP positive infections compared to HH-RACD (3 versus 1).

- In this setting, there are important factors that are shared by SB-RACD screened individuals and index cases, potentially highlighting important risk factors for malaria infection. These include: 1) men having a higher likelihood of being an index case and being screened during SB-RACD; 2) being between 30-45 years of age; 3) occupation of logging, mining or other outdoor labor compared to farming or other; 4) having slept in the forest or family members with forest exposure in the past 60 days; 5) and having a traditional housing type as their main residence. All these factors may be important to consider for improved targeting of high-risk populations for malaria.

5.4 Study IV: Acceptability and feasibility of RDA in Thailand

This was a sub-study from the parent RCT to evaluate the effectiveness of RDA, targeting high-risk villages and forest workers, compared to standard RACD for reducing incidence and prevalence of malaria in Thailand. This qualitative study aimed to: 1) evaluate the acceptability and feasibility of RDA among key public health staff, village health volunteers (VHV), and community members in two provinces in Thailand; and 2) to identify the necessary improvements in RDA activities and operational considerations required for future scale-up.

The parent RCT was conducted from November 2020 – November 2021, while study IV was conducted from December 2021 – February 2022. Target areas for this study were the two provinces with the highest number of RDA events conducted during the parent RCT (Tak and Kanchanaburi). A total of 13 KIIs and 8 FGDs were completed with 61 participants from the study areas (13 KII and 48 FGD). Dedoose qualitative analysis software (version 9.0.62) was used to perform the content analysis.

Key takeaways from this study

- In this study setting, RDA targeting within and around the household and forest-going co-workers/travellers was well accepted by the individuals that participated and the VHVs and HPH staff who implemented RDA. Community participation was driven by: 1) a fear of contracting malaria; 2) the individual- and community-level prophylactic protection of the malaria medicines; and 3) the increased access to health care in the community. In low transmission settings, a low perceived threat of malaria may outweigh risk of taking medicines, although refusals to enroll in this study were low (1.1%). The reasons provided by individuals who refused RDA include: 1) not wanting to have their blood drawn for G6PD testing or to take medicines, particularly without symptoms; or 2) they did not recently visit the forest and typically stay near the household residence.

- VHV and HPH staff noted the feasibility of implementing RDA. However, staff highlighted that it would be important to transfer some of the malaria-related duties from the HPH staff to the VHVs to optimize public health operations. The VHVs are closer to the communities that they serve, and therefore by transferring some of the malaria-related roles it could provide potential cost-savings by needing less transport by the HPH staff to go to the field and could support building greater capacity in disease control and elimination. Furthermore, a quicker response may be feasible as HPH staff have other public health priorities and there may be greater trust among the community to receive the intervention if done by VHVs. That would require more training be provided to the VHVs on malaria-related duties, including on-the-ground practice to support integration of malaria activities into community-based activities led by VHVs. With appropriate training for VHVs, HPH staff could transition to more of a supervisory role.

- More community and healthcare provider education and sensitization was perceived to be important, particularly on the purpose of RDA due to concerns raised about being asked to take malaria medicines without

having illness. Effectiveness of an intervention such as RDA depends largely on target population coverage, strong community engagement, and adherence to the full treatment course. In low transmission areas, where infections are largely asymptomatic and low-density, providing information in an appropriate format (such as radio, public health service announcements, local theatre, through village leaders, etc) for the target populations may support the uptake of RDA.

- Participant safety was a concern highlighted, particularly when underlying diseases (e.g., hypertension) exist. G6PD testing prior to administration of any 8-aminoquinilines was shown to be feasible, but VHVs noted fear of mis-reading the G6PD test results and were not confident in their abilities to manage this activity without further training. Providing more training and having the HPH staff provide supervision of G6PD testing and medicine dispensation may support the uptake of RDA.

6 Conclusions

- Improving the quality of the RACD activities being implemented can enhance its effectiveness by increasing the completeness and timing of follow-up and improve the knowledge and practices of those conducting the RACD activities. Malaria programs conducting case investigation and RACD, or those that are considering implementing or scaling-up these activities, should establish a minimum set of reporting and coverage indicators and simple-to-follow standard operating procedures to monitor and evaluate the performance of surveillance activities. In doing so, malaria surveillance personnel can improve reporting practices and provide timely reporting of malaria data. Furthermore, setting up policies for mandatory reporting and monitoring systems, including possibly providing incentives, may support uptake and improve reporting and follow-up practices. Ensuring an appropriate and complete target population is screened during RACD, particularly those most at-risk for malaria, will support effective implementation of RACD.
- To increase malaria infection detection and potential treatment, the use of molecular diagnostics in RACD, particularly in non-*falciparum* and low transmission settings, should be considered by malaria programs where feasible. Inclusion of molecular diagnostics will increase the detection of additional infections in RACD, making RACD a more effective approach at identifying infections around an index patient. Although there are additional costs to consider for molecular methods, such as PCR or LAMP, their inclusion in low transmission settings may accelerate malaria elimination efforts by providing more information on where sub-patent infections exist within persistent malaria foci or among certain high-risk populations. This additional information can be used for improved targeting of interventions. If diagnosis and treatment can be provided quickly, it may help to reduce the infectious reservoir and accelerate progress towards elimination.
- To help characterize local epidemiological transmission patterns, particularly in non-*falciparum* and low transmission settings, the use of genotyping in RACD should be considered by malaria programs where feasible. The inclusion of genotyping in RACD can provide important surveillance information to distinguish imported from locally transmitted infections,

characterize parasite flow between geographic regions, and identify important drivers of transmission. The additional costs of including genotyping should be considered. However, when genotyping is used in residual malaria foci or in areas that have recently eliminated malaria, the additional information may be useful to determine an appropriate response (if any is needed). Genetic intelligence, in combination with other epidemiological surveillance, may help to focus a response, whether it is targeted in a geographic area or among individuals with shared risk factors such as high-risk behaviors.

- Targeting sociobehavorial risk factors with RACD compared to household RACD only, may likely yield a greater number of malaria infections detected by microscopy. The demographic and behavioral characteristics of index cases and their socially- or occupationally-related contacts may highlight important shared factors that increase the risk for malaria infection and can be targeted. There will likely be additional costs to consider when targeting these sociobehavorial-related individuals, such as at their work sites in remote locations. But in doing so, malaria programs may identify additional infections therefore making RACD a more effective intervention. Also, malaria programs may be able to provide other interventions such as ITNs or hammock nets, or targeted IRS to reduce the opportunity for future infection. By including a molecular diagnostic, such as LAMP or PCR when targeting forest-goers and travellers associated with an index patient, an even greater number of infections may be detected, making SB-RACD even more effective and may possibly eliminate important reservoirs of infection among individuals with a higher risk of infection.

- Implementation of a drug-based strategy such as RDA when following-up a positive index case is feasible to be conducted by VHVs and acceptable by the household and community members nearby to the index case in this study setting. If RDA were to be implemented in a program setting, a rigorous safety monitoring system should be implemented, including adequate training to be provided to VHVs to maximize the safety and acceptability of RDA among participants and malaria-endemic communities. Supervision of the local VHVs by trained public health professionals should be incorporated into the patient safety monitoring system. While there are important risks associated with taking malaria medicines, implementing an RDA strategy using community- and village-based health workers/volunteers may help to streamline public health activities, increase trust among the community to receive the intervention, save costs on transport to the communities being served, build capacity at the community-level, and increase treatment adherence if DOT was included.

7 Personal reflections and future perspectives

Based on the research outlined above, future areas of research to improve the ways in which RACD is conducted and to explore how RACD may be optimized, include:

1. Better understanding of why and when RACD as part of a case investigation can be introduced in different malaria endemic settings, particularly in medium-burden catchment areas as they transition toward elimination. What surveillance information is gathered during RACD, how is it used, and can this detail be improved in different settings. This intel should help programs find out more about who to target. Furthermore, establishing the importance of monitoring and evaluation systems, program tools, and indicators to monitor practices and knowledge of these activities among those involved. Considerations should include the malaria case incidence and prevalence, human resources available, and capability of surveillance system and reporting requirements.

2. More research elucidating and evaluating sociobehavorial-related strategies for RACD in different settings to identify infections in the community and gather more surveillance information (such as demographic and behavioral risk factors) to improve targeting of interventions. This research would consider and evaluate the heterogeneity within high-risk populations in different settings and the different sociobehavorial-related strategies for RACD to be most effective at detecting infections. Can a RACD response be better targeted to focus on certain demographic or behavioral factors or ecological settings to increase the potential for additional infections to be detected?

3. In low transmission non-*falciparum* species setting, is there value in conducting RACD at all, given the increased likelihood of an infection being a relapse infection? How can reactive infection detection strategies be improved to better target non-*falciparum* malaria settings? Can serial testing with molecular diagnostics in persistent malaria foci help to understand and better target malaria in these areas?

4. More research on the utility of molecular diagnostics in RACD in a program context, particularly in low transmission and mixed-species settings, given the limitations of detecting low-density and asymptomatic infections with standard diagnostics (microscopy and RDTs). Inclusion of molecular diagnostics, such as in areas with persistent malaria foci, can identify sub-patent infections, thereby providing more surveillance information (such as demographic and behavioral risk factors) to improve targeting of interventions among the population. And if those individuals were treated quickly, it may help to reduce the infectious reservoir.

5. More research on the benefit of incorporating genotyping in RACD in a program context, particularly in low transmission and mixed-species settings to characterize local epidemiological transmission patterns. The inclusion of genotyping can help to provide more surveillance data and evidence to further improve targeting of interventions by understanding parasite relatedness and flows between regions, distinguishing local versus imported malaria, and identifying important drivers of transmission. In low transmission settings where research institution capacity exists, can a genetic surveillance system be combined with epidemiological information to improve program activities by better targeting of interventions?

6. Should RDA be found effective at reducing malaria within a programmatic setting in Thailand, further evaluating the safety, acceptability, and feasibility of implementing drug-based strategies for malaria reduction, like RDA, particularly in mixed-species settings will be critical. With the epidemiological and genetic surveillance and evidence on the most at-risk populations better understood, RDA can be targeted to those individuals for greater impact on transmission reduction. Including forest-goers and MMPs in RDA will be essential due to the challenges associated with testing and returning for treatment. Evaluating the safety and use of different 8-aminoquiniline treatment regimens will be important.

7. In areas already conducting RACD, including more programmatic costing data would be helpful to better understand and compare the cost-effectiveness of different RACD strategies, including household- versus sociobehavorial-based responses. Some evidence on the costs of RACD exists, but costing and cost-effectiveness data would be helpful for malaria program managers and policy makers when deciding to implement (or not implement) RACD and how the RACD strategy could be optimized. This would apply to settings considering RDA as a response strategy as well. There is very little costing and cost-effectiveness evidence available on the use of molecular diagnostics in RACD and none on RDA in a program setting.

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