Title

Surveillance and response for malaria high-risk populations: moving from routine to tailored approaches to reach persistent pockets of transmission

Author

Gallalee, Sarah

Publication Date

2023

Peer reviewed|Thesis/dissertation

Surveillance and response for malaria high-risk populations: moving from routine to tailored approaches to reach persistent pockets of transmission

by
Sarah Gallalee

DISSERTATION
Submitted in partial satisfaction of the requirements for degree of
DOCTOR OF PHILOSOPHY

in

Global Health Sciences

in the

GRADUATE DIVISION
of the
UNIVERSITY OF CALIFORNIA, SAN FRANCISCO

Approved:

DocuSigned by:
Jennifer Smith — Jennifer Smith
E25419F30E104D1...
Chair

DocuSigned by:
Adam Bennett — Adam Bennett
[illegible]744A...

DocuSigned by:
Roland Gosling — Roland Gosling
[illegible]428...

DocuSigned by:
Bryan Greenhouse — Bryan Greenhouse
3FD59F1CBAFD444...

Committee Members

Acknowledgments

There are countless people to thank for making this work possible. An enormous thank you goes to my dissertation committee for their expert mentorship. Thank you to my advisor Jenny Smith for continual guidance throughout my dissertation process and answering every question, big and small. Thank you to Adam Bennett for championing my career before I had even joined UCSF and ever since. Thank you to Roly Gosling for always helping me see the big picture and providing many pep talks. Thank you to Bryan Greenhouse for welcoming me into your lab and for your guidance. Thank you to my PhD program directors, Elizabeth Fair and Ali Mirzazadeh, for your constant support since the first day of the program. It has been of immeasurable value to have you to rely on through every twist and turn. I also cannot thank my PhD cohort enough: Lucía Abascal, Canice Christian, Jane Fieldhouse, and Johnson Lymio – thanks for your friendship and unwavering encouragement. I certainly could not have done it without you.

Thank you to MEI colleagues and leadership (Allison Tatarsky, Michelle Hsiang, Cara Smith Gueye, Jenny Smith) for welcoming me onto the team, providing a great team environment, and supporting my PhD work! A special shoutout to Katie Geissler for constant encouragement. I also want to thank the IGHS community and our class professors: an admirable and dedicated community of global health experts. Thank you to Jaime Sepulveda, George Rutherford, and Jeremy Alberga for your leadership and thoughtful advice throughout the past four years. Endless thanks to Darlene Mergillano, Tammy Spath, Inez Bailey, Avery Seefeld, and Minerva Lee for your diligent support through each stage of the program. I also want to thank Ray Care for your advice and mentorship. Thank you to the caring friends who have supported me throughout my training and all the wonderful mentors I have had during my career. Lastly, thank you to my incredible family (in particular, my parents and brother). I am continuously inspired and buttressed by each of you.

Contributions

The research in this dissertation was funded by the Bill and Melinda Gates Foundation.

The research in Chapter 2 was conducted by the University of California, San Francisco's Malaria Elimination Initiative (MEI) and the Eijkman Institute for Molecular Biology. Chapter 2 was written in collaboration with the following co-authors: Iska Zarlinda, Martha G Silaen, Chris Cotter, Carmen Cueto, Iqbal R.F. Elyazar, Jerry O Jacobson, Roly Gosling, Michelle S. Hsiang, Adam Bennett, Farah N. Coutrier, and Jennifer L. Smith. The authors extend their gratitude to the Aceh Provincial Health Office, the Aceh Jaya and Aceh Besar District Health Offices, the Indonesia Malaria Control Program, and health facility staff in the study subdistricts. The authors are especially grateful to the patients and study participants who contributed samples and provided their time to the study.

The research in Chapter 3 was conducted by MEI and the Center of Malariology, Parasitology, and Entomology (CMPE) in Lao PDR. Co-authors include Emily Dantzer, Francois Rerolle, Jennifer Smith, Roly Gosling, Andrew Lover, Bouasy Hongvanthong, and Adam Bennett. We thank the study team and the study participants.

Chapter 4 was conducted by the MEI, PATH, and the PNLP of Senegal. The co-authors for this study include Demba Kande, Tidiane Thiam, Henry Ntuku, Caterina Guinovart, Laura Merriman, Abiboulaye Sall, Moustapha Cissé, Aichatou Barry Diouf, Mamadou Diop, Baba Camara, Niene Seck, Faith De Amaral, Roly Gosling, Bryan Greenhouse, Yakou Dieye, Jennifer Smith, and Adam Bennett. The authors extend their gratitude to the Saraya District Health Office, the Kaolack District Health Office, and the study health facility staff. The authors all also grateful to all the study participants for participating in the study.

Surveillance and response for malaria high-risk populations: moving from routine to tailored approaches to reach persistent pockets of transmission

Sarah Gallalee

Abstract

Malaria high-risk populations (HRPs) are groups at increased risk of malaria due to common characteristics that lead to increased exposure to malaria-transmitting mosquitoes. While addressing malaria among these populations is essential to elimination, they present a persistent challenge to malaria control programs globally because HRPs often have limited access to health services and lower intervention coverage. This dissertation explores methods for identifying malaria HRPs and targeting them with effective interventions across contexts. First, a case-control study is used to identify risk factors for malaria, characterize malaria HRPs, and identify intervention points in Aceh, Indonesia. Second, augmented routine surveillance data are utilized to identify risk factors for malaria in Champasak, Lao PDR. Third, the effectiveness of a package of interventions for two HRPs in Senegal (gold miners and Koranic school children) is assessed. The findings of this research contribute to our understanding of malaria HRPs, methods to characterize them, and approaches to target interventions to these vulnerable populations in order to decrease morbidity and mortality and eliminate malaria.

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List of Abbreviations

ACT	Artemisinin-based combination therapy
ANC	Antenatal care
aOR	Adjusted odds ratio
API	Annual parasite index
CHIRPS	Climate Hazards Group InfraRed Precipitation with Station data
CHW	Community health worker
CI	Confidence interval
CMPE	Center for malariology, parasitology, and entomology
DAG	Directed acyclic graph
DBS	Dried blood spots
DID	Difference in difference
DSDAARA	Dispensateur de soins à daara (CHW at a boarding school)
DSDOM	Dispensateur de soins à domicile (CHW)
DSDOR	Dispensateur de soins à mine d'or (CHW at gold mine)
EVI	Enhanced vegetation index
GMS	Greater Mekong Subregion
HF	Health facility
HIV	Human immunodeficiency virus
HRP	High risk population
IRS	Indoor residual spraying
Lao PDR	Lao People's Democratic Republic
LLIH	Long-lasting insecticide treated hammock
LLIN	Long-lasting insecticide treated net
LST	Land surface temperature

MERFAT	Malaria Elimination Risk Factor Assessment Tool
MODIS	Moderate Resolution Imaging Spectroradiometer
NGO	Non-governmental organization
NMCP	National malaria control program
nPCR	Nested polymerase chain reaction
OR	Odds ratio
PCA	Principal component analysis
PCR	Polymerase chain reaction
PECADOM	Prise en charge à domicile (home-based management of malaria)
Pf	*Plasmodium falciparum*
Pk	*Plasmodium knowlesi*
PNLP	Programme national de lutte contre le paludisme (NMCP)
PSE	Population size estimation
Pv	*Plasmodium vivax*
QA	Quality assurance
qPCR	Quantitative polymerase chain reaction
RACD	Reactive case detection
RadR	RDS-adjusted RTM
RCT	Randomized controlled trial
RD	Risk difference
RDS	Respondent driven sampling
RDT	Rapid diagnostic test
rRNA	Ribosomal ribonucleic acid
RTM	Reverse tracking method
SBCC	Social and behavior change communication

SD	Standard deviation
SDG	Sustainable development goals
SES	Socioeconomic status
SMC	Seasonal malaria chemoprevention
UCSF	University of California, San Francisco
varATS	*var* gene acidic terminal sequence

Chapter I

Introduction

Malaria continues to kill over 600,000 people annually and is responsible for 2.6 percent of the global burden of disease; in 2021 there were 247 million cases in eighty-four countries [1,2]. Global malaria incidence declined by 27% between 2000 and 2015; however, the rate of decline has slowed with incidence declining less than 2% between 2015 and 2019 [3]. Progress towards elimination has also been substantial: since the year 2000, twenty-four countries have been newly certified as malaria free by the World Health Organization [1]. While many countries have achieved large declines in malaria incidence, global progress has stalled, and countries are working to identify the geographies and populations where malaria transmission continues and address the causes of on-going transmission in order to eliminate malaria. As countries shift from control to elimination, national malaria control programs (NMCPs) must deal with changing epidemiology as malaria clusters in geographic areas and among specific demographic populations [4-6].

Malaria transmission across geographies and populations is heterogenous: malaria endemic countries range from high-transmission contexts where the majority of the population is at risk of malaria, to lower endemic contexts where as few as 1% of the population are at risk and the country or subnational area is aiming to eliminate malaria (Figure 1.1). When countries achieve reductions in malaria transmission, the degree in heterogeneity of malaria transmission often increases [7,8]. Because malaria is naturally heterogeneous across geographies, progress towards elimination occurs at varying rates within a country due to differences in the effectiveness of interventions and various socioeconomic and environmental changes over time [9]. In addition to the increasing spatial heterogeneity of transmission, decreasing malaria burden leads to more focal transmission among specific populations [9]. Across transmission settings, these malaria high-risk populations (HRPs) are formed due to variable access to malaria services and interventions in combination with specific exposures to malaria-transmitting mosquitoes. Malaria HRPs are groups of individuals at higher risk of malaria infection due to shared characteristics such as demographics, behavior, occupation, or geographic location [10]. While HRPs are context specific within and across countries, there are overlapping themes that define these populations including limited access to prevention, diagnosis, and treatment for malaria in combination with behavior that leads to exposure outside of the household [4,5].

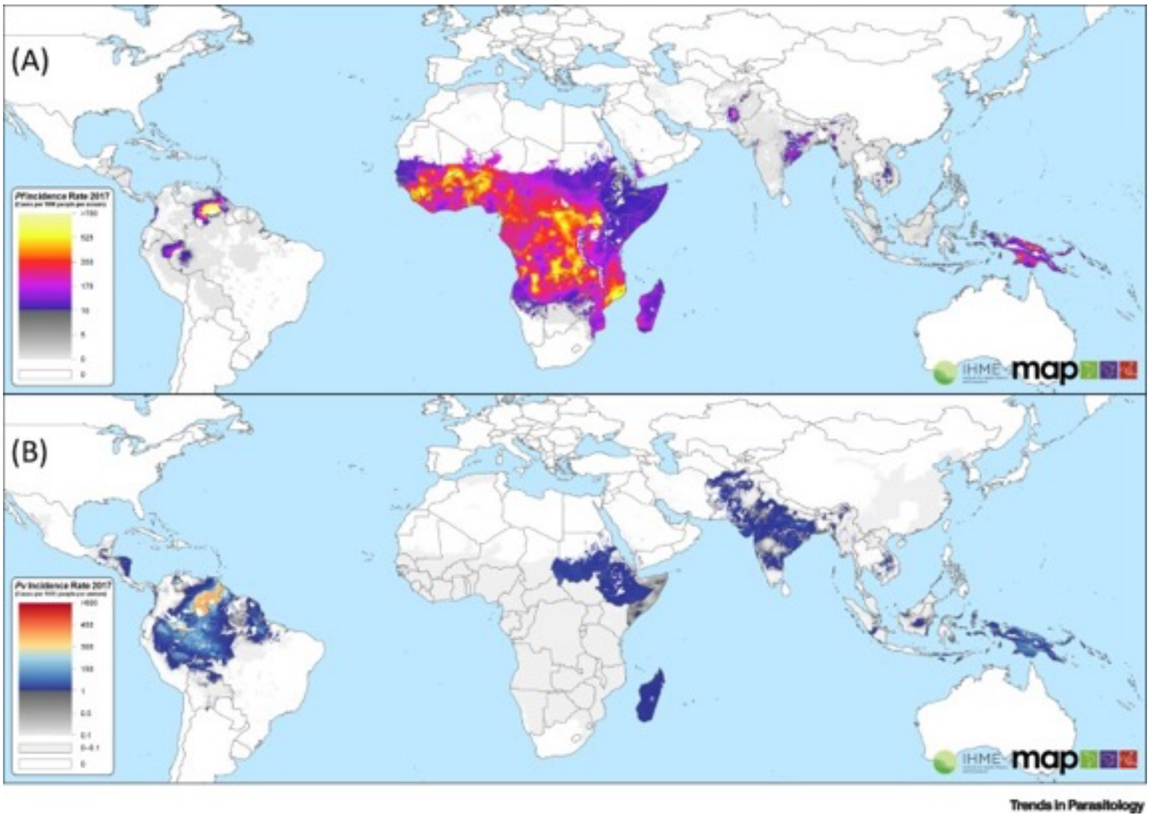

Figure 1.1: *Plasmodium falciparum (Pf)* and *Plasmodium vivax (Pv)* incidence rates, 2017 [10]

Across transmission settings, those at high risk of contracting malaria may be challenging populations in border areas, groups defined by geographic risk factors such as proximity to health facilities and/or vector breeding sites, or vulnerable populations who have been removed from society or face stigma [4,11]. HRPs may also include groups whose behaviors result in the ineffectiveness of standard control measures; for example, sleeping outdoors at night when the temperature is hot rather than inside under a protective bed net [12]. In low-endemic contexts, local transmission may only continue to occur among HRPs; HRPs can act as a reservoir of infection with highly mobile groups driving seasonal transmission or re-introducing the parasite to geographies where it has been eliminated [13]. Therefore, interventions among HRPs can have a disproportionate effect on transmission in elimination contexts and reaching these populations is key to malaria elimination [13].

A number of challenges hinder programs from addressing malaria among HRPs. The status quo of malaria control in many settings is to focus approaches on vector control interventions such as bed net and indoor residual spraying campaigns, based on the assumption that individuals are exposed to night-biting mosquitoes when they are inside at their home residence [4,6,14,15]. In some cases, donors and the establishment do not consider HRPs or that exposure to mosquitoes may be happening outside of the community. Additionally, where program capacity and donor funding are already stretched, addressing transmission in these populations may seem out of reach, too challenging, or to be less of a priority compared to interventions that benefit the general population [4,16].

However, improved coverage of malaria services and interventions in HRPs may have a disproportionate impact on transmission in both high and low burden settings [4,9,17]. Early engagement with approaches to address malaria HRPs and integrating those approaches into routine surveillance strategies, as much as possible, is likely to accelerate efforts to control malaria, ensure equity, and help to prepare programs for a shift towards malaria elimination strategies [16,17]. Conventional tools for malaria control are often unable to reach these populations or are unsuitable in their specific context; more tools are needed to identify malaria HRPs, engage directly with the HRPs to design interventions, and improve surveillance and response efforts to control and prevent malaria in their settings [4,17-19].

In recent efforts to develop methodologies for identifying and targeting malaria HRPs, leveraging approaches from other disease areas such as human immunodeficiency virus (HIV) has been valuable [4,11]. HIV control programs have long dealt with the challenges of transmission occurring in a concentrated manner among populations that are not successfully identified via household surveys. For malaria programs to be able to identify HRPs, a mechanism is needed to reorient surveillance approaches in a manner that can measure and address malaria among these populations. **Figure 1.2** depicts an ideal surveillance cycle for malaria HRPs; this cycle was adapted by Jacobson et al from HIV guidelines for Second Generation Surveillance [4,20]. In an ideal surveillance cycle for targeting malaria HRPs, there are four steps: conducting a pre-surveillance assessment, confirming risk factors and characterizing high-risk groups, implementing ongoing surveillance, and improving and targeting interventions (**Figure 1.2**) [4,20].

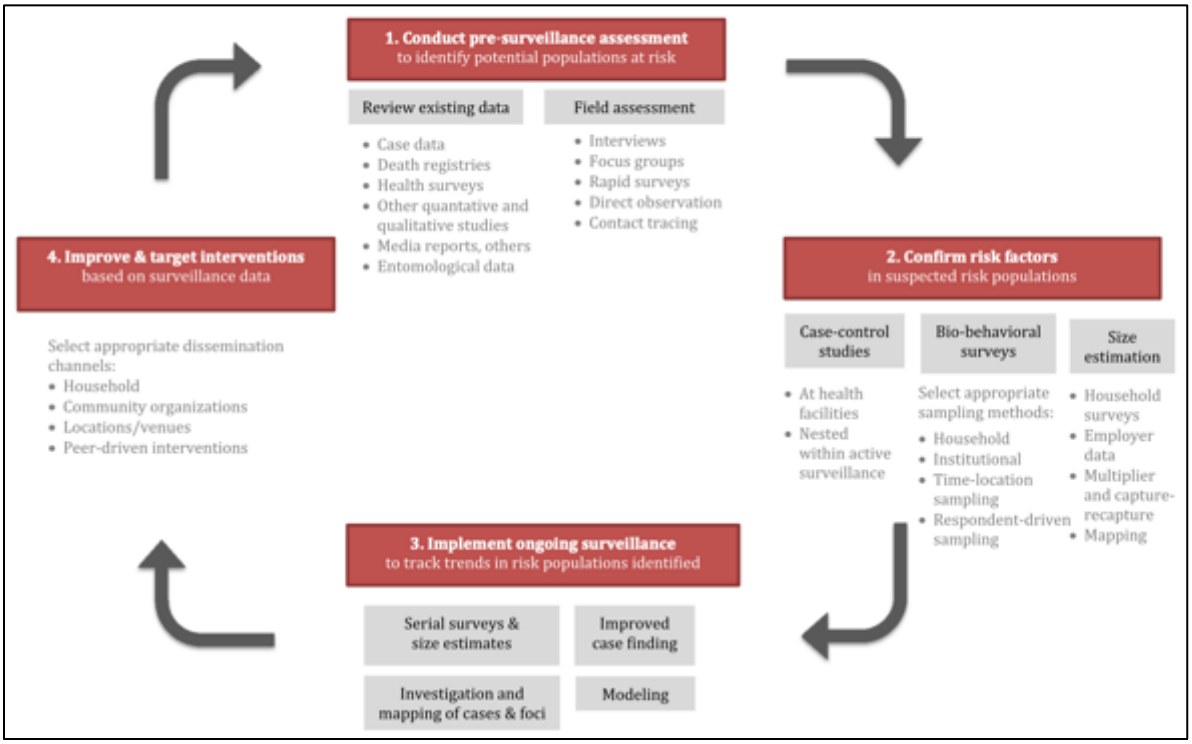

Figure 1.2: *Surveillance cycle for malaria high-risk populations* [4,20]

The research chapters of this dissertation fall into two steps of the surveillance cycle: Chapters 2 and 3 entail characterizing malaria high-risk populations and confirming risk factors in Indonesia and Lao PDR (Step 2 in **Figure 1.2**). In Chapter 2, a case-control methodology is utilized; in Chapter 3 routine surveillance data are utilized. Chapter 4 falls under the fourth step: assessing the efficacy of a package of interventions for high-risk populations in Senegal. The objective of this dissertation is to contribute to ongoing research and practice to address malaria among HRPs and reach the final persistent pockets of malaria transmission.

Understanding and defining context and diversity within HRPs

Across settings, malaria risk factors are shaped by environmental influences on anopheline mosquitos, species and location specific vector behavior, local health systems and level of access to them, context-specific human behavior, and economic forces influencing human movement and occupational activities [4]. While these influences are complex and context specific, malaria HRPs have some broad overlapping characteristics in lower endemic settings including tendency to be male gender, mobility and seasonality in their work, lack of access to healthcare, exposure to outdoor biting mosquitoes, occupational risk, and being adults or school-age children [11]. In some regions there are consistent high-risk occupational activities that have been defined; for example, forest-going across South and Southeast Asia and gold mining in Latin America [4,21-24]. Other examples of malaria HRPs that have been defined include military personnel in Indonesia and Lao PDR, plantation workers Malaysia, cross-border migrants in Thailand, and young male travelers in Namibia [25-29]. However, in many settings there is still a need for HRPs to be better defined, or redefined if the malaria context has evolved [4]. Developing an in-depth understanding of HRPs in greater detail is essential for national malaria control programs to design and target effective interventions for these populations.

Amongst HRPs, some groups are easy to reach and accessible but have not been prioritized or identified as high-risk, while others are not only high-risk but also hard-to-reach (Figure 1.3). For example, in Senegal, boarding school students have been identified as high-risk populations, but have not typically been targeted with specific interventions although they are largely stationary populations and many of the schools are easy to access in urban areas [30,31]. In contrast, groups such as gold miners and laborers who spend extended time working deep in the forest can be classified as mobile-migrant populations that are harder to reach [17,21]. Hard-to-reach and mobile HRPs are particularly prone to lack access to care and be missed in passive surveillance systems [4,5,11,13,17]. Even when these groups have been successfully identified by routine surveillance, the available information is often not detailed enough to aid in the comprehensive characterization of these populations [4].

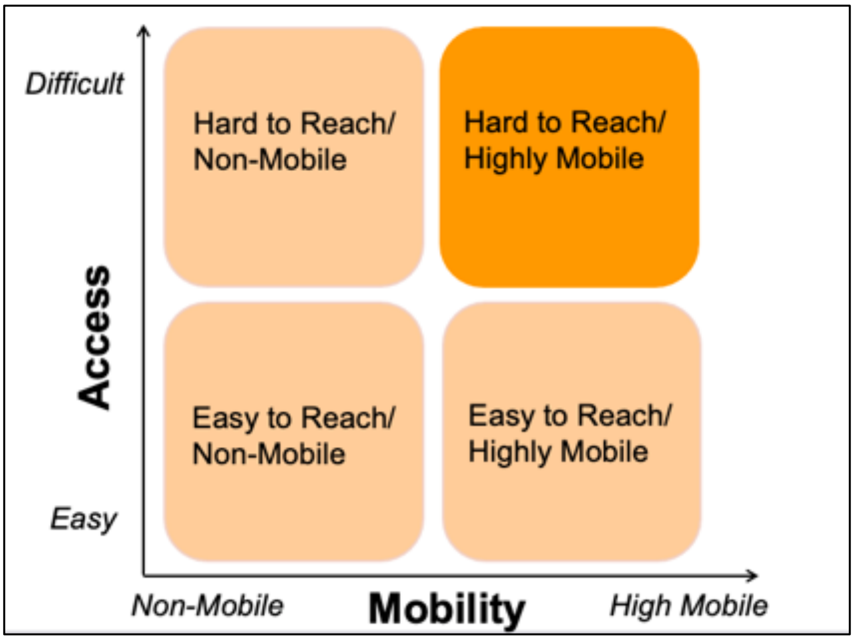

Figure 1.3: *HRP diagram (Source: Malaria Elimination Initiative)*

Rigorous approaches are needed to improve the identification and characterization of HRPs in order to target effective interventions to them. One of the simplest ways to collect data is to ask front line health workers who often have important intelligence that is not formally collected through data systems, such as site specific knowledge and access to local networks [32,33]. Additionally, subnational programs frequently hold knowledge about intersectionality between these characteristics in local populations and can identify key challenges around achieving coverage across all populations [33,34]. It is critical to access existing knowledge and data around HRPs as a first step to identifying operational research gaps [35]. A central malaria control program may not have insight into the diversity of different high-risk population characteristics (e.g., a wide variety of sub-national working or sleeping patterns) [33]. NMCPs must strengthen subnational units to identify HRPs and engage HRPs to choose interventions that can address specific malaria control challenges [35-37].

One method for better characterizing HRPs and identifying risk factors is utilizing case-control studes; a case-control study is a classic study design in epidemiology that has been used for identifying risk factors for malaria across settings in sub-Saharan Africa and Southeast Asia [29,38-43]. Case-control studies compare the characterisitcs of individuals with a confirmed malaria infection (cases) to individuals without malaria (controls), which allows for the controlling of a number of exposure variables that may be related to malaria transmission. Case-control studies can be particularly efficient for identifying risk factors for malaria in low transmission settings where there may be relatively few cases; they are also often faster and less expensive than cohort studies [29,44]. Chapter 2 of this dissertation utilizes a case-control study design with two control series (health facility and community controls) to characterize malaria high-risk populations in Aceh Province, Indonesia. The methods used in this chapter were developed at UCSF's Malaria Elimination Initiative, and were later published as part of the Malaria Elimination Risk Factor Assessment Tool (MERFAT), which provides a detailed framework for implementing case-control studies within programmatic contexts for malaria risk factor assessments [45].

Another approach to detecting HRPs is to adapt routine surveillance systems: tailoring routine surveillance approaches to include data on suspected risk factors can improve the ability of passive surveillance systems to detect malaria HRPs [4]. Although robust passive surveillance systems are the backbone of malaria surveillance across all transmission settings and essential to malaria elimination, they can be inadequate for identifying HRPs if they do not gather the data needed to identify risk factors related to behavior or occupation [4]. Case investigation and active surveillance are methods utilized to further characterize and identify cases beyond the capabilities of routine surveillance [9]. Additionally, if routine systems are specifically tailored to identify HRPs (based on previous research, formative assessments, and stakeholder knowledge), routine surveillance can be utilized to improve our understanding of risk factors and detect HRPs. This approach is particularly applicable if there are already suspected risk factors that need to be confirmed and if few questions are needed to confirm them. Programs can tailor data collection activities to level of knowledge and existing data, with a focus on understanding who the HRPs are and issues around access within regions and communities. Chapter 3 of this dissertation utilizes augmented passive surveillance data to identify risk factors for malaria in southern Lao PDR.

Leveraging networks to overcome barriers of community engagement and effectively target interventions to HRPs

Major challenges to improving access to care and the delivery of interventions to malaria HRPs include high population mobility and limited group organization (e.g., no leadership or social hierarchy) [46]. Even when HRPs are identified and characterized in detail, they can still be missed by routine interventions due to their behavior or occupation [13]. Populations that have low access and uptake of services may also be poorer, socially marginalized, and vulnerable to stigmatization due to legal or socioeconomic concerns [47]. Even in well-integrated populations, such as school children, there are often gaps in intervention coverage that require novel solutions [48].

Leveraging places that connect HRPs or are hotspots of transmission can be useful to cost-effectively deliver services to people with shared malaria risk factors. Existing networks in place often link people: these include places of employment, transportation hubs, locations where travelers congregate or pass through, schools, and shops [49-51]. Such populations require specific and sensitive approaches to engage them; processes to identify and engage gatekeepers allow for the collection of information on access points and barriers to uptake [52,53]. Multisectoral approaches are also important to consider, e.g., complementary strategies to include other platforms such as Ministries of Education to ensure high coverage among students for at-risk periods [35,54]. These approaches form a critical part of planning for targeted delivery and mobilization of support.

Recent studies have applied strategies from other disease areas to target malaria HRPs. These include utilizing peer navigators to increase access to case management in Lao PDR [55], engaging employers of forest goers in Indonesia to participate in survey recruitment [56], and mapping out strategies to characterize mobile and migrant populations in Nepal [19], among others [45]. Chapter 4 of this dissertation assesses the efficacy of a package of interventions for malaria HRPs in Senegal, including the novel approach of utilizing peer-CHWs within two HRP groups: gold miners and Koranic school children.

Prioritizing HRPs: why empirical evidence and translational research is still needed

Addressing malaria among HRPs is essential to global equity [57]. Many malaria HRPs do not have equal access to prevention, diagnosis, and treatment for malaria: there are equity benefits to improving methods to reach these populations. Vulnerable populations are those who experience marginalization, prejudice, and unequal rights; these populations are at higher risk of malaria as well as severe malaria and death [7,58]. Additionally, in elimination settings, malaria becomes increasingly clustered in disadvantaged communities [7,58]. Targeting HRPs has the benefit of reducing disparities in access to care and prevention of malaria; these efforts advance global health and development goals such as the Sustainable Development Goals (SDGs) [7].

Malaria elimination will not be possible without engaging all populations at risk. The global malaria community including NMCPs, non-governmental organizations (NGOs), foundations, and other partners have begun to take strides to reorient programs towards addressing HRPs with targeted intervention approaches including improved surveillance. However, evidence and translational research are still needed on effective methods and approaches. The research in this dissertation contributes to addressing malaria among HRPs by focusing on methods for measuring and targeting malaria high-risk populations across three pre-elimination and elimination contexts in Indonesia, Lao PDR, and Senegal (Figure 1.4). The second chapter is a case-control analysis in Aceh, Province, Indonesia that supports the evidence base for methodologies to characterize HRPs in low transmission settings and identifies actionable risk factors to inform programmatic action. The third chapter utilizes augmented routine surveillance data to detect risk factors for malaria in Southern Lao PDR. The fourth chapter assesses the efficacy of a package of interventions for two high risk populations in Senegal: gold miners and Koranic school children. These findings contribute to our understanding of malaria HRPs, methods to characterize them, and approaches to target interventions to these vulnerable populations.

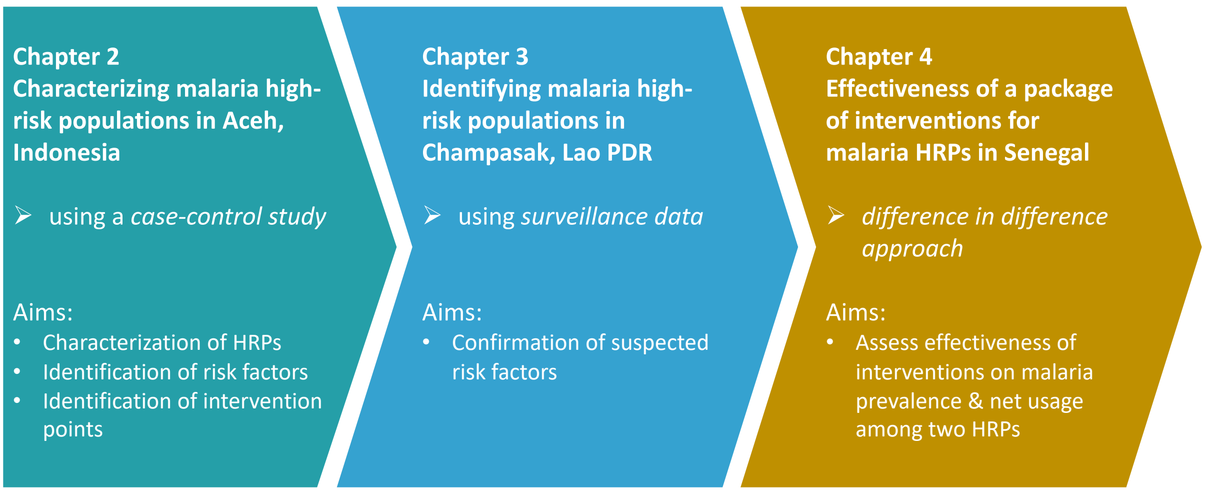

Figure 1.4: *Overview of research chapters in this dissertation*

Chapter II

Forest-goers as a heterogeneous population at high-risk for malaria: a case-control study in Aceh Province, Indonesia

Abstract

Background: A major challenge to malaria elimination is identifying and targeting populations that are harboring residual infections and contributing to persistent transmission. In many near-elimination settings in Southeast Asia, it is known that forest-goers are at higher risk for malaria infection, but detailed information on their behaviors and exposures is not available.

Methods: In Aceh Province, Indonesia, a near-elimination setting where a growing proportion of malaria is due to the species *Plasmodium knowlesi*, we conducted a case-control study to identify risk factors for symptomatic malaria, characteristics of forest-goers, and key intervention points. From April 2017 to September 2018, cases and controls were recruited and enrolled in a 1:3 ratio. Cases had confirmed malaria infection by rapid diagnostic test or microscopy detected at a health facility (HF). Gender-matched controls were recruited from passive case detection among individuals with suspected malaria who tested negative at a health facility (HF controls), and community-matched controls were recruited among those testing negative during active case detection. Multivariable logistic regression (unconditional for HF controls and conditional for community controls) was used to identify risk factors for symptomatic malaria infection.

Results: There were 45 cases, of which 27 were *P. knowlesi*, 17 were *P. vivax*, and one was not determined. For controls, 509 and 599 participants were recruited from health facilities and the community, respectively. Forest exposures were associated with high odds of malaria; in particular, working and sleeping in the forest (HF controls: adjusted odds ratio (aOR) 21.66, 95% CI 5.09-92.26; community controls: aOR 16.78, 95% CI 2.19-128.7) and having a second residence in the forest (aOR 6.29, 95% CI 2.29-17.31 and 13.53, 95% CI 2.10-87.12). Male forest-goers were a diverse population employed in a variety of occupations including logging, farming, and mining, sleeping in settings such as huts, tents, and barracks, and working in a wide range of group sizes. Reported use of protective measures such as nets, hammock nets, mosquito coils, and repellants was low among forest-goers and interventions at forest residences were absent.

Conclusions: Second residences in the forest and gaps in use of protective measures point to key malaria interventions to improve coverage in forest-going populations at risk for *P. knowlesi* and *P. vivax* in Aceh, Indonesia. Intensified strategies tailored to specific sub-populations will be essential to achieve elimination.

Background

Global achievements in reducing the burden of malaria have inspired many countries to aim for malaria elimination [7]. In countries nearing elimination, residual transmission among high-risk populations sustains transmission; however, these groups are often difficult to reach and not captured by routine malaria surveillance [17]. In many countries nearing elimination in Asia and Latin America, risk of malaria infection is linked with spending time in forests where individuals are exposed to malaria-transmitting mosquitoes [24,59]. Identifying these risk factors, characterizing forest-going populations, and pinpointing intervention gaps are essential components of malaria elimination in these settings.

Indonesia has eliminated malaria in over half of the country's districts and most remaining transmission occurs among forest-going populations who are difficult to identify and reach with interventions [56,60]. In Southeast Asia, forest activities including logging, agriculture, and mining have been identified as high-risk activities with transmission occurring among adult males who have increased exposure to outdoor-biting and forest dwelling mosquitoes [6,42,61-63]. Forest workers in Indonesia are also highly mobile populations who have limited access to care and may facilitate movement of malaria between high and low burden areas [56]. The presence of the simian malaria parasite *Plasmodium knowlesi* presents additional challenges to elimination because of the zoonotic reservoir, diagnostic difficulties, differing risk factors, and a need for rigorous evaluation of interventions for this parasite species [62,64].

Current regional efforts to address this high-risk population of forest-goers include research on targeted chemoprophylaxis, vector control methods to target outdoor transmission such as insecticide-treated hammock nets, assessing and improving active surveillance, and efforts to understand zoonotic crossover of simian malaria [56,59,65]. It is critical to identify additional details about subpopulations within these forest-going groups in order to tailor approaches in Indonesia and similar settings in Southeast Asia.

A case-control study was carried out in Aceh Province, Indonesia, to determine the most important risk factors for symptomatic malaria. The risk factors examined in this study include demographic variables, potential risk factors related to community exposures, and forest exposures (including human and simian interaction and the types and duration of activities). A subgroup analysis among forest-goers was also conducted in order to delineate high-risk sub-groups in more detail and identify key intervention points.

Methods

Study Area

Aceh Province is located on the island of Sumatra and is the northwesternmost province in Indonesia (Figure 2.1). An estimated half of the land area in Aceh is forested and the average temperature in this region ranges from 26°C to 28°C [56,66]. Major industries in Aceh Besar and Aceh Jaya include agriculture, manufacturing, mining (such as gold mining), service, and trade [56]. Malaria transmission varies across Aceh province with high malaria transmission season typically occurring from January to July [56].

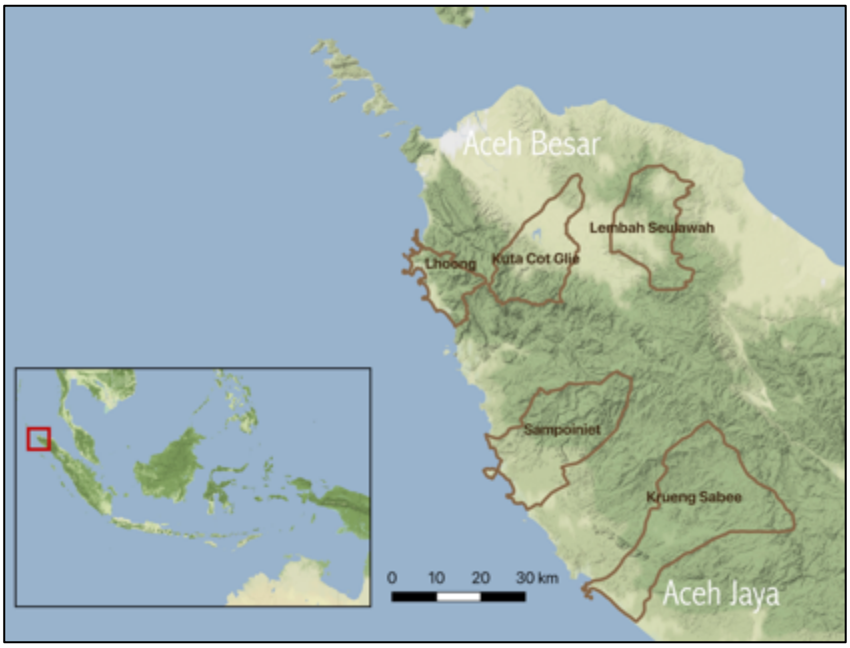

Figure 2.1: *Selected study sub-districts in Aceh Besar and Aceh Jaya, Sumatra, Indonesia. Background Terrain: Stamen Design* [67].

This study took place in five sub-district level health facility sites (Krueng Sabe, Kuta Cot Gli, Lembah Seulawah [Saree], Lhoong, and Sampoiniet) of Aceh Besar and Aceh Jaya districts (Figure 2.1). Other than Sampoiniet, these were the subdistricts with the highest incidence in their district in 2016 and three of them (Kuta Cot Gli, Lhoong, Saree) were included in a study of reactive case detection (RACD) previously published [62,64,68]. Sampoiniet was added in July 2018 with the goal of capturing additional cases to improve statistical power. The annual parasite index (API, cases per 1,000 population) among the Aceh Besar subdistricts in 2016 ranged from 0.26 and 0.50 in Saree and Lhoong, respectively to 1.44 in Kuta Cot Gli [69]. The reported study population of these three subdistricts of Aceh Besar in 2016 was 32,822 [69]. In Aceh Jaya, API was 0.29 in Sampoiniet and 4.08 in Krueng Sabe [70]. The reported study population of these two subdistricts in 2016 was 15,799 [70].

Study Design

A prospective case-control study on risk factors for symptomatic malaria was conducted in Aceh Province from April 2017 to September 2018. The study population was drawn through malaria passive case detection (patients presenting with suspected malaria to study primary care facilities or district hospitals) and active case detection (testing of largely asymptomatic individuals in the community). Patients presenting to a health facility with suspected malaria were eligible to participate as a case if they were confirmed positive for malaria by rapid diagnostic test (RDT) or microscopy and were eligible to participate as a health facility control if they tested negative. Willingness and availability to participate in the study and residence in selected subdistricts were also inclusion criteria.

The purpose of the health facility control series was to capture individuals who had similar selection factors to cases recruited into the study through the passive surveillance system, including a similar likelihood of reporting to a health facility for care. Age and gender are known confounders for many occupational and travel-related exposures; additionally, a formative assessment in Aceh showed that cases rarely occur in people younger than fifteen and occurred more often in males [56]. In order to avoid a large imbalance and allow for the analysis of risk factors, controls were selected from RDT negative individuals aged 15 years and older and frequency matched by gender.

To balance the potential limitations of only using health facility controls, we also included neighborhood matched community controls. These individuals provided a population-based comparison population and were recruited from individuals who tested negative by microscopy during RACD of all subjects residing within 500 meters of an index case detected in passive case detection. These controls were matched by neighborhood to minimize case ascertainment bias due to variations in geographical accessibility to the health clinics and to control for socioeconomic factors.

Original sample size calculations determined that a minimum sample size of 177 cases and 531 controls (1:3 case to control ratio) was needed to detect a difference with 80% power for the matched case-control study (correlation = 0.3). Due to declining transmission over the study period, the total number of participants recruited included 45 cases, 509 health facility controls, and 599 community controls which allows us only 45% power to detect an effect size of 2.25 and 80% power for an effect size of 3.47.

Data Collection

A survey instrument informed by formative work carried out in May-July 2016 was developed in English and translated to Bahasa Indonesia and back translated by a fluent bilingual health expert before field testing. Any ethnic minority language interviews were conducted in the appropriate language and translated into Bahasa Indonesia. The survey questions were field tested prior to data collection and administered using handheld tablets.

For health facility cases and controls, all individuals matching a recruitment profile and diagnosed in selected health facilities as confirmed positive by RDT or microscopy (cases) or confirmed negative by RDT or microscopy (controls) were interviewed by a health facility staff member on the same day. Neighborhood controls were individuals identified during household RACD activities. These individuals were screened for inclusion, tested for malaria, and if negative by RDT or microscopy, enrolled as community controls.

Laboratory Methods

RDT and microscopy were performed in health facilities and during RACD. Quality assurance (QA) of microscopy performed at health facilities was conducted by certified microscopists at the provincial lab in Aceh according to national guidelines.

Dried blood spots (DBS) were collected using Whatman 3MM paper and sent to the Malaria Pathogenesis Laboratory at the Eijkman Institute in Jakarta. All RDT- or microscopy-positive samples (and 10% of negative samples) underwent polymerase chain reaction (PCR) testing. Molecular testing was performed using chelex-extracted DNA from the DBS [71]. Genus-specific PCR targeting the mitochondrial *cytb* gene followed by *Alu*I enzyme digestion was used for species identification as described elsewhere [64,72]. Additional methods were used including nPCR testing targeting the 18S rRNA gene [73] and *P. knowlesi*-specific nPCR [74]. If PCR results were not available for a case, microscopy was used to determine the species.

Data Processing

Data entry was carried out using PC and tablet computers and uploaded daily to two centralized servers. Data management, cleaning, and analysis were conducted using STATA 16.1, R 4.1.2, and QGIS 3.12.3. Six observations were dropped due to missing data for prior night net use, and four observations were dropped due to missing data for household IRS (indoor residual spraying). Additionally, because there was only one case that was older than sixty years old and over one hundred controls that were over 60 years old, the dataset was restricted to participants under sixty years. Categories within variables that were conceptually similar were recombined to avoid cells smaller than five; for indoor residual spraying (IRS) and subdistrict, it was not possible to regroup the categories further.

Occupation was grouped into three categories: 1) farming and plantation work; 2) logging, mining, and other outdoor labor such as rattan collector, forest ranger, and elephant work; 3) other non-outdoor labor such as teaching, professional or government work, driver, or factory labor. A categorization of occupation with a larger number of groups was not possible due to small cell sizes; therefore, more detailed analyses of occupation groups were descriptive. To represent socio-economic status (SES), we conducted a principal component analysis (PCA) of household-level binary assets (e.g. electricity, radio, refrigerator, car, motor bike, cell phone, and water heater) and a categorical fuel variable [75]. To define traditional home (for primary residence), we adapted the approach by Tusting et al [76] and defined a traditional home as a building with wooden walls, partially wooden walls, or no walls. Sleeping in the forest was defined as someone who reported working in the forest or forest fringe (outside of any village) in the past sixty days and sleeping in the forest during these work trips. These potential risk factors for malaria were grouped into three broad categories: demographic variables, community exposures (at the participant's main residence), and forest-related exposures. Additional survey questions were asked only of the forest-goers about their most recent trip to the forest. Sleeping structure in the forest was classified as tent (tent or plastic makeshift tent), hut (hut, house, or other), or barracks. The variable "used protection against mosquitoes" was defined as a binary variable with 'yes' indicating reported use of at least one valid method (bed net, hammock, repellant, coil, wearing covering clothes, or chemoprophylaxis).

Data Analysis

Associations between risk factors and the outcome of malaria infection were explored using unconditional logistic regression for the health facility control series and conditional logistic regression for the community control series. Model building was informed by a directed acyclic graph (DAG) conceptualizing relationships between the primary forest exposure (working and sleeping in the forest), the outcome of malaria infection, and potential confounding and mediating variables [77]. We then assessed the bivariate relationship of the variables identified as potential confounders (age, gender, occupation, education, SES, net use, IRS, traditional house, and family member with forest exposure) between the main exposure and the outcome. Crude odds ratios (ORs) with 95% confidence intervals were estimated in bivariate logistic regression; variables associated with the outcome (significant at p-value 0.05) or those that changed the main effect OR by more than 10% in the final model were included.

The model building process was conducted separately for each control series. The health facility control series used an unconditional logistic regression model with fixed effects at the subdistrict level and a dummy variable for gender because the controls were frequency matched by gender (therefore ORs for subdistrict and gender are not presented). In the conditional logistic regression community control series, the community identifier was used as the grouping variable and gender was included as an *a priori* confounding variable. Adjusted odds ratios (aOR) and 95% confidence intervals for the variables included in the final models for each control series are presented. The SES measure was tested as a continuous and categorical measure. The second component of the PCA was included as a continuous measure for health facility controls and the first component of the PCA was included for community controls.

A descriptive subgroup analysis was conducted among forest-goers, i.e., participants who reported working and sleeping in the forest in the previous sixty days to identify risk factors specific to this group and descriptively assess the characteristics of this population. A logistic regression model was used to confirm key exposures using the health facility control series. The community control series was excluded from model building as it required use of the grouping variable to account for matching, which was not possible in the small subgroup. A sub-group analysis by species was not possible due to the limited number of cases.

Results

Descriptive Analysis

Between April 2017 and September 2018, 45 positive cases were identified and enrolled in the study along with 509 health facility controls and 599 community controls. Species identification by PCR was available for forty (89%) of the forty-five cases, and by microscopy for the remaining cases. Twenty-seven cases (60%) were *P. knowlesi,* 17 (38%) were *P. vivax*, and for one case the species was undetermined. Case recruitment was highest during a 7-month period from April 2017- October 2017 (Figure 2.2).

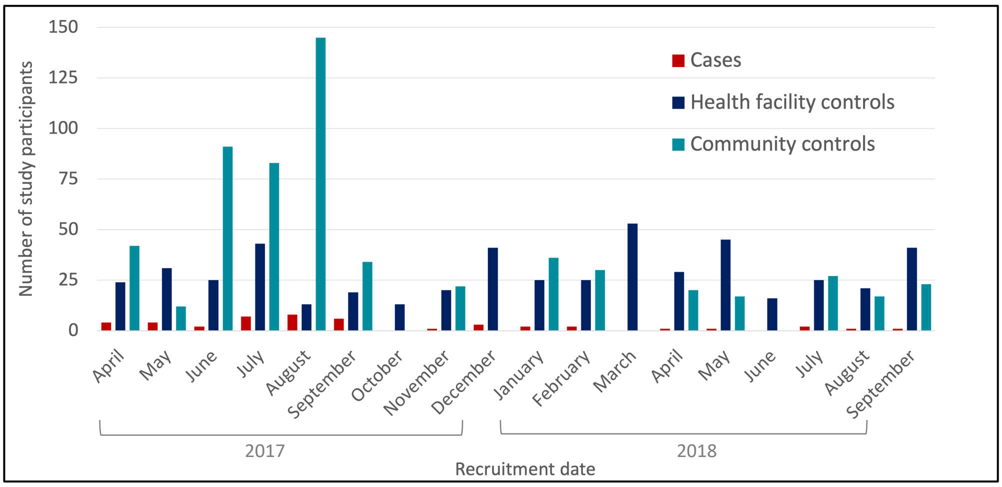

Figure 2.2: *Temporal recruitment of cases, health facility controls, and community controls between April 2017 and September 2018*

Table 2.1: *Distribution of potential risk factors among index cases, health facility controls, and community controls. HF Health Facility. *Socioeconomic status (SES) measure: represented as the lowest quartile of the first component of the principal component analysis (PCA)*

	Variable	Index cases n=45 (%)	HF controls n=509 (%)	Community controls n=599 (%)	Total n=1,153 (%)
Demographic variables	Age category (years)				
	15-29	9 (20%)	141 (28%)	201 (34%)	351 (30%)
	30-45	26 (58%)	264 (52%)	298 (50%)	588 (51%)
	>45	10 (22%)	104 (20%)	100 (17%)	214 (19%)
	Male	42 (93%)	461 (91%)	248 (41%)	751 (65%)
	Post-elementary or higher education	27 (60%)	370 (73%)	426 (71%)	823 (71%)
	Occupation				
	Farming	14 (31%)	197 (39%)	277 (46%)	488 (42%)
	Logging, mining, or other outdoor labor	26 (58%)	136 (27%)	27 (5%)	189 (16%)
	Other	5 (11%)	176 (35%)	295 (49%)	476 (41%)
	Lowest quartile SES*	13 (29%)	110 (22%)	175 (29%)	298 (26%)
	Subdistrict				
	Krueng Sabe	19 (42%)	339 (67%)	205 (34%)	563 (49%)
	Kuta Cotgli	2 (4%)	36 (7%)	19 (3%)	57 (5%)
	Lhoong	12 (27%)	86 (17%)	235 (39%)	333 (29%)
	Sampoiniet	3 (7%)	9 (2%)	51 (9%)	63 (5%)
	Saree	9 (20%)	39 (8%)	89 (15%)	137 (12%)
Community	Slept under net the previous night	23 (51%)	361 (71%)	317 (53%)	701 (61%)
	Main residence ever sprayed	4 (9%)	31 (6%)	115 (19%)	150 (13%)
	Main residence is traditional house	9 (20%)	87 (17%)	57 (10%)	153 (13%)
Forest exposures	Slept in the forest in the past 60 days	39 (87%)	97 (19%)	33 (6%)	169 (15%)
	Second residence in the forest	29 (64%)	24 (5%)	24 (4%)	77 (7%)
	Frequency of monkey sightings				
	None	20 (44%)	402 (79%)	340 (57%)	762 (66%)
	Less than once a day	15 (33%)	86 (17%)	200 (33%)	301 (26%)
	At least once a day	10 (22%)	21 (4%)	59 (10%)	90 (8%)
	Family member with forest exposure	6 (13%)	9 (2%)	40 (7%)	55 (5%)

The distributions of demographic, community, and forest exposure information for participants are presented in (Table 2.1). The majority of cases were male (93%) and between 30-45 years old (58%), which was similar to frequency matched facility controls (91 and 52%). Community controls had a more balanced age distribution (41% male) and were similarly aged (50% between 30-45 years old). Cases were more likely to work in logging, mining, or other outdoor jobs (58%) compared to both health facility and community controls (27 and 5%) and had a similar or lower education level and SES compared to controls.

In the category of community exposures, cases reported a lower or similar prior night net use than health facility and community controls (51% vs. 71 and 53%). Most cases (91%) and controls (94 and 81%) reported their main residence had never been sprayed with IRS or unknown spray status. A minority of cases (20%) and controls (17 and 10%) reported living in a traditional house with wooden walls.

Forest exposures strongly differentiated case and control populations. Most cases (89% of *P. knowlesi* cases and 88% of *P. vivax* cases) reported working and sleeping in the forest in the last sixty days (Supplementary Table), compared to less than 20% of controls. Furthermore, 64% of cases reported having a second residence in the forest or forest fringe, compared to a small minority of controls (5 and 4%). Cases were also more likely to report frequent encounters with monkeys (at least once a day) and having a family member with a forest exposure.

Table 2.2: *Unadjusted and adjusted odds ratios for bivariate and multivariable unconditional logistic regression (health facility controls) and conditional logistic regression (community controls). OR unadjusted odds ratio, aOR adjusted odds ratio, CI confidence interval, IRS indoor residual spraying, SES Socioeconomic Status. *Socioeconomic measure is the second component of the principal component analysis (PCA) for health facility controls and the first component of the PCA for community controls, included as a continuous measure.*

	Variable	Health Facility Controls				Community Controls			
		OR (95% CI)	P value	aOR (95% CI)	P value	OR (95% CI)	P value	aOR (95% CI)	P value
Demographic variables	Gender					Female as reference			
	Male	—	—	—	—	10.38 (3.09-34.85)	<0.0001	1.25 (0.19-7.99)	0.81
	Age categories								
	15-29	Reference		—	—	Reference		—	—
	30 - 45	1.50 (0.66-3.40)	0.33	—	—	1.59 (0.60-4.22)	0.35	—	—
	45 - 59	1.33 (0.49-3.61)	0.57	—	—	1.12 (0.30-4.21)	0.87	—	—
	Education								
	None or elementary	Reference		Reference		Reference		Reference	
	Post-elementary or higher	0.45 (0.23-0.89)	0.022	0.98 (0.40-2.40)	1.00	0.56 (0.21-1.53)	0.26	0.51 (0.08-3.45)	0.49
	Occupation								
	Farming	Reference		Reference		Reference		Reference	
	Logging, mining, or other outdoors	4.59 (2.07-10.18)	<0.0001	1.20 (0.41-3.53)	0.73	32.90 (8.23-131.51)	<0.0001	12.04 (0.89-162.5)	0.061
	Other	0.54 (0.18-1.63)	0.27	1.10 (0.26-4.75)	0.90	0.43 (0.12-1.50)	0.19	0.99 (0.12-8.26)	0.99
	SES								
	Higher SES*	0.80 (0.65-0.99)	0.044	0.76 (0.57-1.02)	0.068	1.46 (1.06-2.00)	0.020	1.66 (0.95-2.89)	0.073
Community Exposures	Net use prior night								
	No	Reference		Reference		Reference		—	—
	Yes	0.47 (0.24-0.92)	0.026	1.70 (0.71-4.12)	0.24	1.99 (0.70-5.68)	0.20	—	—
	Household sprayed with IRS								
	Never sprayed or do not know	Reference		—	—	Reference		—	—
	Ever sprayed	0.61 (0.16-2.33)	0.47	—	—	0.36 (0.08-1.54)	0.17	—	—
	Traditional house								
	No	Reference		—	—	Reference		Reference	
	Yes	1.36 (0.57-3.20)	0.49	—	—	1.55 (0.48-5.06)	0.46	0.13 (0.01-1.64)	0.120
Forest exposures	Sleep in the forest								
	No	Reference		Reference		Reference		Reference	
	Yes	60.01 (17.34-207.8)	<0.0001	21.66 (5.09-92.26)	<0.0001	78.02 (22.68-268.4)	<0.0001	16.78 (2.19-128.7)	0.007
	Second residence in forest								
	None or not in forest	Reference		Reference		Reference		Reference	
	In forest/fringe	34.12 (15.61-74.53)	<0.0001	6.29 (2.29-17.31)	<0.0001	72.75 (20.86-253.7)	<0.0001	13.53 (2.10-87.12)	0.006
	Frequency of monkey sightings								
	None	Reference		Reference		Reference		—	—
	Less than once a day	3.31 (1.59-6.88)	0.001	1.19 (0.45-3.14)	0.72	1.40 (0.34-5.69)	0.64	—	—
	At least once a day	8.49 (3.15-22.86)	<0.0001	1.16 (0.32-4.15)	0.81	2.70 (0.49-14.83)	0.25	—	—
	Family member with forest exposure								
	No	Reference		Reference		Reference		Reference	
	Yes	9.19 (2.84-29.75)	<0.0001	4.16 (0.69-25.11)	0.12	2.53 (0.60-10.62)	0.21	0.31 (0.03-2.90)	0.31

Risk Factors for Malaria

Table 2.2 presents unadjusted and adjusted odds ratios with 95% confidence intervals for potential risk factors among health facility controls and community controls.

Demographic risk factors

The adjusted odds of malaria among participants who work in logging, mining, or another outdoor job were higher than those who work in farming in both control series; this relationship was stronger and approached significance among the community control series (aOR 12.04 95% CI 0.89-162.5) (Table 2.2). While higher SES was associated with lower odds of malaria among the health facility controls (aOR 0.76 95% CI 0.57-1.02), higher SES was associated with higher odds of malaria among the community controls (aOR 1.66 95% CI 0.95-2.89).

Community exposures

The findings suggest that community exposures at the main residence of the participants were not independent risk factors for malaria after controlling for other covariates; these include net use the prior night, main residence sprayed with IRS, and living in a traditional house with wooden walls or no walls. In the unadjusted analysis using health facility controls, net use the prior night was associated with half the risk of malaria (OR 0.47 95% CI 0.24-0.92). However, this relationship did not remain present in the adjusted model nor among the community control series (Table 2).

Forest exposures

Forest exposures were consistently and strongly associated with risk in this population. These exposures included sleeping in the forest and having a second residence in the forest or forest fringe. Participants who reported working and sleeping in the forest in the last sixty days had 21.66 times the adjusted odds of malaria (95% CI 5.09-92.26) than those with no forest exposure, using health facility controls. Similar results were found using community controls (OR 16.78 95% CI 2.19-128.7). Those who reported having a second residence in the forest/forest fringe had 6.29 times the adjusted odds of malaria (95% CI 2.29-17.31) compared to those who did not have a second residence in the forest (using health facility controls; OR 13.53, 95% CI 2.10-87.12 using community controls).

A higher frequency of monkey sightings and having a family member with a forest exposure were associated with higher odds of malaria in the unadjusted analysis among health facility controls, but not in the adjusted analysis with either control series.

Sub-group Analysis of Forest Goers

The subgroup analysis of forest goers who reported working and sleeping in the forest in the past sixty days included 37 cases and 92 health facility controls (Supplementary Table 2), all of whom were male and the majority aged 30-45 years (57% of cases and 53% of controls). Amongst forest-going cases, 59% were *P. knowlesi* and 41% *P. vivax*. The two subdistricts in Aceh Jaya district (Krueng Sabe and Sampoinet) had a greater proportion of *P. vivax* cases, while two of the three districts in Aceh Besar had 100% *P. knowlesi* cases (Figure 2.3). Other characteristics in terms of education and SES were similar amongst both cases and controls. There was one key finding in the adjusted subgroup analysis: those who reported having a second residence in the forest had 8.8 times the adjusted odds (95% CI 2.4-32.3, $p<0.01$) of malaria than those who did not have a second residence in the forest. Among those who had a second residence in the forest, eighty percent reported the main reason was to be close to a work location and coverage of interventions was uniformly low (discussed below).

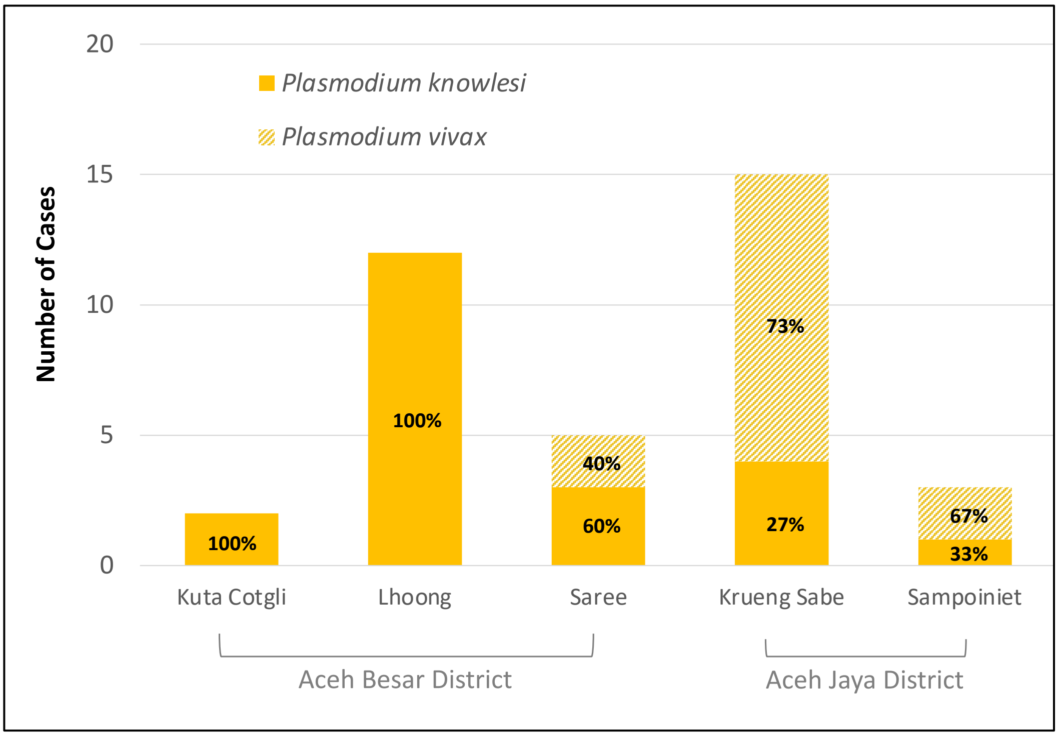

Figure 2.3: *Species of malaria parasite among forest goers by subdistrict*

Occupation differed between forest-going cases and controls (p<0.001): 51% of cases and 82% of controls worked in logging or mining while 38% and 10% worked in farming or plantation work (Supplementary Table 2). There were four occupations in the "other outdoor labor" category that appeared only for cases including elephant work, entomology, hole digger/ rattan collector, and forest ranger. Sleeping structures differed between cases and controls (p<0.001); the most common sleeping structure among cases was a hut (62%) and the most common sleeping structure among health facility controls was a tent (42%). Forest-going miners more commonly slept in barracks or tents while loggers reported sleeping in huts and tents.

Forest travel of both cases and controls were mainly confined to their home subdistrict (92% and 98%) although cases reported staying longer in the forest (49% and 26% over two weeks, p<0.05). Forest worksite locations ranged widely and were on average twelve kilometers away from the nearest road (min: 1, max: 60, median: 10); overall a higher percentage of controls reported a forest location of more than 10 kilometers from the road ($p<0.05$).

Overall, seventy-six percent of forest goers reported using at least one effective protective measure against mosquitoes during this trip; 17% used a bed net, 33% used mosquito repellant or coil, and 50% wore covering clothes (Supplementary Table 2). Only one person reported using a hammock net while sleeping in the forest and none of the forest goers reported use of chemoprophylaxis. It was common to have zero bed nets at the second residence (83% of cases and 73% of controls) and 100% of forest goers with a second residence reported that it had never been sprayed (85%) or they did not know if it had ever been sprayed (15%). The households at the second residences were primarily open construction, e.g., 69% had gaps in between the roof and walls of the structure. A higher percentage of cases (54%) than controls (17%) reported seeing monkeys at least once a day around the forest location ($p<0.001$); it was also more common among *P. knowlesi* cases than *P. vivax* cases to report monkey sightings at least once a day.

Discussion

The key findings from this study support the understanding that forest-going men are at highest risk of malaria in Aceh Province, Indonesia and provide specific details that can aid the design of tailored surveillance and response strategies targeting forest malaria. Cases had twenty-two times the odds of working and sleeping in the forest and six times the odds of having a second residence in the forest than controls (HF control series). Community exposures (e.g., prior night net use at main residence) were not salient risk factors; consistent with exposures that are mainly occurring in the forest. The findings in this study are relevant to other countries nearing elimination in Asia and Latin America, where forest-going is often a risk factor for malaria infection [22,24,59,78].

Our findings are well aligned with literature in Indonesia and greater Southeast Asia that has identified high-risk populations as predominantly adult males who are exposed to outdoor biting mosquitoes via work that brings them to the forest or forest fringe for multiple days at a time [56,63,79]. However, there is heterogeneity within this broad group and a better understanding of subgroups, and their exposure gaps will improve targeted interventions. This study identified specific characteristics of forest-going groups associated with increased risk of malaria, including having a second residence in the forest and working in occupations such as logging, mining, and farming. In addition, we highlight the heterogeneity in forest-going populations in terms of occupation, sleeping and travel habits, worksite characteristics, and malaria parasite species, which reinforces a need for tailored intervention approaches to address forest transmission. With the potential for differing risk factors between *P. vivax* and *P. knowlesi*, gaps in the use of prevention among forest goers, and clear diversity amongst forest-going populations, these results can help to distinguish groups and prioritize behaviors and intervention targets to be explored in designing appropriate and acceptable interventions for these high-risk populations.

A critical finding of this study is that second residences in the forest were common among forest goers, associated with higher risk of malaria, and had a low coverage of malaria interventions. Among the full study population with both control series and in the sub-group analysis of forest goers, those who reported having a second residence in the forest had significantly higher odds of malaria than those who did not. Anecdotal information and some limited research suggest that forest goers in Aceh and wider Southeast Asia often sleep in makeshift or simple housing away from their primary residence [56,63,79]; this study provides evidence that having a second residence in the forest is a risk factor for malaria. Because participants reported that these residences have not been sprayed with IRS and there is low usage of bed nets, these second residences provide key vector control intervention targets that may help address transmission among those at highest risk of infection.

Male forest goers in this study population were a heterogenous group: their occupations varied across farming, logging, and mining, they slept in a variety of settings including barracks, huts, or tents, and they may have worked in the forest alone or at a worksite with a large group. The majority (98%) of forest-goers travelled and worked in a group, which represents a potential opportunity to access these populations through peer networks and/or at worksites. The group size of forest-going farmers and loggers was generally smaller (17% of farmers reported one other worker, 65% of farmers and 83% of loggers reported 2-5 other workers) than amongst miners, in whom it was equally common for the group to be less than five or from five to fifty people. Forest-goers were also diverse in age, level of education, and socioeconomic status. This is consistent with previous research describing the heterogeneity of forest goers in Aceh and Southeast Asia [17,26,56,79,80]. These diverse sub-groups require tailored strategies: our findings indicate specific intervention points to target these high-risk populations and the potential to use network structure to improve outreach and access.

The majority of forest goers slept in a structure such as a barrack or hut when working in the forest, and only 17% reported using bed nets (one of whom used a hammock net). Studies in other countries in Asia Pacific have found hammock nets to be acceptable to forest goers; hammock nets should be piloted in Aceh with close attention to acceptability, feasibility, and effectiveness [65,81]. There were large gaps in utilization of mosquito repellants and coils or wearing covering clothes (i.e., clothes that cover arms and legs). The gaps identified present intervention points where forest goers could be targeted with forest packs with nets and repellants along with education on malaria protective measures [17,23]. Additionally, the 37% of worksites with groups of five or more could be targeted with reactive case detection (RACD) and social and behavior change communication (SBCC) interventions by community health workers, or screening posts at places such as transportation hubs that can provide intervention packages, malaria case management, and gather traveler information routes [17,68]. Though RACD is widely adopted in malaria elimination settings, more information is needed on the effectiveness of RACD among forest-going populations in terms of case-finding and impact on transmission [9,82,83]; one study was conducted recently in Aceh to aid in answering these questions [Bennett et al, *manuscript in preparation*]. Chemoprophylaxis was not used by any forest goers; recent studies have shown that chemoprophylaxis can be effective and acceptable for reducing malaria among forest-going populations in Southeast Asia [59,84,85].

Most cases in this study were *P. knowlesi,* a simian malaria parasite that was recently identified in Aceh Province by Herdiana et al [86]. Our descriptive findings suggest that risk factors for *P. knowlesi* may differ and require specific prevention strategies than *P. vivax.* Forest-goers with *P. knowlesi* were more likely to have a second residence in the forest than those with *P. vivax*, were less likely to report using protective measures against mosquitos in the forest, and more commonly slept in huts, worked in farming, and reported sightings of monkeys. Other studies have noted that risk factors for *P. knowlesi* may differ: Grigg et al found that farming occupation increased risk for *P. knowlesi* infection but not risk of other *Plasmodium* species in Malaysia [26], and Herdiana et al reported species specific differences in Aceh, Indonesia [62]. A growing body of literature suggests that effective strategies to deal with this parasite species likely differ from approaches used for *P. vivax* and *P. falciparum* [87-

[90]. One of the areas in which novel tools are needed is diagnostics for *P. knowlesi*; studies have found laboratory difficulties around species distinction which can lead to misdiagnosis [64,91], which has important implications for appropriate treatment regimens [92]. We did not find evidence that increased number of monkey sightings was associated with higher odds of malaria but note that monkey sightings during trips to the forest were universal among forest-goers and individuals positive for *P. knowlesi* reported more frequent monkey sightings than those with *P. vivax*. More research is needed on addressing the zoonotic reservoir to reduce spillover and understanding the behavior of vectors of *P. knowlesi* given that the importance of *P. knowlesi* is likely to increase as other malaria species decline in the region and coincide with the combination of relaxed control measures, possible waning cross-species immunity, and continued encroachment into forested areas where the zoonotic reservoir resides [59,89].

This study had several limitations. The first is recall bias for self-reported measures; for example, individuals who tested positive might have been more likely to remember forest-going behavior. Second, cases were detected passively and therefore some high-risk populations who do not seek care at health facilities or malaria cases that spontaneously resolved would have been missed. Third, statistical power was limited by the small number of cases during the data collection period, leading to wide confidence intervals and a need to consolidate categories to avoid small cells. The limited cases were potentially due in part to mining restrictions and a logging ban in early 2018 but also likely reflect declining transmission. Due to this low number of cases, it was not possible to do a subgroup analysis by species. The strengths of the study include two sets of control series to address different types of bias and the results with both series of controls were similar, adding to the evidence to support the conclusions of this study. Implementation via routine surveillance was another strength: the study was conducted through the provincial national malaria program in Aceh Province, including the subdistrict health facilities and health facility staff.

The final persistent pockets of transmission among high-risk populations will be the hardest to eliminate [59,60]. We identify malaria risk factors in Aceh Indonesia that represent exposure gaps and intervention targets that should inform collaborative design and evaluation of acceptable interventions in an iterative fashion to halt transmission [45]. Preventing the continued rise of *P. knowlesi* will also require iterative work including the collection and incorporation of entomological surveillance data to complement epidemiologic data and inform in more detail the design of effective vector control interventions.

Conclusions

Malaria high-risk populations in Aceh, Indonesia are predominantly forest-goers who are a diverse population; the findings of this study delineate sub-groups among forest-goers and outline gaps to be targeted with interventions. The recommended interventions target second residences in the forest and aim to complement forest-going behavior. This study also highlights the threat of *P. knowlesi*, which has increased in priority and threatens to reverse many years of progress towards malaria elimination in Indonesia and Southeast Asia. Additional research on effective methods for preventing and mitigating this parasite species among forest-goers will be a key challenge. Rigorous targeting of effective interventions to these populations is essential to achieve malaria elimination in Indonesia and other settings challenged by forest malaria.

Supplementary information

Supplementary Table 1: *Distribution of demographic variables of cases by species. *Socioeconomic status (SES) measure: represented as the lowest quartile of the first component of the principal analysis.*

	Variable	*P. knowlesi* N=27 (%)	*P. vivax* N=17 (%)	Total N=44 (%)	p-value
Demographic variables	Age Category (years)				0.2
	15-29	5 (19%)	4 (24%)	9 (20%)	
	30-45	18 (67%)	7 (41%)	25 (57%)	
	>45	4 (15%)	6 (35%)	10 (23%)	
	Male	24 (89%)	17 (100%)	41 (93%)	0.15
	Post-elementary or higher	18 (67%)	9 (53%)	27 (61%)	0.36
	Occupation				0.26
	Farming	11 (41%)	3 (18%)	14 (32%)	
	Logging, mining, or other outdoor labor	13 (48%)	12 (71%)	25 (57%)	
	Other	3 (11%)	2 (12%)	5 (11%)	
	Lowest quartile SES*	9 (33%)	4 (24%)	13 (30%)	0.49
	Subdistrict				<0.001
	Krueng Sabe	5 (19%)	13 (76%)	18 (41%)	
	Kuta Cotgli	2 (7%)	0 (0%)	2 (5%)	
	Lhoong	12 (44%)	0 (0%)	12 (27%)	
	Sampoiniet	1 (4%)	2 (12%)	3 (7%)	
	Saree	7 (26%)	2 (12%)	9 (20%)	
Comm-unity	Slept under net the previous night	12 (44%)	10 (59%)	22 (50%)	0.35
	Main residence ever sprayed	2 (7%)	2 (12%)	4 (9%)	0.62
	Main residence is traditional house	3 (11%)	5 (29%)	8 (18%)	
Forest exposures	Slept in the forest in the past 60 days	24 (89%)	15 (88%)	39 (89%)	0.95
	Second residence in the forest	19 (70%)	10 (59%)	29 (66%)	0.43
	Frequency of monkey sightings				0.55
	None	13 (48%)	7 (41%)	20 (45%)	
	Less than once a day	7 (26%)	7 (41%)	14 (32%)	
	At least once a day	7 (26%)	3 (18%)	10 (23%)	
	Family member with forest exposure	6 (22%)	0 (0%)	6 (14%)	0.036

Supplementary Table 2*: Distribution of potential risk factors among forest-goers. *Socioeconomic status (SES) measure: represented as the lowest quartile of the first component of the principal component analysis (PCA). **Occupation categories grouped differently than in main analysis (all occupations were outdoor occupations).*

Variable	Index case n=37 (%)	Health facility controls n=92 (%)	p value
Age category (years)			0.93
15-29	8 (22%)	21 (23%)	
30-45	21 (57%)	49 (53%)	
> 45	8 (22%)	22 (24%)	
Lowest quartile SES*	11 (30%)	24 (26%)	0.67
Post-elementary or higher education	24 (65%)	47 (51%)	0.15
Subdistrict			<0.001
Krueng Sabe	15 (41%)	78 (85%)	
Kuta Cotgli	2 (5%)	4 (4%)	
Lhoong	12 (32%)	2 (2%)	
Sampoiniet	3 (8%)	4 (4%)	
Saree	5 (14%)	4 (4%)	
Trip destination in the same subdistrict	34 (92%)	90 (98%)	0.11
Number of nights in the forest			0.044
< 7	11 (30%)	43 (47%)	
8 to 15	8 (22%)	25 (27%)	
> 15	18 (49%)	24 (26%)	
Distance to nearest road (km)			0.011
1 km or less	6 (16%)	8 (9%)	
1-10 km	15 (41%)	18 (20%)	
10-20 km	10 (27%)	53 (58%)	
>20 km	6 (16%)	13 (14%)	
Sleep structure			<0.001
Barracks	7 (19%)	28 (30%)	
Hut/Other	23 (62%)	25 (27%)	
Tent	7 (19%)	39 (42%)	
Type of forest work**			<0.001
Farming or plantation work	14 (38%)	9 (10%)	
Logging or mining	19 (51%)	75 (82%)	
Other outdoor occupation	4 (11%)	8 (9%)	
Number of other workers			0.62
0-5 other workers	25 (68%)	56 (61%)	
5-10 other workers	7 (19%)	25 (27%)	
>10 other workers	5 (14%)	11 (12%)	
Frequency of monkey sightings in the forest			<0.001
At least once a day	20 (54%)	16 (17%)	
Less than once a day	15 (41%)	75 (82%)	
Missing	2 (5%)	1 (1%)	
Standing water present	32 (86%)	79 (86%)	0.93
Mosquitos present	36 (97%)	91 (99%)	0.5
Use protection against mosquitoes	26 (70%)	72 (78%)	0.34
Used bed net	4 (11%)	18 (20%)	0.23
Used mosquito repellant or coil	12 (32%)	31 (34%)	0.89
Wore covering clothes	18 (49%)	47 (51%)	0.8
Second residence in the forest	27 (73%)	21 (23%)	<0.001

Chapter III

Forest-going as a risk factor for confirmed malaria cases in Champasak Province, Lao PDR

Abstract

Background: Lao People's Democratic Republic (Lao PDR) has made significant progress in reducing malaria in recent years and is targeting malaria elimination by 2030. The aim of this study was to utilize routine surveillance data to assess risk factors for malaria cases in a relatively higher burden province of Lao PDR.

Methods: Routine passive surveillance data were extracted from health facilities in three districts of Champasak Province from August 2017 to December 2018; additionally, at the time of presentation, fever cases were asked to report any recent forest travel. The full dataset includes malaria parasite test results, demographic information (age, gender, occupation), date, and forest-going frequency. Multivariable logistic regression was used to assess the relationship between forest-going and malaria infection while controlling for other covariates.

Results: Of 2,933 fever cases with data available on forest-sleeping and malaria parasite diagnosis from twenty-five health facilities, 244 (8%) tested positive; among positives, 82% were male and 88% were older than fifteen. Forty-four percent of malaria cases were *Plasmodium falciparum* and 56% were *Plasmodium vivax.* All but one case (99.6%) and 71% of malaria negative patients reported sleeping at least one night in the forest in the past thirty days. Compared with sleeping 0-2 nights in the forest in the last thirty days, sleeping 3-7 nights in the forest was associated with 9.7 times the odds of malaria infection (95% CI: 4.67-20.31, $P<0.001$) when adjusting for gender, occupation, and season.

Conclusions: Forest-going, especially longer trips, is associated with increased risk for malaria in Southern Lao PDR. Targeted intervention efforts are needed to protect this population and keep Lao PDR on the path to elimination.

Introduction

The Greater Mekong Subregion (GMS), including Cambodia, Lao PDR, Myanmar, Thailand, Vietnam and the Yunnan province of China, has become a focal point of global malaria work in recent years because drug resistance to Artemisinin-based combination therapies (ACTs) has spread in the subregion and threatens global malaria control efforts; making malaria elimination urgent and critical [93]. Despite these challenges, the countries in the region reduced the regional reported cases of malaria by 74% between 2012-2018 [94]. Lao PDR has made significant progress in reducing the malaria burden in recent years; confirmed cases fell 81% from 46,202 in 2012 to 8,913 in 2018[95]. In the context of increasing resistance to ACTs in the subregion, the country aims to eliminate all species of malaria by 2030.

In the malaria elimination setting of the GMS, there is substantial evidence that the population at greatest risk for malaria is adult males who travel and work in forested areas [6,42,61,96]. These individuals are often exposed to the predominantly outdoor-biting, forest-adapted malaria vectors in the region through work such as logging, agriculture, foraging, and industrial work [61]. Traditional malaria control interventions such as long-lasting insecticide-treated nets (LLINs) and indoor residual spraying (IRS) do not protect individuals who sleep outside at night, and long-lasting insecticide treated hammocks (LLIHs) are often not widely available [97,98]. Additionally, some of these workers are highly mobile or migrant, which often leads to decreased access to healthcare [81,99]. It has been a continuing challenge in the region to understand the best interventions to protect these populations from malaria transmission. Determining who is at risk, characterizing the populations in more detail, and assessing which interventions are most effective for them are priorities for elimination [100].

In Lao PDR, forest-based activities are a major part of the economy in many places, and between 2009 and 2014 (during a period of heavy forest activity and a major outbreak), the proportion of reported malaria cases among males five and older increased from 46% to 86%, suggesting the occurrence of occupational exposures [96]. Despite the growing consensus in the GMS that adult forest-going men are at the highest risk for malaria, it is still essential to delineate specific sub-regional patterns and risk factors. A better understanding of the role of sleeping in the forest on malaria risk, as compared to the role of village-based risk could provide improved evidence for choosing targeted interventions to minimize malaria transmission in Lao PDR. This study sought to assess whether sleeping in the forest was a risk factor for confirmed malaria cases diagnosed through the health system in a relatively higher burden province of Southern Lao PDR.

In this study, routinely collected malaria surveillance data were augmented to include potential risk factors related to forest-going in order to determine if there was an association between sleeping in the forest and malaria risk in Champasak Province, Lao PDR.

Methodology

Study Area

Champasak Province is located in southern Lao PDR and is bordered by Thailand, Cambodia, and three other provinces in Lao PDR (**Figure 3.1**); as of 2018, 95% of the country's malaria burden occurs in the five southernmost provinces in Lao PDR [101]. Champasak is a heavily forested province that is moderately mountainous with an economy mainly reliant on agricultural and forest-based work [102]. The study area included three districts: Mounlapamook, Panthampone, and Sanamsaboun, which were selected for a randomized controlled trial (RCT) to assess the effectiveness of active case detection in southern Lao PDR [103]. In 2015, the respective population and annual parasite indices (API) of Mounlapamook, Panthampone, and Sanamsaboun were 38,800; 61,252; 69,338 and 66.8; 83.0; and 26.8 per 1,000 population.

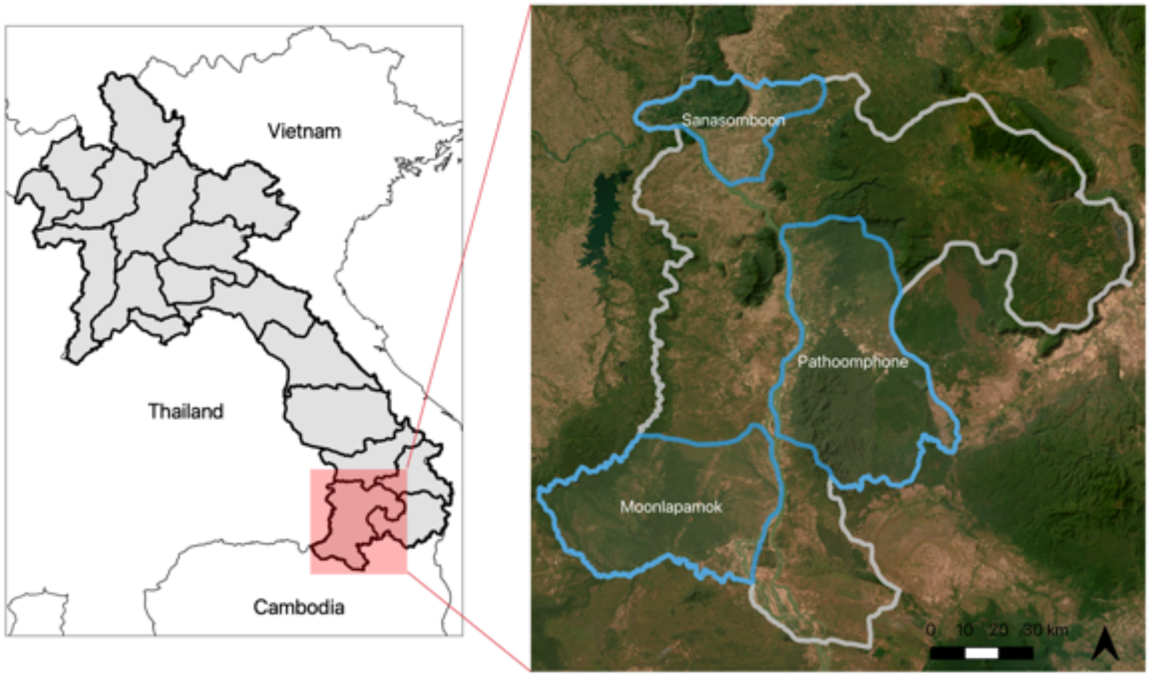

Figure 3.1: *Three study districts in Champasak Province, Lao PDR*

Study Design

During the study period of the RCT on active case detection [103], routine malaria surveillance data were augmented to include additional variables related to forest behavior. All patients with suspected malaria and who received a malaria test at twenty-five health facilities were included in the study. The study population was drawn from suspected patients tested for malaria at twenty-five selected health facilities that were part of the study in three districts. Patients that tested positive for malaria by rapid diagnostic test (RDT) or microscopy were considered a case; patients that tested negative for malaria by RDT or microscopy were considered a non-case.

Data Collection

Outpatient register data from the paper-based passive malaria surveillance system were collected from twenty-five health centers for the period between August 2017 and December 2018. Participants were those who visited health facilities in three districts of Champasak province and were tested for malaria with a diagnostic test (RDT or microscopy). The registries included data on date of diagnosis, species-specific test results, demographic information (age, gender, occupation), village of residence, and the added questions on forest-going behavior (number of days slept in the forest in the past thirty days, suspected place infected with malaria, and travel destination in the past thirty days).

Data Processing

Data cleaning and analysis were conducted using STATA 16.1 and R 4.1.2. Participants were included if they had a malaria test result and available data on the main variable of sleeping in the forest. One percent of participants were missing demographic information and removed from the analysis. One mixed-infection malaria case was included with the *Plasmodium falciparum* species category. Categories within variables that were conceptually similar were recombined to avoid cells smaller than five (e.g., age group and occupation). Occupation was grouped into three groups: farmer, student/child, and other. Other included the categories of housewife, employee, monk, and retired. A categorization of occupation with a larger number of groups was not possible due to small cell sizes. An analysis comparing sleeping in the forest to sleeping no nights in the forest was not possible due to small cell sizes (only one malaria case slept no nights in the forest, so sleeping 0-2 nights was the reference). Seasons were defined as dry season (November – May) and monsoon season (June – October)[104].

Data Analysis

Associations between risk factors and the outcome of malaria infection were explored using unconditional logistic regression with a clustering term at the health facility level. Model building was informed by a directed acyclic graph (DAG) conceptualizing relationships between the forest exposure (sleeping in the forest), the outcome of malaria infection, and potential confounding variables [77]. We then assessed the bivariate relationship of the variables identified as potential confounders (age, gender, occupation) between the main exposure and the outcome. Crude odds ratios (ORs) with 95% confidence intervals were estimated in bivariate logistic regression; variables associated with the outcome (significant at p-value 0.05) or those that changed the main effect OR by more than 10% in the final model were included. The same analysis approach was used to assess the outcomes of *P. falciparum* and *P. vivax* separately. A sensitivity analysis was carried out to evaluate the potential impacts of differences between malaria positive and malaria negative patients in the number of individuals missing responses for forest-sleeping.

Results

Between August 2017 and December 2018, 7,111 patients with a malaria test result were captured by routine surveillance in the three study area districts. 3,306 (24% of cases and 55% of controls) were missing a response for the variable number of nights spent sleeping in the forest in the past thirty days; these were dropped from the analysis. 2,933 patients were included in the analysis: 244 (8%) were positive for malaria. Among malaria positive cases, 44% were *P. falciparum,* 56% were *P. vivax*, and one case was a mixed infection. The distribution of potential risk factors for malaria among participants is presented in **Table 3.1**.

Table 3.1: *Characteristics of patients who visited health centers in three districts of Champasak, Lao PDR during the study period and had data available on forest-going. Percentages are row percentages to depict test positivity prevalence by group. The one mixed infection case was grouped in the* P. falciparum *category.*

Variable	**Overall**	**Malaria Negative**	**Malaria Positive**		
			All Positive	***P. falciparum***	***P. vivax***
	N=2,933	**N=2,689**	**N=244**	**N=108**	**N=136**
District					
Mounlapamoak	691	651 (94%)	40 (6%)	9 (1%)	30 (4%)
Pathoumphone	1,474	1,302 (88%)	172 (12%)	98 (7%)	74 (5%)
Sanasomboun	768	736 (96%)	32 (4%)	0 (0%)	32 (4%)
Age group					
1-15 years	425	396 (93%)	29 (7%)	7 (2%)	22 (5%)
> 15 years	2,508	2,293 (91%)	215 (9%)	100 (4%)	114 (5%)
Gender					
Male	1,984	1,783 (90%)	201 (10%)	86 (4%)	115 (6%)
Female	949	906 (95%)	43 (5%)	21 (2%)	21 (2%)
Occupation					
Farmer	2,294	2,085 (91%)	209 (9%)	102 (4%)	106 (5%)
Student and/or child	511	485 (95%)	26 (5%)	4 (1%)	22 (4%)
Other	128	119 (93%)	9 (7%)	1 (1%)	8 (6%)
Nights in the forest					
0-2	1,724	1,695 (98%)	29 (2%)	12 (1%)	17 (1%)
3-7	1,041	894 (86%)	147 (14%)	66 (6%)	81 (8%)
8-14	139	83 (60%)	56 (40%)	25 (18%)	30 (22%)
> 14	29	17 (59%)	12 (41%)	4 (14%)	8 (28%)
Season					
Dry	1,678	1,508 (90%)	170 (10%)	98 (6%)	72 (4%)
Monsoon	1,255	1,181 (94%)	74 (6%)	9 (1%)	64 (5%)

The majority of participants were over fifteen years old (86%), male (82% of positives and 66% of negatives) and worked as farmers (78%) (**Table 3.1Error! Reference source not found.**). In Sanasomboun district, thirty-one out of thirty-two cases identified as a farmer and 100% of cases were over fifteen years old. Five participants under ten years old had malaria (one of them was under five). The occupation category of "other" included forty-nine participants who identified as housewives (all negative for malaria) and seventy-seven who identified their job as employee (12% were positive). Among cases, 72% of women and 92% of men were over fifteen (among negatives 80% and 87% respectively).

Only one person who was malaria positive (for *P. falciparum*) reported not sleeping in the forest during the last 30 days. Of patients with forest travel data available, 99.6% of positives and 71% of negatives reported sleeping at least one night in the forest in the last 30 days.

Average length of trip was similar among the under fifteen and over fifteen age groups and among men and women among both cases (average 6.4 nights in the forest, standard deviation: 4.7) and non-cases (average 2.3 nights in the forest, standard deviation: 2.6). As shown in Figure 3.2, the number of nights spent in the forest was similar on average during monsoon season (average: 3, SD: 3, min: 0, max: 30) compared to dry season (average 2.5, SD: 2.9, min:0, max: 16; $p<0.005$). The average number of nights spent in the forest did not differ significantly between cases of *P. falciparum* and *P. vivax* (**Table 3.1Error! Reference source not found.**); however, season did: 92% of *P. falciparum* cases occurred during dry season compared to 53% of *P. vivax* cases.

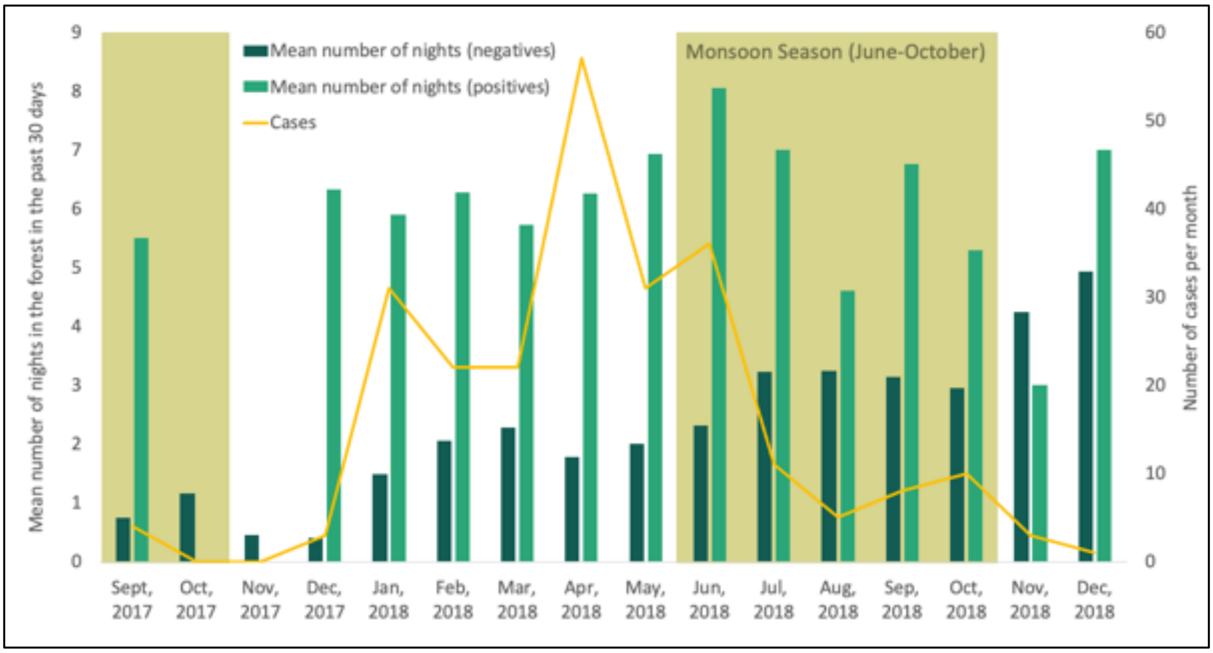

Figure 3.2: *Total cases and average number of nights spent in the forest in the past thirty days among participants who were positive or negative for malaria.*

Table 3.2: *Unadjusted and adjusted odds ratios for bivariate and multivariable logistic regression.*

All Species				
Variable	**OR (95% CI)**	**P value**	**aOR (95% CI)**	**P value**
Age group				
1-15 years	Reference		—	—
> 15 years	1.28 (0.81-2.01)	0.285	—	—
Gender				
Female	Reference		Reference	
Male	2.38 (1.36-4.15)	<0.005	2.23 (1.21-4.08)	<0.05
Occupation				
Farmer	Reference		Reference	
Student and/or child	0.53 (0.31-0.93)	<0.05	0.67 (0.34-1.31)	0.244
Other	0.75 (0.13-4.31)	0.75	0.73 (0.14-3.64)	0.705
Nights in the forest				
0-2	Reference		Reference	
3-7	9.61 (4.68-19.75)	<0.001	9.74 (4.67-20.31)	<0.001
8-14	39.43 (13.09-118.81)	<0.001	38.71 (12.42-120.67)	<0.001
> 14	41.26 (6.25-272.42)	<0.001	43.67 (5.92-322.33)	<0.001
Season				
Monsoon	Reference		Reference	
Dry	1.80 (0.88-3.66)	0.105	1.97 (1.04-3.70)	<0.05
P. falciparum				
Variable	**OR (95% CI)**	**P value**	**aOR (95% CI)**	**P value**
Age group				
1-15 years	Reference		—	—
> 15 years	2.49 (0.88-7.03)	0.085	—	—
Gender				
Female	Reference		Reference	
Male	1.98 (1.13-3.49)	<0.05	1.74 (0.97-3.11)	0.062
Occupation				
Farmer	Reference		Reference	
Student and/or child	0.17 (0.07-0.39)	<0.001	0.21 (0.08-0.53)	<0.005
Other	0.17 (0.03-0.88)	<0.05	0.18 (0.02-1.31)	0.091
Nights in the forest				
0-2	Reference		Reference	
3-7	10.43 (4.24-25.68)	<0.001	11.23 (4.49-28.06)	<0.001
8-14	44.25 (12.62-155.13)	<0.001	41.24 (10.43-163.03)	<0.001
> 14	33.24 (4.32-255.70)	<0.005	31.64 (4.13-242.74)	<0.005
Season				
Monsoon	Reference		Reference	
Dry	7.67 (3.41-17.26)	<0.001	8.7 (4.05-18.70)	<0.001
P. vivax				
Variable	**OR (95% CI)**	**P value**	**aOR (95% CI)**	**P value**
Age group				
1-15 years	Reference		—	—
> 15 years	0.89 (0.46-1.74)	0.743	—	—
Gender				
Female	Reference		Reference	
Male	2.78 (1.42-5.46)	<0.005	2.74 (1.34-5.62)	<0.01
Occupation				
Farmer	Reference		Reference	
Student and/or child	0.89 (0.44-1.80)	0.751	—	—
Other	1.32 (0.20-8.95)	0.775	—	—
Nights in the forest				
0-2	Reference		Reference	
3-7	9.03 (3.83-21.26)	<0.001	8.83 (3.78-20.60)	<0.001
8-14	36.04 (9.55-135.97)	<0.001	37.06 (9.64-142.53)	<0.001
> 14	46.92 (4.61-477.20)	<0.005	44.93 (4.18-482.52)	<0.005
Season				
Monsoon	Reference		Reference	
Dry	0.88 (0.46-1.70)	0.706	—	—

OR unadjusted odds ratio, *aOR* adjusted odds ratio, *CI* confidence interval, *IRS* indoor residual spraying

Compared with sleeping 0-2 nights in the forest in the past thirty days, sleeping 3-7 nights in the forest led to 9.74 times the odds of malaria (95% CI: 4.67-20.31, P<0.001) when adjusting for gender, occupation, and season (Table 3.2). The adjusted odds of malaria were increasingly higher for longer periods of sleeping in the forest, as shown in Table 3.2. The results from the sensitivity analysis suggested that these associations remained unchanged despite differences between cases and controls in the number of responses missing for forest-sleeping. Additionally, in the adjusted model, men had 2.23 (95% CI: 1.21-4.08, P<0.05) times the odds of malaria than women. Patients also had 1.97 (95% CI: 1.04-3.70, P<0.05) times the adjusted odds of getting malaria during dry season (November – May) than during monsoon season.

These trends remained similar when *P. falciparum* and *P. vivax* cases were analyzed separately in subgroup analyses. Those who spent more than two weeks sleeping in the forest in the past thirty days had 32 (95% CI: 4.13-242.74, P<0.005) times the adjusted odds of *P. falciparum* malaria and 45 (95% CI: 4.18-482.52, P<0.005) times the adjusted odds of *P. vivax* malaria than those who slept 0-2 nights in the forest (Table 3.2). Men had 1.74 (95% CI: 0.97-3.11, P<0.062) times the adjusted odds of *P. falciparum* malaria and 2.74 (95% CI: 1.34-5.62, P<0.01) times the adjusted odd of *P. vivax* malaria than women. While dry season was associated with higher odds of *P. falciparum* malaria than monsoon season (aOR: 8.8, 95% CI: 4.05-18.70, p<0.001), for *P. vivax* there was no association (Table 3.2).

Discussion

Sleeping in the forest, especially during longer trips, was associated with increased risk for symptomatic malaria infection in Southern Lao PDR: those who spent more than two weeks in the forest in the past thirty days had 44 times the adjusted odds of malaria (95% CI: 5.92-

322.33, P<0.001) than those who spent two nights or less in the forest. Men had twice the adjusted odds of malaria compared to women (95% CI: 1.21-4.08, P<0.05). These results are consistent with a body of literature suggesting that working-age males who are exposed to outdoor biting mosquitoes while taking extended trips to the forest are at high risk for malaria in the GMS [56,61,63,96,100,105]. Studies have found that forest-going behavior in Lao PDR includes logging, collecting timber or bamboo, hunting, foraging for food or plants, and agricultural work such as rice farming [102,104]. In this study, most participants identified their main occupation as farming. In the limited data available on reason for travel, participants reported going to the forest to cut wood or look for food or fruit. Of the forty-nine women who identified as housewives, none were positive for malaria, and of the sixty-nine children under five years old, only one was positive, which could suggest minimal community transmission of malaria in villages of the study area. Only one malaria case out of 244 did not spend any nights in the forest in the past thirty days; this supports the findings of another recent study on forest-goers in Lao PDR that suggests malaria control programs may have underestimated the magnitude of this population and might be better off targeting certain interventions to forest locations than village locations if it is possible to reach them [104]. In this study we found that trips to the forest were varied in duration and season; this is supported by literature that has noted the heterogeneity in patterns of travel among forest goers [106,107].

Another important finding of this study is the relevance of seasonality to malaria transmission in Lao PDR. Sleeping in the forest was associated with similar odds for both *P. falciparum* and *P. vivax*; however, dry season was associated with higher odds for *P. falciparum* than monsoon season while odds of *P. vivax* malaria were not higher during one season than the other. Additionally, 92% of *P. falciparum* cases were captured during the dry season compared to 53% of *P. vivax* cases. These findings could be explained by the relapsing behavior of *P.vivax* infections arising from the dormant liver stages [10], making it more difficult to determine when malaria was acquired by the patient. The higher risk of *P. falciparum* malaria during the dry season could be related to human behavior of forest-goers (e.g., they are likely able to go deeper into the forest when it is dry), though the relationships are difficult to untangle. In this study, the number of nights spent in the forest was longer during monsoon season than dry season and other studies have found that forest goers in Lao PDR may be more active during the monsoon season, e.g., drawn out to work and spend the night in rice fields [104]. However, the intensity of monsoon season can also lead to flooded and impassible roads, which could indicate less access to testing at health facilities during this season and could explain longer trips when mobility to go back-and-forth for brief stints is limited.

A goal of this study was to determine if risk factors for malaria in Champasak could be assessed utilizing routine surveillance data that had been augmented with additional forest-going questions. As routine data are regularly collected, utilizing them for research does not add additional work burdens to local staff. We determined that this type of study can be effective if the research aim is to confirm or reassess an individual risk factor that can be asked simply. However, for more detailed characterization of risk populations, a more in-depth questionnaire is needed, in this case more information on occupation, behavior, and travel destinations are needed. Future research could develop more detailed characterization of forest-going activities and delineate risk amongst forest-goers depending on risk level of forest-based activity in order to define intervention points [45,104,106].

One limitation of this study is that test positivity rates are highly dependent on treatment seeking behavior at public health facilities and quality of diagnostic testing practices. As a metric for malaria transmission, this indicator may be biased; for example, areas farther away from the clinic likely have less attendance and true incidence may be underestimated because not everyone will visit a public facility for febrile illness. Data on treatment seeking behavior and quality of diagnostic testing were not available to include in the models. Another limitation of this study was missing data: a large percentage of responses for the forest-going variable were missing, and more were missing for negatives than positives. It is likely that health facility workers were more concerned with gathering data on forest going for cases than for non-cases, which could lead to differential misclassification of the exposure. An additional limitation is that variables available for this analysis were limited to those available in Lao PDR's passive surveillance, system; therefore, additional confounding variables may exist that were not available for analysis. It was also not possible to adjust for environmental covariates due to limited geolocation data of the villages and poor quality of the open-response travel destination data.

There is ample evidence that forest-going behaviors have an impact on malaria transmission in the GMS[102]; the findings of this study show the higher risk of malaria associated with longer trips to the forest in Lao PDR and indicate the need for control measures that can be effective for preventing malaria among forest-going populations who spend extended periods of time in the forest. Studies in the region have explored alternative interventions for these populations such as insecticide treated hammocks, protective clothing, mosquito coils, and mosquito repellants [106,108-110]. Enhanced case finding may also be important to target this population, as individuals may be less able to seek care at health facilities; this could include mobile or border health units or community volunteers that are placed at large plantations or forest industry sites [17]. Chemoprophylaxis with anti-malarial medication is also a potential option; recent studies indicate that chemoprophylaxis can be effective and acceptable for forest-goers in Southeast Asia [59,84,85,106]. This study found that trips were highly heterogeneous in season, purpose, and length; this finding supports calls for interventions to be targeted based on the spatio-temporal variability in size and behavior of the forest-going populations [104,106,107].

Conclusion

Characterizing forest-going populations and the malaria risk associated with trips to the forest is essential to malaria elimination in the GMS. The large size of the forest-going populations, the diversity in the duration and nature of forest-going trips, and the hard-to-reach nature of these individuals pose large challenges for targeting these groups with effective interventions for malaria prevention and treatment. Studies utilizing routine passive surveillance data can be useful for confirming suspected risk factors for malaria; however, more extensive questionnaires administered during routine surveillance are needed to characterize forest-goers in enough detail to define intervention points, and additional approaches are needed to incorporate populations that do not reach healthcare at public facilities. Forest-going, especially longer trips, is associated with increased risk for malaria in Southern Lao PDR; targeted and novel control efforts are needed to protect populations that work and sleep in the forest.

Chapter IV

Feasibility and effectiveness of tailored interventions targeting two populations at high-risk of malaria in Senegal: Koranic school children and gold miners

Abstract

Senegal has made significant progress in reducing its malaria burden over the last decade. However, malaria remains a major cause of morbidity and mortality in some regions and key challenges exist among high-risk populations who have high exposure to mosquito bites, but low coverage and use of vector control measures and limited access to healthcare. Two identified high-risk populations are goldminers and talibés (Koranic school children).

We conducted a controlled pre/post study to determine whether targeted malaria interventions, including expansion of active community case management and distribution of LLINs, at mining sites and Koranic schools (Daaras) increased reported LLIN usage and reduced malaria infection prevalence during high transmission season in Senegal. We randomly assigned eight health facility catchment areas to intervention or control groups: four in Kaolack (a city with many Koranic schools) and four in Saraya (a district with gold mining sites). Surveys were conducted pre (Oct 2021; n=1740) and post (Feb 2022; n=2200) delivery of the intervention package to assess intervention coverage and infection prevalence by rapid diagnostic test and qPCR. We compared prevalence and self-reported intervention coverage by group using a difference in difference (DID) framework with binomial generalized linear mixed models.

Among the talibés, the package of interventions was associated with an 11-percentage point relative reduction in malaria prevalence ($p<0.05$, CIs: -2 to -20) and a 35-percentage point

relative increase in reported prior night net use ($p<0.001$, CIs: 27-43) in the intervention group compared to the counterfactual. However, among the gold miners there was no measured association between the package of interventions and malaria prevalence or prior night net use in the intervention group compared to the counterfactual. Targeted interventions resulted in an increase in net usage and reduction in prevalence among Koranic school children but not among the gold miners; these findings support the need for interventions that are specific to different high-risk populations.

Background

The successful implementation of malaria control interventions in Senegal including distribution of long-lasting insecticide treated nets (LLINs), indoor residual spraying (IRS), seasonal malaria chemoprevention, and improved case management has led to a decrease in malaria incidence (from 61 to 42 per 1,000 population) and incidence of malaria deaths (from 5.7 to 0.27 per 1,000 population) between 2010 to 2019 [1]. Since 2019, a reversal of this trend has been witnessed, with malaria beginning to increase again [1,111]. Malaria is unevenly distributed across Senegal and as malaria transmission is reduced, the changing epidemiologic context often leads to more heterogeneous malaria transmission in which malaria clusters among specific high-risk populations (HRPs): groups of individuals at higher risk of malaria infection due to shared characteristics such as demographics, behavior, occupation, or geographic location [7,8]. Recent formative work and a case-control study confirmed that Koranic School Children (talibés) and gold miners are at higher risk of malaria infection in Senegal [31,112].

Talibés are mainly boys aged five to eighteen years old who live in traditional Koranic boarding schools (daaras) under the responsibility of a school master. Talibés often live in poor housing conditions, may sleep outside, and are exposed to mosquitoes while outside in the evenings begging [112]. Other studies in Senegal have found that adolescents are particularly at risk of infection and vulnerable to malaria [30,113] and research in other African countries indicates that while school age children and adolescents experience high burdens of malaria, they are often less likely to be prioritized for interventions than other groups [48,114]. Gold miners are considered a malaria high-risk population globally; despite regional variations in climate, vectors, and populations, malaria is associated with mining operations in three separate continents [21]. Recent studies have found that gold miners are a high-risk population in Senegal that require targeted interventions [31,112]. Gold miners in Senegal include citizens of various neighboring countries (in addition to Senegalese), are highly mobile, have a lack of access to healthcare personnel, and are often missed by routine interventions such as IRS due to the remoteness of worksites or because they sleep in makeshift housing that is not sprayable [112].

While there is evidence documenting that talibés and gold miners are at higher risk of malaria, there has been limited research on the feasibility and effectiveness of specifically targeting interventions to reach these populations. Both groups are highly exposed to mosquitoes and are difficult to address due to congregate living away from households; tailored surveillance and intervention approaches are needed for these populations. This study evaluated the implementation of a package of interventions targeted to talibés and gold miners in Senegal; this included enhanced community case management, targeted routine delivery of long-lasting insecticide treated nets (LLINs), and malaria education.

Methods

Study Area

This study took place among two high-risk populations in two districts of Senegal: talibés in Kaolack district and gold miners in Saraya district (Figure 4.1). While daaras exist throughout the country, Kaolack district has the largest number of daaras and talibés in Senegal [112]. Gold miners are active in multiple districts in southern Senegal, primarily in Saraya district [31]. Anecdotal reports, routine malaria surveillance, a formative report, and a case-control study informed the identification of talibés in Kaolack and gold miners in Saraya [31,112]. Malaria is highly seasonal in both districts, with the typical high season running from July to November (during and just after the rainy season). Malaria is almost entirely due to

Plasmodium falciparum [115]. In 2020, a total of 40,014 malaria cases were reported in Saraya district for a population of about 63,832, or an annual incidence of 627 cases per 1000 population, while Kaolack reported 3,455 cases for a population of approximately 388,285, resulting in an annual incidence of 9 cases per 1000 population [116].

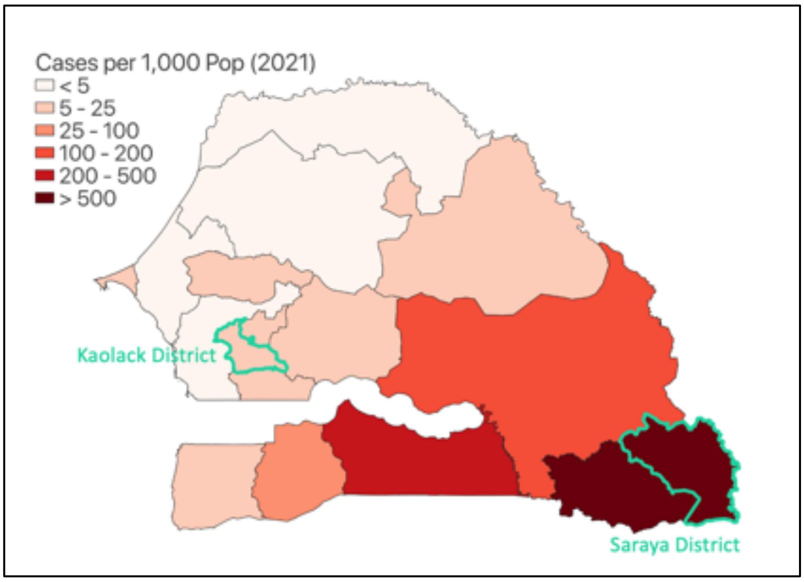

Figure 4.1: *2021 malaria incidence in Senegal and the locations of Kaolack and Saraya Districts*

The study sites include the catchment areas of four health posts in each district: Khossanto, Diakhaling, Mamakhono and Sambrambougou in the rural Saraya health district, and Nimzatt, Ngane, Thioffack and Tabangoye in the largely urban area of Kaolack health district. The total study population was approximately 14,000 and 81,000 in Saraya and Kaolack respectively, within the catchment areas of four health posts in each district. These health posts were selected based on the presence of each high-risk population in their catchments and historically high malaria incidence. The enumerated population targeted for the cross-sectional surveys included gold miners working at gold mining sites selected for the study (estimated population of 5,762) in Saraya district, and talibés living in schools selected for the study (estimated population of 4,118) in Kaolack district.

Study Design

This study was a controlled pre- and post-intervention evaluation to compare the intervention and comparison groups. Randomization was conducted at the health facility catchment level: two health facility catchments in each district were randomly assigned to receive the intervention and two in each district were assigned to be control.

The control group experienced routine malaria control activities including case management with rapid diagnostic tests (RDTs) and artemisinin-based combination therapy (ACT) in health facilities and by community health workers (DSDOMs) at the village level, distribution of LLINs through universal coverage mass campaigns (a campaign occurred in 2022) and through antenatal care (ANC), seasonal malaria chemoprevention (SMC) targeting children aged 3 months to 10 years, and indoor residual spraying (IRS) of insecticide.

Intervention

The intervention group received routine malaria control activities as well as a three-part intervention package. The first item in the package was enhanced community case management in which a peer community health worker was established at the Koranic school (DSDAARA) or the gold mining site (DSDOR), trained and provided with necessary supplies for diagnosis and treatment of uncomplicated malaria. These community health workers were also trained to conduct weekly visits to test patients with fever or history of fever for malaria (PECADOM+). The second item in the package of interventions was targeted routine delivery of LLINs; LLINs were distributed to gold miners and talibés via community health workers during the baseline cross-sectional survey (October 2021) and to any new students or gold miners who arrived after the baseline survey. The third item in the package of interventions was malaria education sessions that were conducted by community health workers throughout the study period on causes, symptoms, and prevention of malaria, the importance of seeking care rapidly after the onset of symptoms, and demonstrations on how to use and hang the LLINs.

Outcomes

The two primary outcomes evaluated in this study were prevalence of *P. falciparum* infection using rapid diagnostic tests (RDTs) and polymerase chain reaction (PCR) and self-reported intervention coverage defined as the proportion of individuals who reported sleeping under an LLIN last night. We also evaluated the descriptive outcome of malaria knowledge.

Sample Size

Sample size calculations for the primary outcomes were powered to detect at least a 10% absolute difference in intervention coverage (LLINs) in target populations between control and intervention groups in each district, assuming 50% coverage in control areas with 80% power at the 5% significance level and allowing for 10% non-response. The sample size was designed to provide 80% power to detect an absolute reduction of 6% in malaria infection prevalence in gold miners between control and intervention groups, assuming 15% prevalence in the control group, and a 5% significance level. In talibés, the sample size was designed to provide 80% power to detect an absolute reduction of 5% in the prevalence of malaria between control and intervention groups, assuming a prevalence of 10% in the control group, and a 5% significance level. After the baseline survey was conducted, the design effect calculated was 1.25; this was adjusted for in the endline survey sample size.

A census listing of sites (gold mining sites and Koranic schools) was used to enumerate schools and gold mines for the study. For the surveys of talibés, sites were selected with probability proportional to size of the school and the number of students to sample at each school was fixed at twenty-two to ensure equal probability of selection at all sights and reach total sample size [117]. For the gold miners, all gold mining sites in operation in the study area at the time of the survey were included. Within each mine, the number of gold miners recruited was proportional to the size of the site (no weighting was used). At each site, participants were selected using a random number table.

Data Collection

A survey instrument informed by formative work in 2020 was developed in English and French. Talibés were eligible for inclusion in the study if at the time of the intervention they were a boarding learner at a selected school within the four health post catchment areas in Kaolack District and they were available and willing to participate in the study. Gold miners were eligible for inclusion in the study if at the time of the intervention they were working as a gold miner at a selected gold mining site within the four health post catchment areas in Saraya District and they were available and willing to participate in the study. Written informed consent was obtained from all participants. Informed consent for participants under the age of 18 was provided by the parent or guardian. Written assent was requested from minors between the ages of 12 and 18, in addition to the consent of the parent or guardian.

The baseline survey for this study occurred in October 2021 and the endline was in January and February of 2022. For the talibés, some questions were asked of the talibés directly, and some survey questions were asked of the school masters, i.e., questions that were the same for the whole school such as how many students live in this school. Participants at baseline and endline were interviewed by study coordinators in the appropriate languages. All participants were tested for malaria by RDT. Dried blood spots (DBS) were collected on filter paper for all participants; a subset of these DBS was available for laboratory analysis.

Data Processing

Data entry was carried out using electronic questionnaires on tablet devices and entries were uploaded to secure cloud-based databases. Data management, cleaning, and analysis were conducted using STATA 16.1, R 4.1.2, and QGIS 3.12.

To represent socio-economic status (SES), we conducted a principal component analysis (PCA) of binary assets (e.g. electricity, radio, television, refrigerator, car, bicycle, motorcycle, mobile phone, air conditioner, water heater, solar electricity) and a categorical variables of water source, toilet facilities, and fuel/energy for cooking [75]. For the talibés, PCA quartiles were used in the regression; for gold miners the quartiles were condensed into two groups (below and above 50th percentile) to avoid small cells. Estimates of average monthly precipitation (rainfall in mm) were obtained from CHIRPS at 1 km spatial resolution [118]. Monthly enhanced vegetation index (EVI) at 1km spatial resolution and monthly average land surface temperature (LST) were obtained from MODIS using the MODIStsp package in R [119-121]. Averages of precipitation, EVI, and LST for the two months leading up to the baseline and endline surveys were calculated and extracted to point for the individual study sites (schools and mines). Euclidean distance from each site to the closest health facility was calculated in QGIS.

Laboratory work was conducted at the University of California, San Francisco. Samples were placed in desiccant and stored as dried blood spots (DBS) on filter paper at 4°C. To process, individual samples were cut into circles using a 6mm hole-punch and placed into 96-well plates. DNA was then extracted using the chelex-based extraction method [122,123] with a final volume of 100uL-150uL, then stored at -20°C. Following extraction, we conducted qPCR to quantify parasitemia of *P. falciparum* using var gene acidic terminal sequence (varATS) assay [124]. Samples were considered positive for *P. falciparum* if the parasite quantity was greater than 1p/uL.

Data Analysis

The two HRPs (talibés and gold miners) were analyzed separately. For the analysis of talibés, basic probability weights were computed; weighted proportions are presented for categorical variables and weighted means (with standard deviation) are presented for continuous variables [125] (for the gold miners, unweighted proportions and means are presented).

The analysis approach for the main outcomes of malaria prevalence (RDT-derived and qPCR-derived) and self-reported intervention coverage was using the difference in difference (DID) estimator with binomial generalized linear mixed models. A DID is a quasi-experimental study design that makes use of serial cross-sectional data collected before and after an intervention in two groups: (1) a sub-population of people where the interventions were acted upon (i.e. treated group) and (2) a sub-population where the interventions were not carried out (i.e. control group) [126]. DID uses data from the control and intervention groups to determine an appropriate counterfactual, which represents the unobserved outcome trend in the intervention group had the intervention not been implemented and is used to estimate a causal effect. This technique has been frequently used to estimate causal effects where a randomized control trial is not possible.

In the generalized linear mixed models, a logit link was used to specify the relationship between the linear combination of coefficients and the binary dependent variable. Covariates were determined a priori and assessed for collinearity (if two variables were collinear, the most interpretable or programmatically useful was kept). The models account for clustered standard errors at the site (school or gold mine) and the adjusted models include fixed effects to adjust for covariates relevant to each HRP. Using marginal means, the risk difference (RD; percentage point change) was interpreted to estimate intervention effect between the intervention group and the counterfactual.

There was a loss in the field of DBS samples, primarily from one health facility catchment: endline survey DBS samples from daaras in the Thioffac catchment (a health facility catchment in the control group) were lost. Therefore, the analysis of malaria prevalence with qPCR among the talibés was restricted to include only daaras that had DBS available at baseline and endline. Among the gold miners, a sensitivity analysis restricted to individuals who reported at endline that they were also present at baseline was conducted to evaluate the impact of turnover at the mine on the main study outcomes.

Results

The total number of participants included 1,739 individuals at baseline and 2,200 at endline. Figure 4.2 depicts the outcomes of the randomization at the health facility level including the number of sites (koranic school or gold mine) in each group during the pre- and post-intervention surveys.

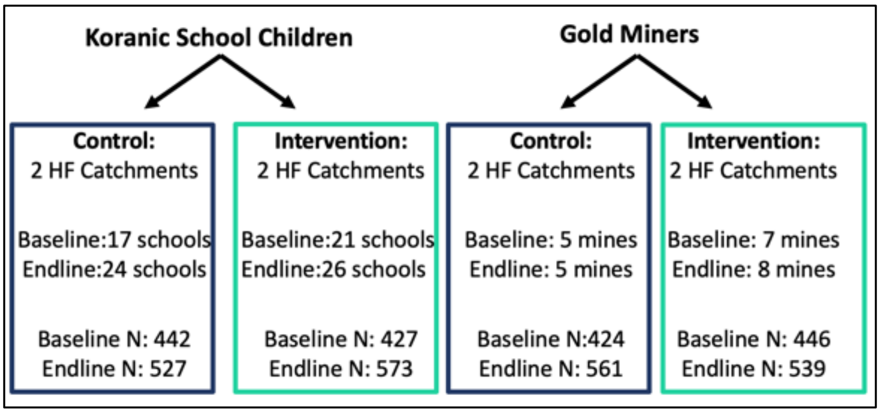

Figure 4.2: *Diagram of randomization at the health facility level and number of sites and participants in each group. HF: health facility*

Talibés

Descriptive analysis

Table 4.1 presents the characteristics of the talibés, environmental variables of the sites, and malaria and bed net usage outcomes of the study population for the control and interventions groups at baseline and endline. The talibés were 98% male overall and mainly in the age group of 10-15 (Table 4.1). The mean number of people living in the schools was 58 (ranging from a minimum of 15 to a maximum of 325).

Based on the weighted analysis, baseline prevalence (RDT) was 5.0% (95% CIs: 3.0-8.1%) in the control group and 4.8% (95% CIs: 3.0-7.5%) in the intervention group; prevalence (RDT) increased to 23.9% (95% CIs: 19.8-28.6%) in the control group and 13.2% (9.9-17.7%) in the intervention group at endline. qPCR results were available for 78.5% of participants (the majority that were not available were from the endline control group). To calculate qPCR derived prevalence, analysis was restricted to schools that had DBS available at baseline and endline. Distribution of characteristics such as age and gender were similar among the population with DBS available and the population missing DBS. Weighted qPCR derived prevalence among this subgroup was 9% (95% CIs: 4-19%) and 15% (95% CIs: 11-19%) among baseline control and intervention groups, respectively. At endline, weighted qPCR derived prevalence increased to 43% (95% CIs: 33-53%) among the endline control group and 28% (95% CIs: 23-34%) among the endline intervention group.

For the outcome of net usage last night, reported net usage decreased in the control group from a weighted 53% (95% CIs: 41-64%) to 40% (95% CIs: 31-50%) between baseline and endline; in the intervention group net usage increased from 65% (95% CIs: 54-75%) to 92% (95% CIs: 85-95%) (Table 4.1).

Table 4.1: *Distribution of model covariates, malaria prevalence, and net coverage among the Talibés. % are weighted. *For continuous variables: mean (standard deviation). CI: confidence interval; SES: socioeconomic status, PCA: principal component analysis; SMC: seasonal malaria chemoprevention; EVI: enhanced vegetation index; HF: health facility.*

	Baseline Control		**Baseline Intervention**		**Endline Control**		**Endline Intervention**	
	N=442 (%*)	95% CI	N=427 (%*)	95% CI	N=527 (%*)	95% CI	N=573 (%*)	95% CI
Health facility								
PS Ngane	136 (29%)	19-42%	—	—	123 (21%)	13-31%	—	—
PS Nimzatt	—	—	231 (57%)	45-69%	—	—	310 (54%)	43-64%
PS Taba Ngoye	—	—	196 (43%)	31-55%	—	—	263 (46%)	36-57%
PS Thioffac	306 (71%)	57-81%	—	—	404 (79%)	69-87%	—	—
Age group								
5-9	168 (38%)	32-45%	131 (31%)	25-38%	173 (34%)	30-39%	146 (25%)	21-29%
10-15	242 (55%)	50-61%	236 (53%)	46-59%	328 (61%)	57-66%	355 (62%)	57-66%
16 and older	32 (7%)	4-10%	60 (16%)	11-23%	26 (5%)	3-7%	72 (13%)	9.8-17.8%
Sex								
Female	33 (7%)	4-12%	5 (2.9%)	0.9-8.6%	36 (7%)	4-11%	8 (2%)	0.8-4%
Male	409 (93%)	88-96%	422 (97%)	91-99%	491 (93%)	89-96%	565 (98%)	96-99%
Number of people living in the Daara	59 (34)	50-67	57 (34)	49-66	58 (25)	52-64	60 (56)	48-72
SES (PCA Quartile)								
0-25	142 (35%)	24-49%	83 (19%)	11-30%	114 (21%)	13-31%	141 (23%)	15-33%
25-50	28 (6%)	2-16%	108 (24%)	15-36%	99 (21%)	13-31%	218 (38%)	29-49%
50-75	84 (18%)	9.6-30%	161 (38%)	27-50%	292 (54%)	43-65%	130 (23%)	15-33%
75-100	188 (41%)	29-55%	75 (19%)	11-30%	22 (4%)	1-12%	84 (15%)	9-25%
Treated with SMC								
No	28 (6%)	4-10%	42 (10%)	6-15%	109 (21%)	17-25%	34 (6%)	4-10%
Ineligible/No response	223 (49%)	43-55%	246 (57%)	51-64%	254 (46%)	41-52%	371 (65%)	60-70%
Yes	191 (45%)	38-52%	139 (33%)	27-39%	164 (33%)	28-38%	168 (28%)	24-33%
EVI	0.26 (0.07)	0.24-0.27	0.21 (0.08)	0.19-0.23	0.21 (0.03)	0.20-0.21	0.18 (0.04)	0.18-19
Temperature (F)	85.6 (0)	85-85	85 (0.75)	85-85	95 (0)	95-95	96 (2.6)	95-96
Precipitation (mm)	215 (0)	215-215	217 (9)	215-219	1.3 (0)	1.3-1.3	1.3 (0.03)	1.3-1.3
Distance to nearest HF								
<0.25km	21 (6%)	2-16%	103 (24%)	15-35%	20 (4%)	1-12%	110 (19%)	12-29%
0.25-0.5km	145 (33%)	23-46%	182 (48%)	36-60%	127 (25%)	17-36%	261 (46%)	36-57%
0.5-1km	173 (38%)	26-50%	91 (19%)	12-30%	233 (45%)	35-57%	132 (23%)	15-33%
>1km	103 (23%)	14-36%	51 (9%)	4-18%	147 (25%)	17-36%	70 (12%)	6-20%
RDT result								
Negative	416 (95%)	92-97%	406 (95%)	92-97%	397 (76%)	71-80%	499 (87%)	82-90%
Positive	26 (5%)	3-8%	21 (5%)	3-8%	130 (24%)	20-29%	74 (13%)	10-18%
Slept under net last night								
No	217 (47%)	37-59%	148 (35%)	25-46%	305 (60%)	50-69%	46 (8%)	5-15%
Yes	225 (53%)	41-63%	279 (65%)	54-75%	222 (40%)	31-50%	527 (92%)	85-95%

Intervention effect on malaria prevalence and LLIN usage

Among the talibés, the package of interventions was associated with an 11-percentage point relative reduction in malaria prevalence (RDT) in the intervention group ($p<0.05$, CI's: -2 to -20) compared to the counterfactual (what would have been observed in the intervention group had treatment never occurred) (Figure 4.3). This result remained similar (13-percentage point reduction, $p<0.05$, CI's -3 to -22) after adjusting for age group, number of people living in the school, socioeconomic status, distance to nearest health facility, seasonal malaria chemoprevention, and environmental factors including EVI and temperature (**Table 4.2**). Prevalence measured using qPCR was available for a subset of results; using this measure of the outcome the package of interventions was associated with an unadjusted 20-percentage point reduction ($p<0.001$, CI's: -9 to -31) and an adjusted 13-percentage point reduction ($p<0.05$, -2 to -24) in qPCR derived malaria prevalence compared to the counterfactual.

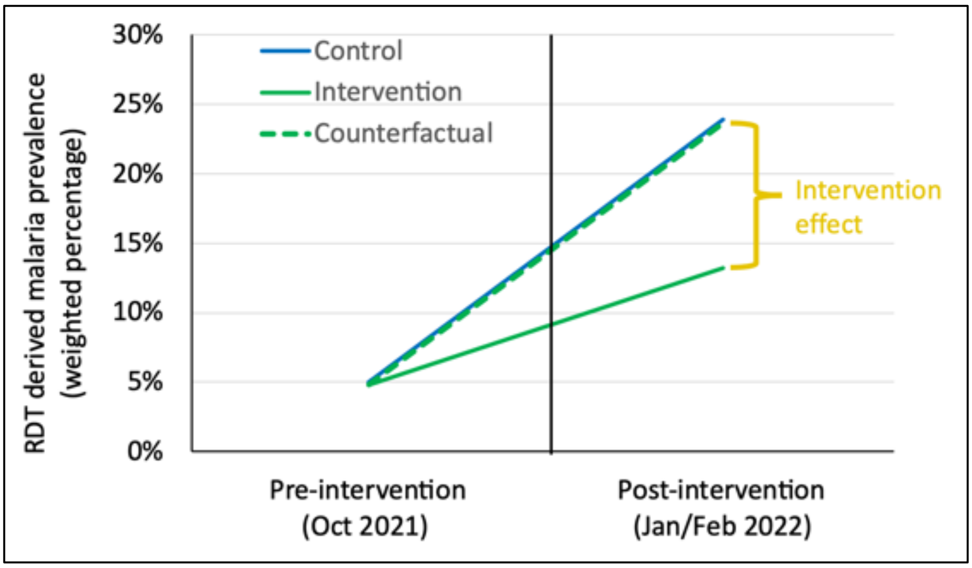

Figure 4.3: *Difference in difference of RDT-derived malaria prevalence among the talibés*

Among the talibés, the package of interventions was associated with a 35-percentage point increase in reported prior night net use in the intervention group ($p<0.001$, CI's: 27-43) compared to the counterfactual. After adjusting for age group, number of people living in the school, socio-economic status, and distance to nearest health facility, the estimated intervention effect was a 44-percentage point increase in reported prior night net use in the intervention group (p<0.001, 95% CIs: 36-52).

Table 4.2: *Difference in difference of main outcomes among the talibés. % are weighted. CI: confidence interval; DID: difference in difference; RDT: rapid diagnostic test; qPCR: quantitative polymerase chain reaction*

Indicator	Intervention		Control		
	Baseline n (%)	Endline n (%)	Baseline n (%)	Endline n (%)	Adjusted DID (95% CI)
RDT-derived malaria					-13* (-3, -22)
Yes	21 (5%)	74 (13%)	26 (5%)	130 (24%)	
No	406 (95%)	499 (87%)	416 (95%)	397 (76%)	
qPCR-derived malaria**					-13* (-2, -24)
Yes	63 (15%)	147 (28%)	11 (9%)	63 (43%)	
No	359 (85%)	384 (72%)	95 (91%)	82 (57%)	
Prior night net use					44* (36, 52)
Yes	279 (65%)	527 (92%)	225 (53%)	222 (40%)	
No	148 (35%)	46 (8%)	217 (47%)	305 (60%)	

*p<0.05
**available for a subset of results

Secondary outcome of malaria knowledge

Malaria knowledge was assessed among the schoolteachers and was high at baseline and endline in both arms of the study. Among schoolteachers with data, at baseline 94% in both groups identified that mosquitoes transmit malaria, 88% in the control group and 100% in the intervention group identified that fever is a symptom of malaria, and 100% in the control group and 94% in the intervention group identified a correct method of malaria prevention. At endline in both groups, 100% of schoolteachers correctly identified mosquitoes as the method of transmission of malaria, fever as a symptom of malaria, and using a bed net as a method of prevention against malaria.

Gold Miners

Descriptive analysis

Overall, the miners were 61% male and 39% female; there was a higher percentage of female miners in the endline control group than in other groups. The majority (61%) of miners were in the age range of 25-49. 66% of gold miners reported having no formal education. Country of citizenship differed substantially between endline and baseline; for example, 52% of gold miners were Senegalese citizens at baseline while only 28% (22% in control and 35% in intervention) were Senegalese citizens at endline. At endline, 207 miners (37%) in control and 192 miners (36%) in intervention reported they were present at baseline.

Table 4.3: *Distribution of model covariates, malaria prevalence, and net coverage among the gold miners.*
**For continuous variables: mean (standard deviation).*
CI: confidence interval; SES: socioeconomic status, PCA: principal component analysis; EVI: enhanced vegetation index; HF: health facility.

	Baseline Control		**Baseline Intervention**		**Endline Control**		**Endline Intervention**	
	N=424 (%*)	95% CI	N=446 (%*)	95% CI	N=561 (%*)	95% CI	N=539 (%*)	95% CI
Health facility								
Mamakhono	127 (30.0%)	26-34%	—	—	165 (29.4%)	26-33%	—	—
Sambrambougou	297 (70.0%)	66-74%	—	—	396 (70.6%)	67-74%	—	—
Diakhaling	—	—	213 (47.8%)	43-52%	—	—	244 (45.3%)	41-49%
Khossanto	—	—	233 (52.2%)	48-57%	—	—	295 (54.7%)	51-59%
Age group								
13-24	120 (28.3%)	24-33%	135 (30.3%)	26-35%	190 (33.9%)	30-38%	189 (35.1%)	31-39%
25-49	285 (67.2%)	63-72%	278 (62.3%)	58-67%	315 (56.1%)	52-60%	323 (59.9%)	56-64%
50 and older	19 (4.5%)	3-7%	33 (7.4%)	5-10%	56 (10.0%)	8-13%	27 (5.0%)	3-7%
Sex								
Male	287 (67.7%)	63-72%	292 (65.5%)	61-70%	259 (46.2%)	42-50%	363 (67.3%)	63-71%
Female	137 (32.3%)	28-37%	154 (34.5%)	30-39%	302 (53.8%)	50-58%	176 (32.7%)	28-37%
Level of education								
No formal education	260 (61.3%)	57-66%	340 (76.2%)	72-80%	384 (68.4%)	64-72%	310 (57.5%)	53-62%
Primary school	41 (9.7%)	7-13%	40 (9.0%)	7-12%	77 (13.7%)	11-17%	108 (20.0%)	17-24%
Koranic school	103 (24.3%)	20-28%	46 (10.3%)	8-13%	74 (13.2%)	11-16%%	78 (14.5%)	12-18%
Secondary or Higher	20 (4.7%)	3-7%	20 (4.5%)	3-7%	26 (4.6%)	3-7%	43 (8.0%)	6-11%
Citizenship								
Burkina-Faso	22 (5.2%)	3-8%	52 (11.7%)	9-15%	69 (12.3%)	10-15%	58 (10.8%)	8-14$
Guinea	105 (24.8%)	21-29%	22 (4.9%)	3-7%	175 (31.2%)	27-35%	26 (4.8%)	3-7%
Mali	58 (13.7%)	11-17%	131 (29.4%)	25-34%	171 (30.5%)	27-34%	250 (46.4%)	42-51%
Other	22 (5.2%)	3-8%	8 (1.8%)	1-4%	25 (4.5%)	3-7%	15 (2.8%)	2-5%
Senegal	217 (51.2%)	46-56%	233 (52.2%)	48-56%	121 (21.6%)	18-25%	190 (35.3%)	31-39%
SES (PCA quartile)								
0-25	151 (35.6%)	31-42%	174 (39.0%)	35-44%	244 (43.5%)	39-48%	184 (34.1%)	30-38%
25-50	59 (13.9%)	11-18%	55 (12.3%)	10-16%	222 (39.6%)	36-44%	200 (37.1%)	33-41%
50-75	132 (31.1%)	27-36%	125 (28.0%)	24-32%	0 (0%)	—	0 (0%)	—
75-100	82 (19.3%)	16-23%	92 (20.6%)	17-25%	95 (16.9%)	14-20%	155 (28.8%)	25-33%
Residence sprayed in last year								
No	392 (92.5%)	90-95%	438 (98.2%)	96-99%	515 (91.8%)	89-94%	454 (84.2%)	81-87%
Yes	32 (7.5%)	5-10%	8 (1.8%)	0.9-4%	46 (8.2%)	6-11%	85 (15.8%)	13-19%
Sleeping structure								
Informal	149 (35.1%)	31-40%	165 (37.0%)	33-42%	375 (66.8%)	63-71%	255 (47.3%)	43-52%
Modern	64 (15.1%)	12-19%	37 (8.3%)	6-11%	40 (7.1%)	5-10%	45 (8.3%)	6-11%
Permanent traditional	211 (49.8%)	45-55%	244 (54.7%)	50-59%	146 (26.0%)	22-30%	239 (44.3%)	40-49%
Household size	4.25 (2.36)	4.0-4.5	4.41 (3.35)	4.1-4.7	3.08 (1.41)	3.0-3.2	2.95 (1.73)	2.8-3.1
EVI	0.438 (0.07)	0.43-0.44	0.477 (0.0705)	0.47-0.48	0.283 (0.0362)	0.28-0.28	0.287 (0.0331)	0.28-0.29
Temperature (F)	81.6 (0.71)	81.5-81.7	80.2 (1.54)	80.0-80.3	98.0 (0.92)	98-98	98.8 (1.54)	98.6-99
Precipitation (mm)	325 (4.67)	325-325	329 (2.67)	329-329	0.159 (0.04)	0.16-0.16	0.125 (0.01)	0.12-0.13
Distance to nearest HF (km)								
0.5 or less	205 (48.3%)	44-53%	117 (26.2%)	22-31	75 (13.4%)	11-16%	83 (15.4%)	13-19%
0.5-1	14 (3.3%)	2-5%	137 (30.7%)	27-35%	48 (8.6%)	7-11%	237 (44.0%)	40-48%
1 or more	205 (48.3%)	44-53%	192 (43.0%)	39-48%	438 (78.1%)	74-81%	219 (40.6%)	37-45%
RDT result								
Negative	290 (68.4%)	64-73%	323 (72.4%)	68-76%	525 (93.6%)	91-95%	505 (93.7%)	91-95%
Positive	134 (31.6%)	27-36%	123 (27.6%)	24-32%	36 (6.4%)	4.5-8.8%	34 (6.3%)	4.4-8.7%
qPCR result								
Negative	133 (31.4%)	27-36%	165 (37.7%)	33-42%	311 (55.4%)	51-60%	314 (58.3%)	54-62%
Positive	291 (68.6%)	64-73%	278 (62.3%)	58-67%	250 (44.6%)	40-49%	225 (41.7%)	38-46%
Slept under net last night								
No	163 (38.4%)	34-43%	183 (41.0%)	37-46%	259 (46.2%)	42-50%	306 (56.8%)	53-61%
Yes	261 (61.6%)	57-66%	263 (59.0%)	54-63%	302 (53.8%)	50-58%	233 (43.2%)	39-47%

Baseline malaria prevalence (RDT) was 32% (95% CIs: 27-36%) in the control group and 28% (95% CIs: 24-32%) in the intervention group. At endline, prevalence (RDT) was 6% in both groups (95% CIs: 4.5-8.8% in the control group; 4.4-8.7% in the intervention group). When defined with qPCR, prevalence was much higher in all groups (Table 4.3).

For the outcome of prior night net usage, reported net usage decreased between baseline and endline in both groups. In the control group, it decreased from 61.6% (95% CIs: 56.7-66.2%) to 53.8% (95% CIs: 49.6-58.0%) and in the intervention group from 59.0% (95% CIs: 54.2-63.6%) to 43.2% (95% CIs: 39.0-47.5%).

Intervention effect on malaria prevalence and LLIN usage

Among the gold miners, there was no measured association between the package of interventions and RDT-derived malaria prevalence (4-percentage point increase in prevalence in the intervention group compared to the counterfactual, p=0.45, 95% CI's: -6 to 14) (**Figure 4.4**). The adjusted model included covariates for age group, gender, citizenship, socioeconomic status, education level, number of people living at the mine, distance to nearest health facility, indoor residual spraying, type of sleeping structure, and temperature; there was no measured association in the adjusted model (aRD: 1-percentage point decrease in malaria prevalence, p=0.66, 95% CI's: -7 to 4) (**Table 4.4**). In the sensitivity analysis, the analysis was restricted to individuals who reported participating at both the baseline and endline surveys, and the results were similar: (aRD: 2.5 percentage-point decrease in prevalence, p=0.55, 95% CIs: -11 to 6).

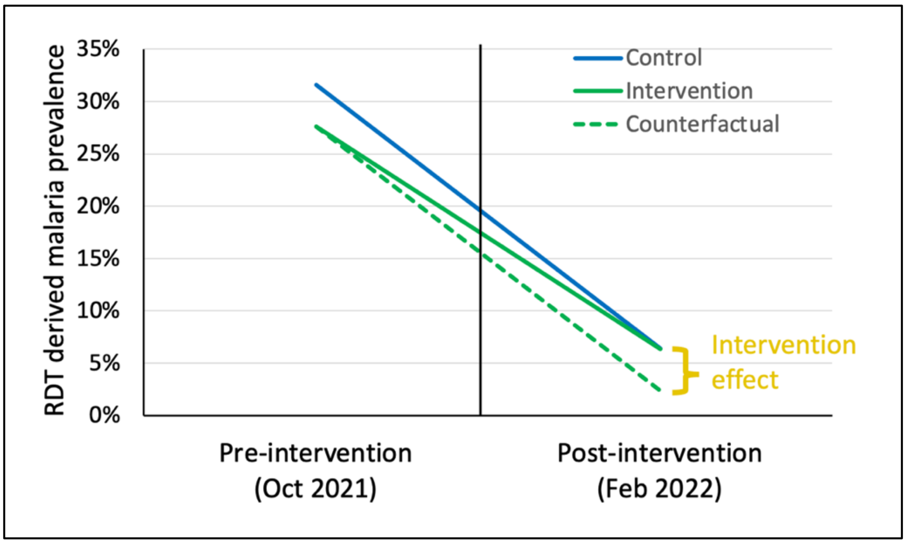

Figure 4.4: *Difference in difference of RDT-derived malaria prevalence among the gold miners*

qPCR results were available for 99.8% of gold miners (Table 4.3); using this measure of the outcome the package of interventions was not associated with a decrease in malaria prevalence (unadjusted 3-percentage point increase, p=0.55, 95% CI's: -7.9 to 14.8; and an adjusted 1-percentage point decrease in malaria prevalence compared to the counterfactual, p=0.84, 95% CI's: -12 to 10). Overall, qPCR-derived prevalence was much higher in all groups than RDT-derived prevalence. Among individuals who reported participating at baseline and endline surveys, the results were similar: (aRD: 0.6 percentage-point increase in prevalence, p=0.07 95% CIs: -16 to 17).

In the unadjusted model, there was no measured association between the package of interventions and reported prior night net use compared to the counterfactual (8-percentage point decrease in reported prior night net use in the gold miner intervention group, p=0.07, 95% CI's -44 to 28). The results remained similar after adjusting for age group, gender, citizenship, education level, socioeconomic status, distance to the nearest health facility, type of sleeping structure, number of people living at the mine, and indoor residual spraying (1.4-percentage point decrease p=0.94, 95% CI's -39 to 36).

Table 4.4: *Difference in difference of main outcomes among the gold miners. CI: confidence interval; DID: difference in difference; RDT: rapid diagnostic test; qPCR: quantitative polymerase chain reaction*

Indicator	**Intervention**		**Control**		
	Baseline n (%)	Endline n (%)	Baseline n (%)	Endline n (%)	Adjusted DID (95% CI)
RDT-derived malaria					1 (-7, 4)
Yes	123 (28%)	34 (6%)	134 (32%)	36 (6%)	
No	323 (72%)	505 (94%)	290 (68%)	525 (94%)	
qPCR-derived malaria					-1 (-12, 10)
Yes	278 (62%)	225 (42%)	291 (69%)	250 (45%)	
No	165 (38%)	314 (58%)	133 (31%)	311 (55%)	
Prior night net use					-1.4 (-39, 36)
Yes	263 (59%)	233 (43%)	261 (62%)	302 (54%)	
No	183 (41%)	306 (57%)	163 (38%)	259 (46%)	

*p<0.05

Secondary outcome of malaria knowledge

At baseline, 99% of all gold miners identified mosquitoes as the mode of transmission for malaria, listed a correct method of prevention, and identified fever as a symptom of malaria. At endline, knowledge of the mode of transmission and an accurate method of prevention remained at 99% and knowledge of fever as a symptom of malaria decreased slightly in both groups to 95%.

Discussion

The purpose of this study was to assess the feasibility and effectiveness of a package of interventions including enhanced community case management, targeted routine delivery of LLINs, and malaria education among two high-risk populations in Senegal: talibés living in boarding schools in Kaolack district and gold miners in Saraya district. The package was associated with an adjusted 13-percentage point reduction in malaria prevalence and an adjusted 44-percentage point increase in reported net usage among talibés. Among the gold miners, the package of interventions was not associated with a difference in malaria prevalence or reported net usage in the intervention group compared to the counterfactual. The dissimilarity in effectiveness of the intervention package among the accessible student populations at urban boarding schools compared to the highly mobile, diverse, and hard-to-reach gold mining populations in remote regions highlights the need for individualized approaches for different malaria high-risk populations.

Talibés are a population at high-risk of malaria infection in Senegal; this study indicates that delivering routine interventions in a targeted package can be an effective approach to reaching this vulnerable population. Other studies have also called for increased attention on interventions for school-age children: there is evidence in Senegal and other African countries that despite an increased risk of malaria among school aged children and adolescents, this population is often overlooked for targeted interventions and there is a great need for interventions specifically tailored to this group [30,48,113,114,127]. An important component of this package of interventions was LLIN distribution via a new approach: through peer community health workers. Targeted LLIN delivery as part of the package was associated with an increase in reported net usage (despite higher temperatures at endline) and reduction in malaria prevalence in talibés. This finding is consistent with the literature: previous research in Senegal has identified that non-use of LLINs is associated with increased odds of malaria among children and adolescents and has called for increased access to healthcare interventions such as LLINs among this population [30]. This result is consistent with findings on bed net usage among children and adolescents in other countries such as Kenya and Rwanda; ownership and usage of LLINs was associated with lower odds of malaria among these age groups [128,129]. In the formative research that preceded this study, respondents indicated the daaras often have insufficient quantities of nets and in these situations, the older students get to use the nets (and the younger do not) or no talibés are allowed to use the nets to prevent disagreements over them [31]. The targeted LLIN delivery in this study may have increased usage simply by increasing access to nets in tandem with education.

This study also provides evidence that increased case management via a peer community health worker in the school was part of an effective package of interventions for talibés. Other studies have found that schools are a good place to deliver health interventions and that effective diagnosis and treatment of malaria can be improved via provision of case management at schools [30,130]. Additionally, while this study found high levels of malaria knowledge among the schoolteachers, malaria knowledge among individual talibés was not assessed. Other studies in Senegal have found low levels of malaria knowledge among children and adolescents and have called for interventions to improve disease knowledge; education in tandem with interventions such as LLIN distribution is an effective approach for increasing LLIN use [111,130].

The results of this study were different among the gold miners: we did not find evidence that the package of interventions was effective in reducing malaria prevalence or increasing reported net usage in this population with high rates of turnover. Formative research among this population indicated that the use of LLINs is not routine among gold miners in Senegal [31]; we did not find evidence that this package including LLINs and education increased net usage among this population. Although research on gold miners in Senegal is limited (often due to the remoteness of the mine and high mobility of the population), research in other regions has indicated that gold miners globally have very limited access to healthcare [131-133]. However, we did not find that this package of interventions including a peer community health worker led to a decrease in malaria prevalence or an increase in reported access to care. Malaria knowledge among gold miners also decreased slightly between the baseline to endline surveys.

It is commonly accepted that gold miners are a group that is difficult to study [21]. Explanations for the lack of effectiveness of the package of interventions among the gold miners include that this population is highly mobile, the population may accept malaria as one of many risks of the industry, and they may face stigma in accessing care or bed nets. The mobility and turnover of the gold miners was a big challenge of the study: at the endline survey, 36% of gold miners (same proportion in both arms) reported that they were present at baseline. The primary nationalities of the gold miners changed greatly between baseline and endline, highlighting the turnover during this period; for example, at baseline the largest contingent of miners were Senegalese while at endline the most common citizenship was of Mali. The DSDORs placed at the gold mines also reported that the mobility of the miners was a great challenge to acting as a CHW. The miners themselves and the mobility patterns of the gold miners are also diverse; formative research found that Senegalese gold miners from regions outside Kaolack return home for holidays and during the rainy season to work in agriculture, while gold miners of other nationalities return home to their families once they have earned enough money and may also return again several times during the year depending on earnings [31]. The influx of gold miners occurs throughout the year, in particular if a new gold vein is discovered. Miners may leave when they hear of a new vein discovered in another region[31]. Populations that are highly mobile have been found to have worse health problems, lower access to care, and low net usage than settled populations [134-136]. Many of those who received the intervention package left between baseline and endline and were not present for the endline survey.

In addition to challenges of mobility and turnover, the formative research and literature suggest that gold miners in Senegal may face numerous risks in the industry of mining; malaria is just one of many occupational hazards and they may be more concerned with where they will sleep, what they will eat, and other risks than with worrying about malaria prevention or treatment [21,31]. Qualitative formative research in Senegal also indicated that lack of time is an obstacle to interventions among gold miners [31]. Stigma or cultural differences among the miners may also be a barrier; for example, indigenous Malinke gold miners live in houses in nearby villages and can accommodate Senegalese gold miners from other parts of the country. In contrast, gold miners coming from other countries are often excluded from the villages and sleep in “Niafa” (precarious dwellings between the villages and the mines that can house hundreds of inhabitants), settlements that are often not sprayable and missed by other routine interventions such as bed net campaigns [31]. There is mistrust among these different gold mining populations, and some communities do not want to integrate with the others [31,137]. Therefore, it is possible that there could be challenges if a peer community health worker is more likely to provide LLINs, education, diagnostics, or treatment to some gold miners and not others, or also that the DSDOR might be easily accessed by some gold miners but not others. This may have led to low utilization of the community health worker at the mine.

There were a few limitations of this study. A difference-in-difference analysis requires the parallel trend assumption; however, two time points does not allow for testing of the parallel trend assumption which asserts that in the absence of the intervention, the difference between the intervention and control groups is constant over the time period [138]. However, randomization and the short time period between the two surveys support the assumption [139,140]. There were also a small number of randomized units: two health facility catchments per arm for each high-risk population. Additionally, among the talibés, only a subset of PCR results was available due to the loss of a package of samples in the field. Some of the questions that were asked of the schoolteacher (e.g., questions on malaria knowledge) likely do not reflect knowledge among the individual talibés themselves. The students and the DSDAARA may have also felt pressured to answer positively about their experience in front of their peers and schoolteacher; this social desirability bias could bias the results away from the null. Among the gold miners, extensive turnover at the mines was a limitation; however, in the sensitivity analysis aimed at assessing the influence of turnover, the results remained similar as to those of the full cohort of participants.

While we did control for environmental variables in the models, it is possible that there are other aspects of seasonality that were not controlled for and may have influenced the results. For example, while the trend among the gold miners of higher prevalence at baseline than endline was consistent with trends in seasonal malaria incidence in the study districts during the same period (via routine surveillance data), the trend of lower prevalence at baseline than endline among the school children was not consistent with trends in seasonal malaria incidence in the study districts during the same period [141]. This could be due to unaccounted for aspects of seasonality that different between Kaolack and Saraya, or other behavior such as travel over the winter holidays (in between the baseline and endline surveys) by the school children.

Conclusions

The findings of this study provide evidence that a package of interventions including targeted LLIN delivery, peer community case management, and malaria education was associated with an increase in reported net usage and reduction in malaria prevalence among talibés in Kaolack District, Senegal. For this high-risk population, routine interventions delivered in a more targeted manner had beneficial effects on malaria indicators; this is highly needed as adolescents and school age children are a group that are often overlooked compared to other groups such as pregnant women and children under five. This package of interventions can be utilized in daaras throughout Senegal and other countries with boarding school populations.

However, there are greater challenges to reaching gold miners who are working in remote rural mines; this package of interventions was not effective among this high-risk population. While talibés spend long periods of time at schools and live in a hierarchy with school teachers who are responsible for them, miners are truly a “hard to reach” population: they are mobile, many are from other countries, heterogeneous in demographics, not organized under a structure within which to target interventions, they are often vulnerable and have many concerns (occupational safety, where to eat and sleep, other disease burdens) and may not know how to access health prevention. Future research among gold miners will require advanced participatory methods to determine interventions that will benefit this population: delivering routine interventions in this highly targeted manner was insufficient.

Chapter V

Conclusion

Malaria high-risk populations (HRPs) are groups at increased risk of malaria due to common characteristics putting them at increased exposure to malaria-transmitting mosquitoes. While addressing malaria among these populations is essential to elimination, they present a persistent challenge to malaria control programs globally because HRPs often have limited access to health services and lower intervention coverage. In countries with weak surveillance systems, HRPs are missed by routine surveillance and aggregate reporting systems do not allow for a detailed understanding of populations at risk amongst those who are captured by surveillance. Even in countries with strong case-based surveillance systems, certain populations may be excluded due to their work, travel, or other behaviors. This dissertation contributes to research on malaria HRPs in three countries: Aceh Province in Indonesia, Champasak District in Lao PDR, and Kaolack and Saraya districts in Senegal. The research on assessing risk factors and characterizing HRPs in Indonesia (Chapter 2) and Lao PDR (Chapter 3) contributes to a greater understanding of these populations and determines gaps to reach them with context-specific malaria interventions. In Chapter 4, the research adds to our knowledge on effective interventions for two HRPs in Senegal: gold miners and Koranic school children.

Characterizing malaria high-risk populations to determine critical intervention points

To ensure optimal effectiveness of interventions for malaria prevention and control, it is essential for malaria programs to delineate in detail the populations at highest risk of infection. The research in this dissertation contributes to our understanding of malaria HRPs as heterogeneous groups that require interventions tailored to the context and population.

In Indonesia, Lao PDR, and many other parts of Asia and Latin America, the populations at highest risk of malaria are often adult males who spend extended time in the forest [42,61,96]. The results in Chapters 2 and 3 are consistent with a body of literature suggesting that working-age males who are exposed to outdoor-biting, forest-adapted mosquitoes while taking trips to the forest are at high risk for malaria in Southeast Asia [56,61,63,96,100,105]. In Chapter 2, participants in Aceh, Indonesia who reported working and sleeping in the forest in the last sixty days had 21.66 times the adjusted odds of malaria (95% CI 5.09-92.26, P<0.0001) compared to those with no forest exposure, and participants who reported having a second residence in the forest/forest fringe had 6.29 times the adjusted odds of malaria (95% CI 2.29-17.31, P<0.0001) compared to those who did not have a second residence in the forest. In Chapter 3 in Champasak, Lao PDR, sleeping 3-7 nights in the forest in the past 30 days was associated with 9.74 times the adjusted odds of malaria (95% CI: 4.67-20.31, P<0.001) compared with 0-2 nights. In both studies, community exposures were not salient risk factors, indicating that exposures are likely occurring away from the home.

Chapters 2 and 3 also indicate that forest-going HRPs in Southeast Asia are a heterogeneous group: forest-goers in Aceh, Indonesia were diverse in age, socio-economic status, and education level, they worked in varied occupations including farming, logging, and mining, they slept in a variety of settings including barracks, huts, or tents, and the size of their worksites ranged from no other individuals to large groups of workers. In Champasak, Lao PDR, most participants identified as farmers and participants reported going to the forest to cut wood or look for food or fruit. In this study we found that trips to the forest were varied in duration and season, indicating that patterns of travel among forest goers are heterogeneous. Delineating gaps in coverage and intervention targets was also an essential aim of the research in Chapter 2 in Aceh, Indonesia. The findings indicated major gaps in net and hammock net use, utilization of mosquito repellants, or covering clothing, and utilization of chemoprophylaxis. The implications of these findings are that second residences in the forest are a critical intervention target in Aceh, Indonesia. Because participants reported that these residences have not been sprayed with IRS and there is low usage of bed nets, these second residences provide key vector control intervention targets that may help address transmission among those at highest risk of infection. Next steps in Aceh should include the piloting of hammock nets [65,81] and chemoprophylaxis for forest-goers Asia [59,84,85] with attention to acceptability, feasibility, and effectiveness. These approaches will be useful among forest-going populations in the region and likely are generalizable to other regions where forest malaria is prevalent, such as other parts of Asia, tropical Africa, and Latin America. In areas where *Plasmodium falciparum* is prevalent among forest-goers, implementation of the RTS,S/AS01 malaria vaccine should also be considered [142].

Chapter 2 of this dissertation supports the evidence base for methodologies to characterize HRPs in low transmission settings: case-control studies are particularly efficient when cases are relatively rare amongst the general population. Population-based surveys (e.g., Malaria Indicator Surveys) or cross-sectional surveys are unlikely to detect sufficient numbers of cases to assess risk factors to direct programmatic interventions to HRPs. Despite very low case numbers in the study area of Aceh Province Indonesia, it was possible to determine HRPs and specific intervention points. Case-control designs also are useful for generating hypotheses on where malaria exposures are occurring which can be tested using additional study designs such as network studies among well-defined HRPs. Even though this study utilized two control series, it is still possible that certain hard-to-reach forest going populations were missed; in particular, individuals who were asymptomatic, had subclinical infection, or were working in in illegal industries or were undocumented, in which case they might have been hesitant to see care and report these illicit occupations [4]. There are also a number of conceptual challenges in case control studies including defining the control population; for example, the selection of inappropriate controls can lead to selection bias [44]. Additionally, in a test-negative design conducted at health facilities, only symptomatic malaria is assessed and populations that do not access facilities may be missed [29].

In Chapter 3, a similar test-negative study design for characterizing risk populations is depicted: utilizing augmented routine surveillance data to assess risk and characterize HRPs in Champasak, Lao PDR. In addition to routine data collection, individuals with fever (both positive and negative for malaria) were asked to report any recent forest travel. This simple approach did not add additional work burdens to health facility staff and this method was effective for the purpose of confirming or assessing an individual risk factor (number of nights sleeping in the forest in the past thirty days). For a more in-depth epidemiological description of risk factors, a more detailed questionnaire would be needed including more information on occupation, behavior, and travel destinations of participants.

Targeting effective interventions to increase intervention coverage and decrease malaria prevalence among high-risk populations

The findings in this dissertation contribute to our understanding of interventions that are effective for reducing malaria prevalence and increasing intervention coverage among high-risk populations and point to areas where more research is needed. In Chapter 4, a package of interventions including enhanced community case management with a peer community health worker, targeted routine delivery of LLINs, and malaria education was assessed among two high-risk populations: talibés (Koranic school children) and gold miners. Among the talibés, the package was associated with an adjusted 13-percentage point reduction in malaria prevalence (CI's -3 to -22, $p<0.05$) and an adjusted 44-percentage point increase in reported net usage (95% CIs: 36-52, $p<0.001$). This package of interventions can be used to effectively reduce gaps in intervention coverage at Koranic schools (including low coverage and limited use of vector control measures) and reduce barriers in access to health services in order to minimize malaria transmission among Koranic school children. This research also impacts the field by demonstrating the feasibility and effectiveness of a new approach at Koranic schools for delivery of bed nets and strengthening of case management: using peer community health workers. The Koranic school children reported they were comfortable with the net intervention and receiving malaria case management from a peer. There is a great need for interventions specifically targeted at school age children across the African continent [30,48,113,114,127]; this peer CHW approach can be piloted and monitored at other daaras in Senegal and in other countries where school age children are at increased risk of malaria.

The findings in this dissertation reinforce the challenge of reaching malaria high-risk populations that may be hidden or difficult to reach. In Chapter 4, the package of interventions was not associated with a change in malaria prevalence or reported prior-night net usage among the gold miners. Only 36% of gold miners at endline reported they were present at baseline, and the study population changed between baseline and endline (e.g., at baseline, 52% of the gold miners were Senegalese and 22% were from Mali; at endline, 28% were Senegalese and 38% were from Mali), which points to high turnover at the mines. The mobility of the miners and the lack of any formal organization within which to target interventions is a challenge across regions: despite differences in vectors, populations, and geography, gold miners are at higher risk for malaria across tropical Africa, Southeast Asia, and South America [21,31]. Gold miners are also vulnerable populations that face occupational risks in the hard labor of mining and the challenges of being a highly mobile population;

these include worse health problems, basic concerns over where they will eat and sleep, lower access to care, practices of self-medicating for malaria, and risk of consequences of illegal activities in some cases [21,31,133-136]. Worrying about malaria prevention or treatment can be a low priority or accepted as an occupational hazard. Standard malaria control approaches are insufficient for those populations that are both high-risk and hard-to-reach.

Research is ongoing to determine effective interventions for gold mining populations. A recent study in French Guiana on "Malakit", a self-testing and self-treatment kit designed for increasing effective therapy among hard-to-reach populations, found that when associated with training and provision of kits, gold miners were able to self-manage their symptoms and the proportion of patients reporting proper drug regiments increased [143]. Potentially useful interventions for these populations overlap with those for forest-goers in some settings, e.g., hammock nets, travel packs, and the RTS,S/AS01 malaria vaccine for *Plasmodium falciparum*.

However, to overcome the complex challenges of reaching malaria HRPs who are difficult to find, operational solutions such as active community engagement via participatory methods will be the most essential component for determining interventions that will be effective and site-specific. Community-driven research has been utilized in other global health areas including HIV, environmental health, and women's health to ensure the community has a voice in the research affecting them [52,144]. It is essential to engage and empower different segments within HRPs to develop community-generated ideas and execute effective interventions. For example, what barriers prevented the gold miners in Chapter 4 from using the bed nets they received during targeted interventions? Additional in-depth knowledge on the needs of hard-to-reach subpopulations is essential. Studies have found that effective community engagement needs to begin with early involvement of the community and remain an iterative process [52]. A recent report from UCSF's Malaria Elimination Initiative also has identified three key actions to improving community engagement: determining a common definition of community engagement in malaria with funders and stakeholders, instituting requests from funders to include detailed community engagement plans in proposals, and linking existing community structures to disease control programs with systematic community engagement in regional planning [145]. There is great potential to improve the effectiveness of malaria interventions via additional incorporation of community engagement as opposed to vertically imposing interventions [146]. These efforts also align with a global call for the decolonization of global health: this call is a recognition that the field has always been mired in inequitable partnerships in which high-income countries largely impose agendas on low-income countries [147]. Improving community engagement and country ownership is critical to ensuring malaria elimination efforts are equitable, ethical, and effective.

Another area of future research to improve the delivery of malaria interventions is methods for determining the size of HRPs in countries and regions. Successful planning and financing for targeted strategies requires programs to know the size of the population and when they can be accessed; the latter is particularly important for mobile populations. For example, a recent study in Lao PDR found that malaria control programs may have underestimated the magnitude of the forest-going population to which the malaria control program is targeting malaria interventions [104]. Accurately estimated denominators are required to determine coverage and stratified incidence. Methods on population size estimation (PSE) can be integrated into existing services by combining different points of access for capture-recapture or multiplier estimates [148]. These efforts should be rolled out in tandem with qualitative data collection and community engagement with gatekeepers and members of the population to map out when and where to deliver interventions. Future research in malaria can also incorporate advanced methods from HIV for population size estimation such as RDS-adjusted RTM (RadR), a novel method that includes venue mapping with respondent driven sampling (RDS) [149].

Facing future challenges to addressing malaria among high-risk populations: an issue of equity in global health

The challenges to malaria eradication are complex and ever-changing, they include intersections with a changing climate, complex emergencies, ongoing and emerging health crises, and the evolving resistance among malaria parasites and vector species to drugs and insecticides which threaten previous gains. Reaching the persistent pockets of transmission among malaria HRPs is a particularly significant challenge to malaria control programs across regions.

To tackle malaria among HRPs, it is essential for programs and the malaria community to improve malaria operations to target data-driven interventions, incorporate novel methods to find and measure these groups and include them in routine surveillance, and significantly improve community engagement with HRPs and the communities they move between. Areas of future research and recommendations are presented in Table 5.1. These steps require continued investment in research and implementation science to enhance program effectiveness.

Malaria has a long history of affecting the most vulnerable. Populations around the world that are disadvantaged are at higher risk of malaria, severe malaria, and death. It is an issue of global health equity to improve access to prevention, diagnosis, and treatment for malaria high-risk populations. This dissertation contributes to our knowledge on malaria HRPs and highlights the utility of strategies for improving the characterization of these populations and effective methods for targeting malaria interventions to them. Harnessing the continued global momentum towards malaria eradication to address malaria among HRPs can prevent extensive suffering and death and contribute to global health and development goals.

Table 5.1: *Areas of future research and recommendations. HRPs: high-risk populations*

Areas of future research	**Recommendations**
How to best access malaria HRPs across contexts	*Utilization of community driven research to* • Harness the knowledge of subnational malaria control programs • Identify and engage gatekeepers who allow for the collection of information on access points and barriers to uptake • Leverage existing networks linking people; these include places of employment, transportation hubs, locations where travelers congregate or pass through, schools, and shops
What interventions are effective for malaria HRPs	*Consider the incorporation of new and improved tools such as* • Utilizing chemoprophylaxis or targeted drug administration for forest goers and gold miners • Assessing how malaria vaccines that have been approved and are in the pipeline could play a role for HRPs • Implementing vector control interventions that target outdoor transmission such as topical repellants and insecticide-treated clothing • Enact enhanced case finding through methods such as mobile or border health units among others
How to accurately track population size, malaria transmission, and intervention coverage	*Incorporating additional methods from other fields including* • Sampling hard to reach populations; e.g., with respondent driven sampling or time location sampling • Population size estimation for hard-to-reach or hidden populations; e.g., with reverse-capture method, capture-recapture, generalized network scale-up or Bayesian synthesis with anchored multiplier

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