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# Identifying malaria risks amongst forest going populations in Mondulkiri province and Kampong Speu province, Cambodia: a large cross-sectional survey

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## Abstract

**Background** Cambodia strives to eliminate all species of human malaria by 2025, requiring that foci among forest-exposed populations in remote settings be addressed. This study explores malaria risks amongst forest-exposed groups in Mondulkiri and Kampong Speu Provinces, Cambodia as part of a multi-stage study on novel mosquito bite prevention tools (Project BITE).

**Methods** A serial cross-sectional survey explored the demographics, housing structure openness, mosquito bite prevention habits, and protection from malaria amongst three target groups: forest goers who work in the forest, forest dwellers who live in the forest, and forest rangers who patrol forested regions. Malaria prevalence data was collected at three time points using rapid diagnostic tests (RDTs) for febrile individuals and qPCR for all participants. Infection locations and travel patterns of *Plasmodium falciparum*-infected individuals were analysed for clustering and the potential movement of infections.

**Results** 2935 participants were enrolled between October 2022 and February 2023, consisting of 1093 (37%) forest goers and 1787 (61%) forest dwellers across both provinces, and 55 (5%) forest rangers in Mondulkiri province. Most worked outdoors as farmers, day labourers, and forest collectors, and reported going to the forest five to seven days a week. For housing, 29% and 39% of participants reported living in partially open primary and secondary structures, respectively. The main methods of mosquito bite protection used were insecticide-treated nets, wearing long sleeves, and burning mosquito coils, with limited protection during the daytime and outside at night. All febrile individuals had negative RDT test results. For qPCR, 24 *P. falciparum* infections (< 1%) were detected among forest goers and dwellers, clustered in Pu Trom and Pu Nhav villages in Mondulkiri Province, and Banteay Roka and Banteay Roka Kirisenchey (M) villages in Kampong Speu Province. *Plasmodium vivax* cases were detected (216 cases, 5%) across all enrolled villages. Only two infections were found in forest rangers.

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**Conclusion** Malaria elimination strategies for forest-exposed populations in Cambodia should focus on vector intervention strategies that offer protection during the day and outside at night, and drug-based strategies to clear subpatent infections, targeting forest goers and dwellers in villages where cases are detected.

**Keywords** Malaria, Malaria elimination, Vulnerable population, Forest malaria, Forest dweller, Vector control, Mosquito, Asymptomatic

## Background

The Greater Mekong Subregion has made tremendous progress towards its goal to eliminate human malaria by 2030 [1]. This region has the highest prevalence of *Plasmodium falciparum* parasites that are resistant to artemisinin-class anti-malarial drugs, and intensive efforts to eliminate this species of malaria regionally have contributed to a 97% reduction in malaria deaths, and 77% reduction in all malaria cases between 2012 and 2022 [2]. Within this region, Cambodia, the epicentre of drug-resistant *P. falciparum* parasites, is undergoing last mile efforts for elimination, with only 1384 infections detected in 2023 [3] and no malaria-related deaths reported in country since 2017. Remaining infections are concentrated in remote locations in forested areas, often along international borders [4]. To meet its goals to eliminate human malaria nationally by 2025 [5, 6], Cambodia will need to clear malaria infections among high-risk populations in forested locations where access to healthcare is poor [7–9] and outdoor malaria transmission is common [10–13].

This project is a part of a multi-stage study, Project Bite Interruption Towards Elimination (BITE), on novel mosquito bite prevention tools distributed in a “forest pack” to guide the last mile efforts in Cambodia and potentially other locations challenged by forest malaria transmission. In earlier phases of the project, a pilot study was conducted in Monduliri province investigating malaria risk factors [14], the efficacy of insecticide treated clothing and spatial repellents to prevent mosquito bites [15, 16], and their acceptability among users [14]. These interventions were chosen with the intent of providing continuous protection for forest-frequenting populations when they were not protected by insecticide-treated bed nets. Results were applied to this project, which is a large-scale risk factor assessment to characterize high-risk populations in Monduliri and Kampong Speu provinces. This study intends to provide insight on who is at risk of malaria with regard to demographic characteristics and why they are at risk based on the openness of their housing structures and methods of protection from mosquito bites in use. Results are intended to inform targeting efforts for high-risk populations for malaria, regarding who these people are, the times in which they are most

in need of protection from mosquito bites, and whether they are indoors or outdoors during those times.

Villages included in this study are some of the highest incidence malaria settings remaining in Cambodia [12], where malaria elimination efforts have been challenged by a high prevalence of outdoor-biting vectors including *Anopheles dirus*, *Anopheles minimus*, and *Anopheles maculatus* mosquitoes [7, 8, 11, 12, 17]. In addition to risk factor investigation this study includes malaria diagnosis data using rapid diagnostic tests (RDTs) and qPCR, providing insight on the nature of infections (symptomatic versus asymptomatic), whether they are geographically clustered, and whether human movement is a factor affecting transmission patterns. Additional outcomes from the parent study, including the rollout and user acceptability of mosquito bite prevention tools to this cohort, will be reported elsewhere. The BITE study results intend to inform the evidence base on eliminating forest malaria in Cambodia and in locations with similar risks of malaria.

## Methods

### Study design

This was a serial cross-sectional survey among forest-exposed individuals. Data was collected at three timepoints: October 2022 (Timepoint 0; T0) for baseline data collection, and two follow-up timepoints in December 2022 (T1), and February 2023 (T2). Due to loss to follow-up, additional participants were recruited in T1. Most risk factors were identified from the baseline survey for new participants recruited at T0, such as their demographics, housing structures, previous history of malaria infection, and malaria prevention tools in use prior to study initiation. New participants recruited in T1 had basic demographic data collected, and for some variables used results collected in T1 and T2, for example time spent in the forest, to gain further insight on whether those risk factors changed throughout the malaria season. At all three timepoints, malaria diagnosis was also conducted using RDTs for febrile individuals and dried blood spots collected for all individuals for subsequent identification of parasite infection using qPCR [18]. All positive cases identified were mapped to villages where participants resided, to check for clustering patterns. For

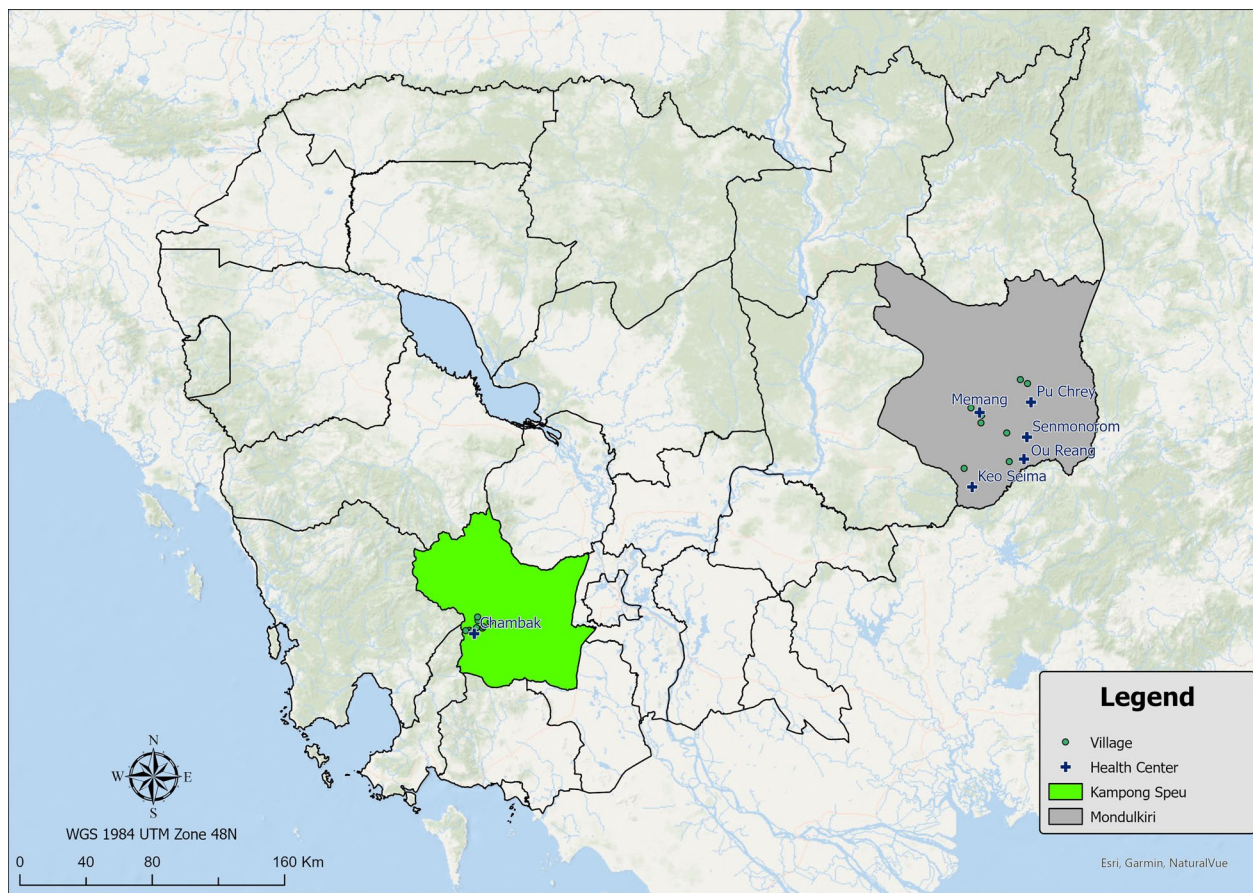
*P. falciparum*, travel patterns were investigated for potential associations between human movement and parasite incidence. This was not done for *Plasmodium vivax* because incident infections could either be new ones or relapses.

**Study location and population**

This study took place in Mondulakiri Province and Kampong Speu Province, Cambodia (Fig. 1), where malaria peak case rates occur during the rainy season from August to January. The targeted study population included three high-risk populations for malaria: forest goers, forest dwellers, and forest rangers [10, 13–17], the first two of which were defined during the pilot phase of this study [14]. Forest goers were individuals who lived at least 1 km from the forest, travelling to the forest regularly for seasonal farming, hunting, or foraging (mushrooms, vegetables, and resin) or seasonal migration for gem mining, logging, and plantation work [11, 14]. Forest dwellers survived on subsistence farming, living in the forest or within 1 km of its fringes in a traditional house in a village for at least part of the year. Many forest

dwellers also had a more open, temporary structure in the farm or forest that they migrated to during planting and harvesting seasons referred to as secondary living structures [14]. Forest rangers were recruited from Mondulakiri province only; like all rangers in Cambodia they worked for government or wildlife and conservation agencies that protect the forest and areas near international borders, staying in ranger stations or outdoor hammocks up to 16 nights per month when they were on patrol [14]. This was because the majority of rangers in Cambodia are in Mondulakiri province, with 115 rangers last documented in country. The other province with many rangers is Ratanakiri, with 85 rangers, and additional ones in Steung Treng and Preah Vihear. There are very few rangers in Kampong Speu province.

Mondulakiri and Kampong Speu have very different environmental features (Fig. 2). Mondulakiri, referred to as the Wild East, features Cambodia’s largest remaining evergreen and deciduous forests, rolling hills and mountains, as well as several rivers like the Sekong and Srepok, alongside waterfalls and lakes. The province experiences strong winds, particularly during the



**Fig. 1** Map of Cambodia with study provinces, villages, and health centre locations



**Fig. 2** Typical environmental features in Mondulkiri (left) and Kampong Speu (right)

dry season, and has a tropical climate with hot, humid conditions in the wet season from May to October and cooler temperatures in the dry season from November to April. In contrast, Kampong Speu has a more temperate southern environment characterized by less dense forests due to significant deforestation, rolling hills and plains, and waterways. The winds in Kampong Speu are generally lighter, and while it too has a tropical climate with year-round hot and humid conditions, it tends to be slightly cooler than Mondulkiri because of its lower elevation.

Villages for the study were selected based on consultation with the Cambodian National Center for Parasitology, Entomology and Malaria Control (CNM), who identified locations with actively identified *P. falciparum* foci that would likely benefit from receiving vector control forest packs for the parent study. “Village” refers to geographic regions that contain residential living structures or ranger stations in forested or non-forested locations, which were defined based on an administrative level within the Cambodian governmental system. Households in these villages were randomly selected, and all eligible participants in that household were invited to join the study until the target sample size was reached.

#### Inclusion criteria

Individuals eligible for inclusion had to:

1. Reside in a village selected for the parent study, which required that they:
  1. Were accessible by automobile or motorbike from September through January
  2. Had access to routine malaria data

3. Had leaders with existing relationships with both the government and implementing partner
2. Meet the definition of one of the three target populations at the time of enrolment:
  1. Forest goer: lived outside the forest (more than 1 km from forest edge) with self-reported travel into the forest at least 15% of the time (approximately 1 day a week)
  2. Forest dweller: lived in a village in the forest (or within 1 km of forest edge)
  3. Forest ranger: actively conducted forest patrol activities and was based at a ranger station
3. Be willing to meet study staff on a monthly basis for study follow-up activities, including meetings and the collection of finger prick blood samples for malaria testing
4. Be age  $\geq 3$  years
5. Provide informed consent if aged  $\geq 18$  years, or obtain consent from guardian if  $< 18$  years of age
6. Speak Khmer and/or Bunong language

#### Recruitment

Individuals from the three target populations were recruited using different methods. For forest goers and dwellers, the study team worked closely with the local government, implementing partners, village chiefs, and other influential members of the communities, to gain support for the study and determine the best methods for participant recruitment. To recruit forest rangers, our study team partnered with the Wildlife Conservation Society (WCS), engaging with manager-level forest

rangers in three ranger stations in Mondulkiri province only, because there were very few ranger stations operating in Kampong Speu.

When recruiting prospective participants, the study team introduced themselves and checked if individuals met the study inclusion criteria. Those eligible and interested to partake in the study were provided with detailed information about the study including the purpose of the project, potential risks and benefits of participation, project duration, and expectations of participants for the study. The study team emphasized that individuals could refuse enrolment in the study or remove themselves from the study at any time without repercussion. After these details were provided, informed consent was sought in Khmer or Bunong. Those able to read were given the informed consent form, and those unable to read had the form read aloud to them. Those who provided informed consent were then enrolled in the study. Eligible individuals from each village were recruited until the target number of participants was reached.

#### Data collection and analysis

At T0, a survey questionnaire was administered to all participants capturing village, household, and individual-level demographic information, housing construction, and use of vector control tools at baseline. For household level data, one answer per household was collected, usually by the head of the household or another knowledgeable adult living in the household. For individuals, participants of all age groups were interviewed; parent were present to assist younger children in answering questions if needed. At T1 and T2, follow-up surveys were conducted that included basic demographic data, self-reported travel, and time spent in the forest over the past two weeks. Surveys are in the supplemental information; S1 was for village demographics at all timepoints, S2 was for detailed household and individual demographic information at T0, and S3 was for simpler information at T1. All survey instruments were developed in English, translated to Khmer, and field tested. Written Bunong language was recently developed and very few people can read or write the language, so when conducting surveys in this language the data collector verbally translated each question and recorded answers in Khmer.

Data collection was conducted using the Open Data Kit (ODK), with the ONA cloud service for secure data storage, which helped minimize entry errors through the use of tablets. The data collectors and their supervisors underwent thorough training on both data entry and validation processes. During the collection phase, a tracking form was used for each village, allowing supervisors to verify the data on the tablets and ensure accuracy throughout the process. Before heading into the field, all

tablets were fully charged, and a power bank was brought along to recharge devices if needed. After returning from the field, data collectors uploaded their entries to a cloud server on a daily basis. The data manager then cross-checked the uploads against the tracking forms provided by supervisors to confirm that all collected data had been successfully uploaded. Each day, investigators reviewed the summary information from the field on the ONA dashboard. Once data collection was completed, the entire database was downloaded onto password-protected computers for analysis by a member of the study team using Stata (StatCorp Version 14). Data were analysed descriptively, with results stratified by target populations as these may have different risk profiles for malaria. Data was also analysed by province, and where differences were found results were separated accordingly. For risk factors that were not expected to vary with time, such as demographic data and housing structure construction, data from all participants in T0 and all new participants in T1 were combined to reflect the backgrounds of all enrolled individuals. For time spent in the forest, data from T1 and T2 were analysed separately as malaria incidence was expected to decrease throughout the study period.

When each survey was conducted, RDTs for malaria were administered to participants who said they had a fever (temperature above 37 °C) during the survey. Dried blood spots were collected from all participants for later diagnosis using qPCR, which entailed extracting and amplifying DNA using a sensitive method that can detect infections below the detection limit of RDTs and microscopy [18]. Positive qPCR-detected infections were speciated, and the characteristics of infected individuals and their village of residence were analysed, as well as self-reported travel patterns for those infected with *P. falciparum* malaria.

#### Sample size calculation

The final sample size implemented was based on requirements for the parent study looking at malaria prevalence at each timepoint following the distribution of various vector control tools and the number of the number of forest packs available and distributed. Overall at least 2100 participants were included at each time point (total of 2935).

#### Results

An overview of villages, households, and individuals enrolled during the study is shown in Table 1. Participant demographics are then described, followed by several risk factors for malaria including housing characteristics, time spent in the forest, and mosquito bite prevention tools used. Next, we summarize individual malaria

**Table 1** Overview of survey data collected at study timepoints

Survey level	Survey timepoint			Total
	T0	T1	T2	
Village	16	2*	0	18
Household	990	313*	0	1303
Individual				
Total	2111	2192	2047	6350
New enrolment	2111	824	0	2935
Survey conducted	Baseline survey	Follow-up survey	Follow-up survey	–

\* New villages and households enrolled at T1

history, cases identified using RDTs and qPCR, the locations of positive identified cases, and travel patterns for incident *P. falciparum* infections.

### Village demographics

The villages enrolled for forest dwellers and goers are described below. A total of 18 villages were enrolled, with nine in Mondulkiri and nine in Kampong Speu province (Table 2). Villages in Mondulkiri had higher average numbers of households (199) and individuals (865) as compared to Kampong Speu, which had an average of 107 households and 375 individuals per village. For accessibility by car, this was lower in Mondulkiri, at 56%, as compared to 100% in Kampong Speu. Accessing high-risk villages in Mondulkiri presented significant logistical challenges, requiring arduous journeys. When car access was not possible, we used motorcycles or specialized forest trucks to reach villages. This required much more effort as compared to Kampong Speu, where accessibility was generally higher. Per inclusion criteria, all villages were accessible by motorcycle during the rainy season when the study took place. The number of Khrom, smaller clusters of households which are often separated from the main village but still included as part the village, was close to three per village for both provinces. Village data was not applicable to forest rangers, who were

recruited from three ranger stations in Mondulkiri province.

### Housing demographics

Forest goers and dwellers enrolled at T0 were asked about their household amenities, and findings were similar between the two risk groups and provinces. For primary water source the most common answer as bottled water used in approximately 30% of households, almost half (47%) had flush toilets while approximately one third (34%) had no toilet, and more than half (61%) of households had electricity (Table S1).

### Participant demographics

The study enrolled 2,935 individuals, including 1,093 (37%) forest goers, 1,787 (61%) forest dwellers, and 55 (2%) forest rangers (Table 3). Demographics were similar when comparing provinces, except that Mondulkiri Province had 59% of individuals of the Bunong ethnic group and 1% of other ethnicities while in Kampong Speu, all participants were Khmer (Table S2). The predominant ethnic group overall was Khmer, although approximately a third of forest goers and dwellers were Bunong, with a small number of participants in each group being from other minority ethnic groups. The majority of participants were ages 26–45, with an average age of 33. Forest goers and rangers

**Table 2** Village summary data

Village characteristic	Location	
	Mondulkiri	Kampong Speu
Villages enrolled	9	9
Number of households per village (average)	199	107
Village population (average)	875	374
Villages accessible by car during the rainy season (%)	56%	100%
Villages accessible by motorcycle during the rainy season (%)	100%	100%
Number of Khrom associated with village (average)	2.8	3.2

**Table 3** Participant demographics

Characteristic	n (%)	Risk group (%)		
		Forest Goer	Forest Dweller	Forest Ranger
<i>Basic demographic data collected on all new participants (T0 and T1)</i>				
Total individuals				
n (%)	2935 (100%)	1093 (37%)	1787 (61%)	55 (2%)
Province				
Mondulkiri	1510 (51%)	59	45	100
Kampong Speu	1425 (49%)	41	55	0
Age				
< 18	517 (18%)	11	22	0
18–25	540 (18%)	17	20	2
26–45	1253 (43%)	49	37	85
46–65	563 (19%)	21	18	13
> 65	62 (2%)	2	3	0
Gender				
Male	1434 (49%)	59	41	96
Female	1493 (51%)	41	59	4
Other/not specified	4 (< 1%)	< 1	0	0
<i>Detailed demographic data collected from new participants at T0 only</i>				
Total individuals				
n (%)	2111 (100%)	730 (35%)	1,339 (63%)	42 (2%)
Ethnic group				
Khmer	1444 (68%)	70	67	81
Bunong	650 (31%)	28	33	17
Other	17 (1%)	2	< 1	2
Languages				
Khmer				
Understand spoken	2100 (99%)	99	99	98
Speak fluently	2014 (95%)	95	95	100
Reading	1259 (60%)	55	61	100
Writing	1210 (57%)	53	58	100
Bunong				
Understand spoken	753 (36%)	33	37	43
Speak fluently	673 (32%)	30	33	38
Reading	139 (7%)	4	8	7
Writing	107 (5%)	3	6	2
Household position				
Head of household	879 (42%)	54	34	91
Spouse of head (husband/wife)	434 (35%)	17	23	0
Child of head (son/daughter)	668 (54%)	24	36	5
Parent of head (father/mother)	26 (2%)	1	2	0
Other	104 (9%)	5	5	5

were predominantly male (59% and 96%, respectively), whereas dwellers reflected a smaller proportion of males compared to females (41% males). For position in their household, the vast majority of rangers were head of household, as well as more than half of the forest goers enrolled. For those who were not

head of household, approximately half of participants were adults who were the son or daughter of the head of household, and results were similar for each target group.

Participants enrolled at T0 were given a list of options on their sources of income. The main income sources

reported by participants required spending time outside in the forest, with the most common income source being a farmer, which was represented by almost half of all participants (47%), including 26% of forest rangers who sometimes had more than one job (Table 4). For forest dwellers and rangers, other common sources of income included day labourers, which could include work in unskilled construction (e.g., rubber industry, rice mills), and forest collectors or foragers who gathered supplies from the forest.

#### Household amenities and structure openness

Participants in all risk groups enrolled at T0 were asked about how open their living structures were, to determine their vulnerability to mosquito bites when spending time indoors. Primary living structures were similar across provinces (table S4) and across risk groups, with the majority of structures (71%) being closed with walls and a ceiling or roof (Table 5). The next most common answer (28%) were partially open structures with two to three walls and a ceiling. When asked whether participants had a secondary structure in the forest or farm, this was higher (57%) in Mondulkiri province as compared to Kampong Speu (20%) (table S3), and different between risk groups with most (95%) of forest rangers having a secondary structure, as compared to half

**Table 4** Participant sources of income (T0)

Income sources*	Total (%)	Risk group (%)		
		Forest Goer	Forest Dweller	Forest Ranger
Total individuals	2111 (100%)	730 (35%)	1339 (63%)	42 (2%)
Farmer	1687 (47%)	85	79	26
Day labourer	644 (18%)	36	28	0
Forest collector/forager	578 (16%)	37	23	0
Logging	180 (5%)	17	4	0
Market trader	149 (4%)	7	7	14
Unemployed	59 (2%)	< 1	4	0
Driver/motorbike Taxi	11 (< 1%)	0	1	0
Retired	9 (< 1%)	< 1	< 1	2
Handicrafts (basket weaving, etc.)	3 (< 1%)	< 1	0	0
Other	200 (6%)	4	12	19

\* Individuals may list more than one source of income

**Table 5** Living structure characteristics (T0)

Structure characteristics	Total	Risk group (%)		
		Forest Goer	Forest Dweller	Forest Ranger
Total individuals n (%)	2111 (100%)	730 (35%)	1339 (63%)	42 (2%)
<i>Primary living structure</i>				
Enclosed room with walls and a ceiling or roof	71%	74	70	64
Ceiling and 2–3 walls	28%	25	30	36
Only ceiling	< 1%	1	0	0
Completely open	< 1%	< 1	0	0
<i>Secondary living structure</i>				
Have secondary structure in forest or farm?	39%	52	31	95
Enclosed room with walls and a ceiling or roof	33%	25	44	3
Ceiling and 2–3 walls	5%	7	3	0
Only ceiling	44%	50	39	50
Completely open	17%	17	14	48



(52%) of forest goers and 31% of forest dwellers. Most of these only had a ceiling (44%), posing risks of getting mosquito bites, with the next most common structure being enclosed (33%). Structure characteristics were similar when comparing risk groups.

#### Time spent in the forest

To understand the risk factors for getting malaria in the forest, participants at T0 were asked how often they go to the forest during the dry and rainy seasons. Results were similar in both provinces, with an average of approximately six days per week. This was approximately seven days a week for forest dwellers in both dry and rainy seasons (as they most often lived directly inside the forest), five to six days per week for forest goers with slightly higher frequency during the rainy season, and approximately five days a week for forest rangers.

During follow-up surveys at T1 and T2, participants were asked how many days they spent in the forest in the past week (Table 6). Results were similar between provinces (Table S5) and timepoints, with 85% of participants reporting going to the forest during the past week, with higher frequencies seen for forest rangers (98%) compared to forest dwellers (93%) and forest

goers (71%). Those who went to the forest spent an average of 5 days in the forest every week, with 88% of forest dwellers reporting that they went to the forest daily, as compared to 59% of forest rangers and 34% of forest goers.

#### Baseline mosquito bite prevention tools used

At T0, participants were asked about the mosquito bite prevention tools they used, not including the tools that were provided as part of the parent study after this survey. At a household level for forest dwellers and goers, almost all (97%) owned a bed net, most of which were treated with insecticides (79%) (Table 7). More than half (66%) of households enrolled also owned at least one hammock net of which most (84%) were treated with insecticides. Bed net and hammock ownership were very similar when comparing forest goers and dwellers.

Participants at T0 were also asked about which mosquito bite prevention tools they used indoors or outdoors, during the day and night. Almost all participants reported using protective measures inside at night. When outside at night, protection was often used, especially for rangers (91%) as compared to forest dwellers (74%) and goers (66%). During the daytime, less protection from mosquito bites was used, with similar levels seen indoors

**Table 6** Time spent in the forest (T1 and T2)

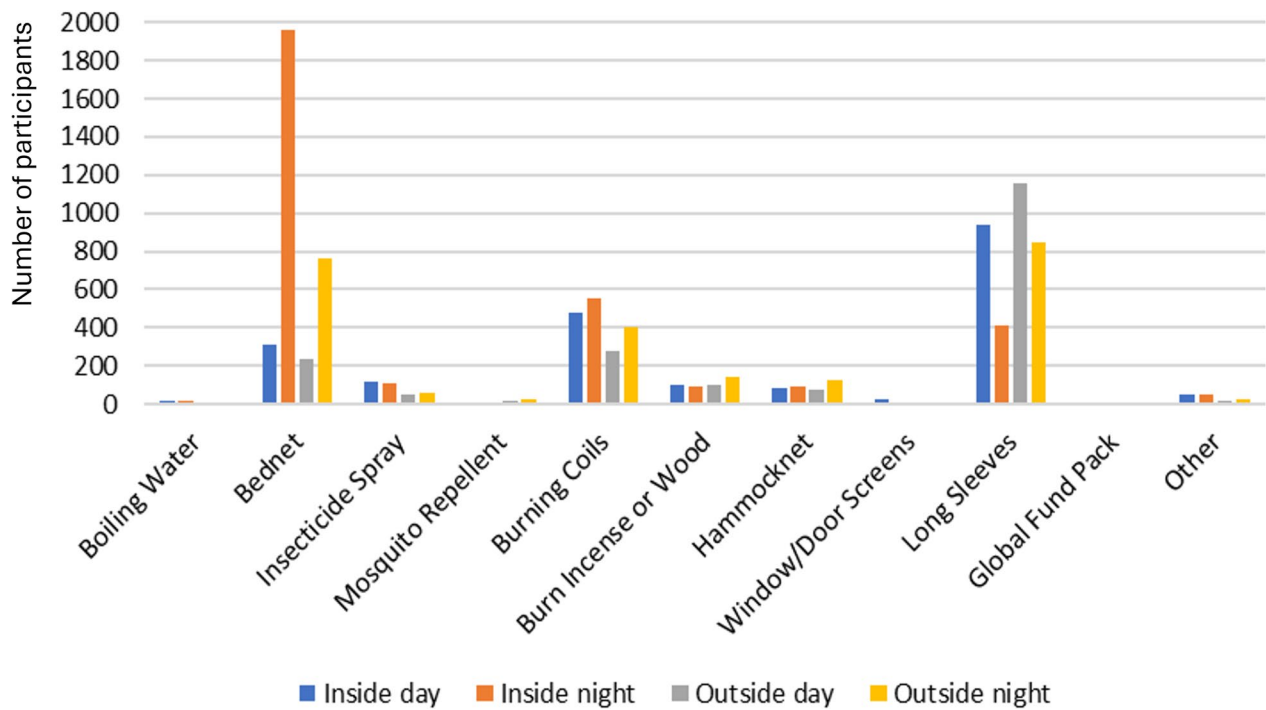
Time spent in the forest in past week	Total (%) (n = 4239)	Risk group (%)		
		Forest Goer (n = 1522)	Forest Dweller (n = 2622)	Forest Ranger (n = 95)
Did not go to forest	632 (15%)	29%	7%	2%
Went to forest every day	2889 (68%)	34%	88%	59%
Went to forest but not every day	716 (17%)	37%	5%	39%
Average number of days*	5.1	5.2	4.4	6.4

\* For those who went to the forest

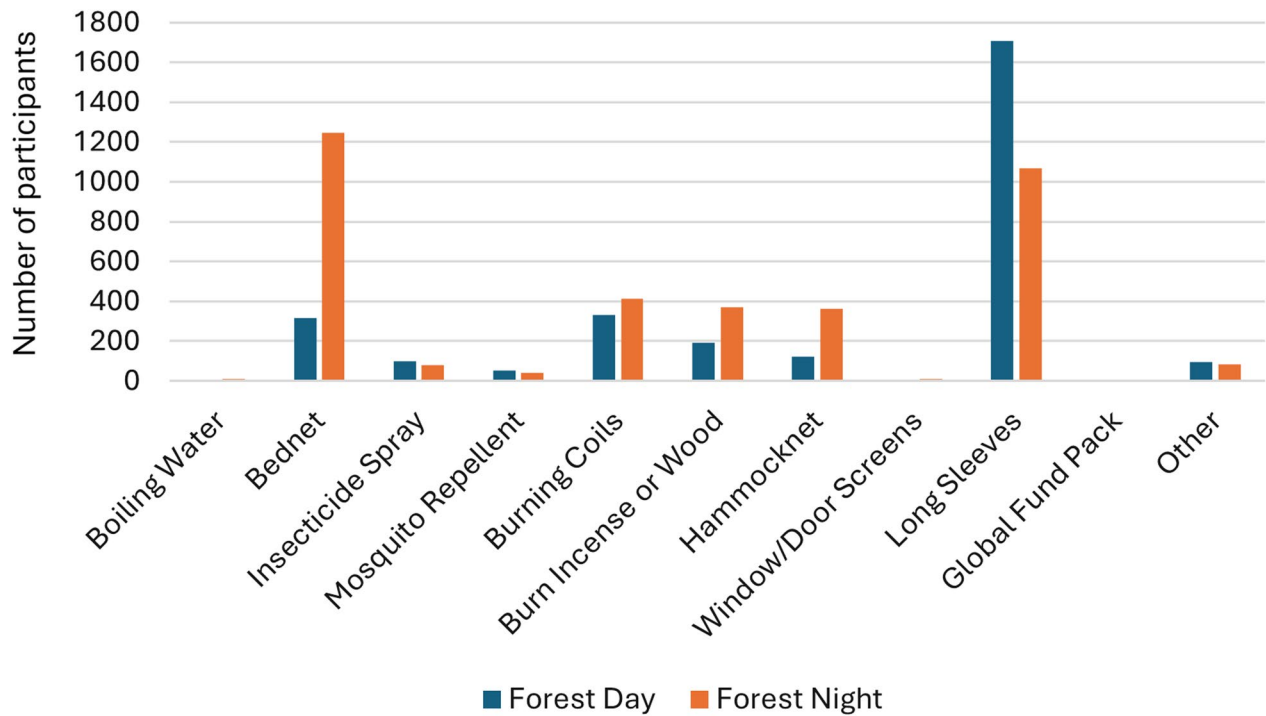
**Table 7** Bed nets and hammocks owned by households

Tool ownership	Total (n = 1303)	Risk group	
		Forest Goer (n = 711)	Forest Dweller (n = 592)
Bed net			
Yes	97%	96.5%	97.5%
How many	2.5	2.4 (range 0–10)	2.6 (range 1–10)
Treated*	79%	74.8%	83.9%
Hammock net			
Yes	66%	64.3%	68.4%
How many	1.5	1.5 (range 1–5)	1.6 (range 0–9)
Treated*	84%	81%	89%

\* Refers to treatment with insecticides (self reported)



**Fig. 3** Mosquito prevention methods used in villages



**Fig. 4** Mosquito prevention methods used in the forest

and outdoors amongst all risk groups. Forest rangers had more than 80% protection outdoors, while dwellers had closer to 70% and goers around 55%.

When asked about specific tools used at different times and locations, results were similar when comparing their use in villages (Fig. 3) and in the forest (Fig. 4) across all target groups. Sleeping under insecticide-treated nets was the most common method of protection, both indoors and outdoors at night, while wearing long sleeves in all circumstances except for being inside at night, when bed nets were presumably preferred. The third most common method reported was burning coils. A Global Fund pack comprised of an insecticide-treated hammock net and topical repellent distributed by health workers and funded by the Global Fund to fight AIDS, Tuberculosis and Malaria.

**Travel patterns**

To understand general levels of mobility and travel, participants at T0 were asked how far they travel to buy necessities. Most participants reported having purchasing activities within the range of 500 m from their primary residency location, especially forest dwellers. Forest rangers generally reported having a greater range of travel distance, with 45% of them reporting buying things from places located more than 5 km from their residency location (Table 8).

At T1 and T2, participants were asked whether they travelled to other villages. Only a small proportion of forest goers and dwellers (18 to 21%) reported travelling to other villages in the past 30 days (Table 9). This was a bit higher for forest rangers, 35 to 43% of whom reported travelling to other villages within that time-frame likely due to their work entailing travel throughout the forest, where the area of their primary ranger station was defined as their home “village.” Participants were also asked about whether they had travel companions; almost all individuals travelled with people from the same villages. Travel patterns between timepoints was similar.

**Malaria prevalence**

Malaria prevalence was assessed at each timepoint. RDTs were administered to participants who reported having an active fever, which was a total of 43 RDTs throughout the test period, all of which were negative. qPCR was conducted on dried blood spots collected from each participant at all three timepoints. This revealed a number of *P. falciparum* and *P. vivax* asymptomatic infections that are described sequentially below and mapped in supplemental Figs. 1 and 2.

***Plasmodium falciparum* prevalence**

The prevalence of asymptomatic molecularly determined *P. falciparum* infections was similar at approximately 0.5% at both T0 and T1, which dropped to 0.2% in T2,

**Table 8** Travel patterns for purchasing necessities (T0)

Distance travelled	Total (n = 2111)	Risk group (%)		
		Forest Goer (n = 730)	Forest Dweller (n = 1339)	Forest Ranger (n = 42)
Under 500 m	1686 (80%)	79	84	19
500 m to 2 km	166 (8%)	11	5	33
2 to 5 km	75 (3%)	3	4	2
More than 5 km	184 (9%)	8	7	46

**Table 9** Travel to other villages during T1 and T2

	Total (%)		Risk group					
			Forest Goer		Forest Dweller		Forest Ranger	
	T1	T2	T1	T2	T1	T2	T1	T2
Total individuals	2192	2047	801	721	1345	1277	46	49
Travel to another village in the past 30 days	400 (18%)	406 (20%)	143 (18%)	155 (21%)	237 (18%)	234 (18%)	20 (43%)	17 (35%)
Travel companions								
People from the same village	1858 (85%)	1654 (81%)	610 (76%)	447 (62%)	1228 (91%)	1161 (91%)	20 (43%)	46 (94%)
People from other villages	11 (<1%)	5 (<1%)	4 (<1%)	4 (<1%)	2 (<1%)	1 (<1%)	5 (11%)	0 (0%)
People from the same and other villages	64 (3%)	15 (<1%)	12 (1%)	5 (<1%)	31 (2%)	9 (<1%)	21(46%)	1 (2%)

**Table 10** *Plasmodium falciparum* qPCR infections and their distribution

Timepoint	Total	Province		Gender*		Risk group		
		Mondulkiri	Kampong Speu	Males	Females	Forest Goer	Forest Dweller	Forest Ranger
T0								
n	2111	1104	1007	999	1108	730	1339	42
Pos (%)	11 (0.52%)	8 (0.72%)	3 (0.30%)	6 (0.60%)	5 (0.45%)	5 (0.68%)	4 (0.30%)	2 (4.76%)
T1								
n	2192	1113	1079	1020	1090	801	1345	46
Pos (%)	10 (0.46%)	9 (0.81%)	1 (0.09%)	6 (0.59%)	4 (0.37%)	4 (0.50%)	6 (0.45%)	0 (0%)
T2								
n	2047	1089	958	935	1026	721	1277	49
Pos (%)	3 (0.15%)	2 (0.18%)	1 (0.10%)	0 (0%)	3 (0.29%)	1 (0.14%)	2 (0.16%)	0 (0%)

\* T0: 4 individuals with unknown gender; T1: 82 individuals with unknown gender; T2: 87 individuals with unknown gender. 0 positives of unknown gender at all three timepoints

an expected result due to declining malaria seasonality throughout the study period (Table 10). Prevalence was higher in Mondulkiri province as compared to Kampong Speu, with similar distribution between males and females. While a prevalence of *P. falciparum* of 4.8% was found in forest rangers at T0, no infections were subsequently found in this group. Forest goers and dwellers had roughly the same number of infections across all time points.

When investigating infection locations and travel patterns of infected individuals, *P. falciparum* infections were found to be clustered in villages in both provinces studied. In Mondulkiri province, 67% of cases were concentrated among forest dwellers in Pu Trom and Pu Nhav villages (Table 11). In Kampong Speu, 60% of *P. falciparum* cases were concentrated amongst forest dwellers in two villages as well, Banteay Roka and Banteay Roka Kirsenchey (M). Only three of the nine villages and two of three ranger stations enrolled in Mondulkiri province, and four of nine Kampong Speu villages included in the study had *P. falciparum* infections.

When asked about travel history, none of the individuals with qPCR-positive *P. falciparum* infections reported travelling to other villages within 14 days of positive blood sample collection. In T0 no information was collected about travel to the forest, and this information was only available for some detected cases in T1 and T2, finding that individuals with asymptomatic *P. falciparum* malaria often travelled to the forest. Travel to villages was, therefore, not a risk factor amongst these cases, while going to the forest was associated with asymptomatic *P. falciparum* infection.

#### *Plasmodium vivax* prevalence

The prevalence of asymptomatic *P. vivax* infections detected by qPCR-positive infections was higher than

**Table 11** Residence locations of *P. falciparum* positive cases

Province	Residency location (village)	Target Group
<i>Infections detected at T0</i>		
Mondulkiri	Tu Trom	Forest dweller
	Tu Trom	Forest dweller
	DA	Forest goer
	DA	Forest goer
	DA	Forest goer
	Pu Nhav	Forest goer
	Ranger Station 1	Forest ranger
	Ranger Station 2	Forest ranger
	Kampong Speu	Banteay Roka Kirsenchey (M)
Banteay Roka Kirsenchey (M)		Forest dweller
Banteay Roka Kirsenchey (M)		Forest goer
<i>Infections detected at T1</i>		
Mondulkiri	Pu Khav	Forest goer
	Pu Khav	Forest goer
	Pu Khav	Forest goer
	Pu Khav	Forest goer
	Tu Trom	Forest dweller
	Tu Trom	Forest dweller
	Tu Trom	Forest dweller
	Tu Trom	Forest dweller
	Tu Trom	Forest dweller
	Tu Trom	Forest dweller
Kampong Speu	Banteay Roka Kirsenchey (M)	Forest dweller
<i>Infections detected at T2</i>		
Mondulkiri	Tu Trom	Forest dweller
	Pu Nhav	Forest goer
Kampong Speu	Doung Kraong Meanchey (M)	Forest dweller

for *P. falciparum*, with an overall prevalence of 4.1% that decreased throughout the study period (122 infections at T0, 78 at T1, and 61 at T2) (Table 12). When comparing between provinces, Kampong Speu had more *P. vivax*

**Table 12** *Plasmodium vivax* qPCR positive cases and their distribution

Timepoint	Total	Province		Gender*		Risk group		
		Mondulkiri	Kampong Speu	Males	Females	Forest Goer	Forest Dweller	Forest Ranger
T0								
n	2111	1104	1007	999	1108	730	1339	42
Pos (%)	122 (5.8%)	54 (4.9%)	68 (6.7%)	71 (7.1%)	51 (4.6%)	38 (5.2%)	84 (6.3%)	0 (0%)
T1								
n	2192	1113	1079	1020	1090	801	1345	46
Pos (%)	78 (3.6%)	22 (2.0%)	56 (5.2%)	49 (4.8%)	27 (2.5%)	34 (4.2%)	44 (3.3%)	0 (0%)
T2								
n	2047	1089	958	935	1026	721	1277	49
Pos (%)	61 (3.0%)	18 (1.7%)	43 (4.5%)	35 (3.7%)	23 (2.2%)	23 (3.2%)	38 (3.0%)	0 (0%)

\* T0: 4 individuals and 9 positives with unknown gender; T1: 82 individuals and 2 positives with unknown gender; T2: 87 individuals and 3 positives with unknown gender

infections than Mondulkiri province, males had more infections than females, forest goers and dwellers had roughly the same prevalence of infection across all time points, and no *P. vivax* infections were found in forest rangers.

When investigating locations of *P. vivax* infections, less clustering was observed as compared to *P. falciparum* cases. In Mondulkiri province, Pu Trom village, which had 42% of all *P. falciparum* cases in that province, accounted for 23% of all *P. vivax* cases. A large proportion of *P. vivax* cases were also identified in Andong Kraoeng (28%) and Pu Char (21%), with cases detected in all nine Mondulkiri villages included in the study. In Kampong Speu, the highest proportions of cases were found in Rumduol Thmei (27%) and Peam Lvea (21%) villages, with cases detected in all nine Kampong Speu villages included in the study.

## Discussion

This large-scale serial cross-sectional study identified risk factors for malaria amongst forest-exposed populations in Mondulkiri Province and Kampong Speu Province, finding that in transmission foci in these provinces, participants often worked outdoors as farmers, day labourers, and forest collectors, some (28%) of whom also lived in open structures with two to three walls and a ceiling in their primary residence. Some participants (39%) also reported having a secondary structure they lived in, which was often open, 44% of which had only ceilings. The most common malaria prevention tools used were bed nets, wearing long sleeves, and burning insecticide-treated coils. All infections detected during the study were asymptomatic, with clustering in villages observed for *P. falciparum* especially amongst forest dwellers, and no association between cases and self-reported travel to other villages. For *P. vivax* incidence was higher (4% as compared to <1% for *P. falciparum*), and infections were found in all enrolled villages among forest goers and dwellers, the latter who reported

going to the forest more often than other risk groups, with 88% reporting spending time in the forest daily in follow-up surveys. For forest rangers, only two *P. falciparum* cases were detected from one ranger station at the baseline survey, and no *P. vivax* cases were detected. This is likely because rangers are provided with mosquito bite prevention tools including government issued hammock nets, mosquito coils, and topical repellents, sometimes also receiving donated products such as insecticide sprays and topical repellent lotions from their employers, the Ministry of Health, and implementing partners supporting malaria elimination efforts.

These findings can be applied directly to malaria elimination efforts in Cambodia. For *P. falciparum*, forest dwellers in villages where infections are found can be targeted for malaria interventions, including Pu Trom and Pu Nhav villages in Mondulkiri and Banteay Roka and Banteay Roka Kirisenchey (M) in Kampong Speu province. *Plasmodium falciparum* elimination will require that asymptomatic infections be addressed, a topic that will be discussed in a separate study that infers its prevalence through comparison with data from Cambodia's malaria information system (MIS). *Plasmodium vivax* malaria was found in all study villages, revealing it is still a risk for forest goers and dwellers living in transmission foci. These findings suggest that in these locations, the prevention and treatment of infections can be targeted geographically, a result consistent with occupational and spatial clustering found in another study in Cambodia [12]. Geographical movement however did not present as a risk factor in this study, suggesting that travel between villages is not a major contributor of asymptomatic malaria transmission. In addition to the need to target forest dwellers and goers, this study also found that forest rangers, despite high amounts of time spent in the forest, had much lower malaria prevalence including no *P. vivax* infections found. This could be due to occupational

health protective measures provided to rangers as shown by higher reported levels of protection from mosquito bites (80%) compared to other risk groups. Malaria elimination efforts in Cambodia can, therefore, target forest goers and dwellers in foci at the village level.

When compared to other studies, these findings provide context to efforts to eliminate forest malaria in a variety of settings. An earlier study conducted in 17 villages in Monduliri Province from 2017–2018 showed higher levels of PCR-detectable infections, with an incidence of 6.4% for *P. vivax* and 3.0% for *P. falciparum*, approximately two to three times greater than that found in this study [12]. The earlier study also detected hotspots of infection in villages, finding that forest work was associated with malaria. These findings suggest that malaria incidence decreased in the study area since 2017, furthermore confirming that clusters of infection at a village level are a risk factor for malaria in these geographies. This study also provides further insights to those found in a pilot study earlier [14], showing that the use of bed nets, wearing long sleeves, and insecticide-treated coils were the most common malaria prevention methods used, and that gaps in protection mostly take place during the day and outside at night.

Targeting these high-risk populations for malaria can combine two approaches. The first is vector control; an evaluation of the distribution and use of forest packs including a topical repellent, a spatial repellent, and insecticide-treated clothes from the parent study is forthcoming and can further inform the selection of vector control tools that can be useful for these populations to prevent malaria. Hopefully forest pack components can overcome the limitations of wearing long sleeves, which was commonly reported in this study, especially when not sleeping indoors, as other Cambodian populations have reported that mosquitoes can bite through clothing [19]. A second approach is chemoprevention, where medicine can be distributed to forest-frequenting populations as intermittent preventive treatment or targeted drug administration to geographic hotspots [20]. This has shown to be effective for forest-going populations in Cambodia when targeting *P. falciparum* malaria [21], although clearing the dormant stages of *P. vivax* is expected to be more challenging [22]. A third approach for which more data is available in sub-Saharan African settings would be housing improvements [23]. In this context it is unclear whether this intervention strategy is feasible for forest-going populations, particularly when staying in secondary living structures when working on forest farms. Portable interventions offering protection are likely more practical solutions for these individuals.

This study had several limitations. It did not specifically include several risk profiles studied in Cambodia, such as illegal loggers [11] and mobile populations that create

temporary forest encampments [19]. These risk profiles were studied several years ago however, and malaria endemicity in Cambodia has since decreased substantially, affirming the approach taken of intervening where malaria hotspots are identified from recently diagnosed cases. The study was also unable to reach high-risk populations that were not accessible by vehicle, reducing generalizability from this study on trends amongst high-risk populations for malaria. It is possible that those living in even more remote locations than participants in this study likely had even less access to mosquito bite protection and spend more time in the forest, as many of these risks arose from living in remote locations in or near the forest. Detailed demographic data was not available for the 824 participants enrolled at T1 as these individuals were enrolled to meet sample size requirements. Although those enrolled at T1 represented approximately 30% of the study population, detailed demographic data on 2111 participants was collected at T0, which should be sufficient to represent these at-risk populations in the village foci selected for inclusion. The study was also not powered to conduct inferential statistics on risk factors. However, given large sample size, the descriptive statistics provide valuable insights on risks of malaria among individuals that spend time in the forest in these study locations.

To support Cambodia's goals to eliminate malaria, findings from this study should be immediately applied to local malaria elimination strategies. Forest goers and dwellers should be targeted for prevention and treatment in hotspots where infections are detected, a strategy that can be further expanded to any location with forest malaria undergoing last mile efforts. The vector control tools available to these high-risk populations can be expanded, with forthcoming reports on the parent study expected to inform the benefits and challenges on the delivery and uptake of topical repellents, spatial repellents, and insecticide-treated clothing in these locations.

### Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12936-025-05290-0>.

Supplementary Material 1  
Supplementary Material 2  
Supplementary Material 3  
Supplementary Material 4

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### Author contributions

DM, DD, NFL, and AT designed and led the cross sectional study. DD led the implementation of the study, VK, PV, SP, VM, SB, KP, SC, KL, KH, and SS implemented the study, and DJM analyzed survey data. IC and NFL designed the molecular components of the study designed to detect and track the movement of infections. JT, DL, and JC analyzed laboratory samples and parasite movement data. IC, DM, DD, EV, AT, and NFL interpreted the results. IC wrote the first draft of the manuscript. All authors reviewed the manuscript.

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### Availability of data and materials

Data is provided within the manuscript or supplementary information files.

### Declarations

#### Ethics approval and consent to participate

The study protocol was approved by the University of California's Human Research Protection Program Institutional Review Board (IRB 22-36956) and the Cambodia Ministry of Health National Ethics Committee for Health Research (NECHR 296). Informed consent was sought by each participant prior to enrolment.

#### Competing interests

The authors declare no competing interests.

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