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Malaria cross-sectional surveys identified asymptomatic infections of *Plasmodium falciparum*, *Plasmodium vivax* and *Plasmodium knowlesi* in Surat Thani, a southern province of Thailand

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ABSTRACT

Objectives: Malaria cross-sectional surveys are rarely conducted in very low transmission settings. This study aimed to determine the prevalence and risk factors of *Plasmodium* infection in a near-elimination setting in southern Thailand.
Methods: Two cross-sectional surveys were conducted in areas of active transmission in the Surat Thani province of Thailand in January and May 2019. PCR was used to detect *Plasmodium* infection.
Results: The prevalence of *Plasmodium* blood infection was 0.45% and 0.61% in January and May 2019, respectively. The major parasite species was *Plasmodium falciparum* in January and *Plasmodium vivax* in May. Unexpectedly, *Plasmodium knowlesi* infections were also detected. Most infections, including those of *Plasmodium knowlesi*, were asymptomatic. Being male and staying outdoors at night-time were the only significant identified risk factors. Of people infected in January 28.0% were positive in May for the same parasite species, suggesting persistent asymptomatic infections.
Conclusions: Despite the very low incidence rate in Surat Thani, most malaria infections were asymptomatic. Outdoor mosquito biting at night-time is likely an important mode of malaria transmission. Unexpectedly, asymptomatic *Plasmodium knowlesi* infection was found, confirming previous reports of such infection in mainland Southeast Asia.

Introduction

Malaria remains one of the world's major scourges. Currently, countries in the Asia Pacific region are aiming to end malaria by 2030 (The Asia Pacific Malaria Elimination Network, 2014). Over the past 5 years, Thailand has continuously reduced its malaria burden; the number of malaria cases has declined by 84% from 34,611 in 2014 to 5425 in 2019 (Ministry of Public Health, 2019).

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However, to reach the elimination goal, this effort will need to be maintained, if not strengthened, in the coming decade.

It has been reported by many studies that the majority of malaria infections in endemic areas are asymptomatic (i.e. not accompanied by clinical symptoms typically associated with malaria illness such as fever and chills) (Baum et al., 2015; Bousema et al., 2014; Chen et al., 2016; Sáenz et al., 2017; Zhao et al., 2018). These infections are often characterised by very low parasitaemia, below the detection limit of light microscopic examination and malaria rapid diagnostic tests (RDTs), which are the current standard diagnoses. While these infections are commonly called asymptomatic, they represent a hidden burden for society as they are associated with recurrent episodes of symptomatic malaria (Douglas et al., 2013), chronic anaemia (Newton et al., 1997), maternal and neonatal mortality (Cottrell et al., 2015), co-infection with invasive bacterial disease (Mabey et al., 1987), and cognitive impairment (Nankabirwa et al., 2013). Many studies have shown that blood from asymptomatic parasite carriers can infect mosquitoes, suggesting that these people contribute to transmission (Alves et al., 2005; Coleman et al., 2004; Kiattibutr et al., 2017; Martins-Campos et al., 2018; Vantaux et al., 2018).

Factors underlying asymptomatic malaria infection have been reported by several studies (Nguitragool et al., 2017; Okell et al., 2012; Tietje et al., 2014; Zhao et al., 2018). However, demographic and risk factors differ across different settings. For instance, being male and aged <15 years were identified as risk factors for asymptomatic malaria in lower endemicity areas but not in higher endemicity areas (Okell et al., 2012). It is therefore important for malaria control programs to determine the risk factors for each target area to efficiently deliver intervention.

Surat Thani is a province in the south of Thailand. It is an epidemiologically distinct setting in the country. It is one of the few inland provinces with active malaria transmission; most other areas are located along the national border, with Myanmar to the west, Laos and Cambodia to the east, and Malaysia to the south. According to the Thai Ministry of Public Health (Ministry of Public Health, 2019), *Plasmodium falciparum* (*P. falciparum*) was the dominant species in Surat Thani. This is in contrast with most other endemic areas in Thailand where *Plasmodium vivax* (*P. vivax*) had become the most common parasite species. During 2017–2019, the ratio of *P. falciparum* to *P. vivax* cases was 3.9 (81 *P. falciparum*: 21 *P. vivax*) in Surat Thani compared with 0.14 (2,828 *P. falciparum*: 19,733 *P. vivax*) in the entire country (Ministry of Public Health, 2019). The overall incidence rate of clinical malaria in Surat Thani is low (0.033/1,000 in 2019), but there are foci of higher transmission. These hotspots mainly comprise rubber and palm plantations located at the outskirt of Ratchaprapha Lake. The complete absence of the C580Y artemisinin resistant marker of *P. falciparum* (Noisang et al., 2019) found elsewhere in the south of Thailand suggests that the population of *P. falciparum* in Surat Thani be isolated.

There have been few malaria studies in Surat Thani. One study in 2010 using light microscopic examination in a malaria clinic in Vibhavadi District estimated the overall prevalence of malaria infection to be 0.6%, of which *P. falciparum* malaria accounted for 66% (58/88 cases) (Inchana et al., 2013). However, a more recent study from Phanom Hospital reported that 97.5% of malaria cases during 2012–2015 were due to *P. falciparum* (385/395) (Kotepui et al., 2017). To date, there has been no published community survey of malaria infection in Surat Thani. The current study conducted two cross-sectional surveys in 2019 to determine the prevalence of malaria infection and the underlying risk factors in the province.

Materials and methods

Study site

The surveys were conducted in 18 villages in four districts of Surat Thani (Figure 1): Chaiya District (Pak Mak subdistrict), Kirirat Nikhom District (Kapao, Nam Hak and Tha Kanon subdistricts), Vibhavadi District (Takuk Nuea and Takuk Tai subdistricts), and Phanom (Khlong Sok subdistrict). These areas were selected because of their high malaria incidence relative to other areas in the province. Surat Thani is located at 9°8′24″N, 99°19′59″E. It had a population size of 1,068,010 in 2019 (Ministry of Interior, 2019). It shares no border with other countries. The climate is tropical, with an average annual temperature of 27.0 °C and average rainfall of 1850 mm. There is a short dry season from January to April, followed by a long wet season from May to December.

Cross-sectional surveys

Two cross-sectional surveys were conducted: one in January (dry season) and the other in May (rainy season) of 2019. All Thai nationals of both sexes, residing in the study villages, aged ≥6 months were eligible. Individuals who could not communicate or were unwilling to provide informed consent were excluded. The study staff visited every household to obtain informed consent and performed fingerprick blood collection. Data on malaria risk factors were obtained using an interview questionnaire (Supplementary Table S1). During the survey, if the body temperature of the participant was ≥37.5 °C or the participant had fever in the previous 2 days, a malaria RDT (CareStart; CareStart Malaria HRP2/pLDH (Pf/PAN) Combo) was performed. If the test was positive, the participant was referred to the nearest malaria clinic for treatment.

Sample processing and Plasmodium *detection by PCR*

Capillary blood samples (~200 μL each) were collected in microtainers with EDTA as the anticoagulant. Samples were placed in a prechilled cooler box (4–10 °C) and within 4 days of collection each sample was separated into pellet and plasma by centrifugation and placed in dried ice. The frozen samples were subsequently shipped to Mahidol Vivax Research Unit, Faculty of Tropical Medicine, Bangkok, and stored in –20 °C freezers. Parasite DNA was extracted from the blood pellets using the QIASymphony SP automated DNA extraction system (Qiagen). Purified blood DNA was eluted in 100 μL of the standard elution buffer and kept at –20 °C. Cultured *P. falciparum* NF54 strain diluted in whole blood at 10 parasite/μL was used as the positive control for DNA extraction and subsequent molecular detection of parasites by quantitative polymerase chain reaction (qPCR).

A genus-specific qPCR assay (QMAL) targeting the 18S rRNA genes of *Plasmodium* spp. (Wampfler et al., 2013) was used to screen for *Plasmodium* infection. The assay, using 6 μL of purified DNA (equivalent to 8 μL whole blood) as the template, was optimised for Rotor-Gene Q thermocycler with 2x QuantiNova™ Probe PCR Master Mix (Qiagen). All samples positive for *Plasmodium* DNA were subjected to *P. falciparum* and *P. vivax* species-specific qPCR assays to identify the parasite species. The *P. falciparum* assay targets the varATS sequences (Hofmann et al., 2015) and the *P. vivax* assay targets *P. vivax* 18S rRNA genes (Rosanas-Urgell et al., 2010). Both assays were optimised for Rotor-Gene Q thermocycler with 2x QuantiNova™ Probe PCR Master Mix (Qiagen) and 6 μL of purified DNA as the template. All qPCR assays were run with the internal standards prepared by serial dilution of plasmids carrying the target sequence at 10^5–10^1 copies/reaction. All qPCR runs successfully detected 10 plasmid copies/reaction.

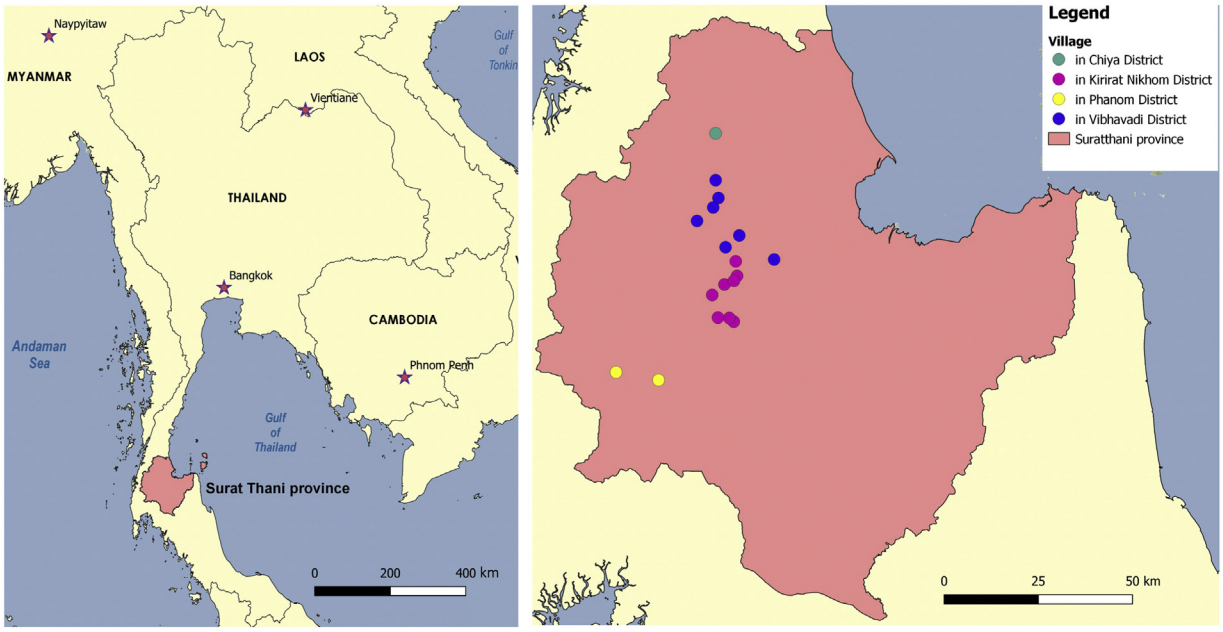

Figure 1. Map of the study site. Left: Surat Thani province. Right: locations of the 18 study villages in Surat Thani, coloured by district.

All samples that were positive for *Plasmodium* infection by QMAL were also subjected to nested PCR, as previously described (Ngernna et al., 2019; Yorsaeng et al., 2019), to identify infection with *Plasmodium malariae*, *Plasmodium ovale* and *Plasmodium knowlesi* (*P. knowlesi*).

Statistical analysis

The prevalence of malaria infection in each survey was presented as percent. Univariate logistic regression was used to first identify potential risk factors ($p < 0.2$) to enter multivariate analysis. The final multivariate logistic regression was performed using SPSS (version 18.0). *p*-Values were based on two-sided test results, and a value of ≤ 0.05 was considered to indicate statistical significance.

Ethical consideration

Written informed consent was obtained from all participants and/or guardians for juveniles aged ≤ 17 years. This study was approved by the Ethics Committee, Faculty of Tropical Medicine, Mahidol University (Submission Numbers TMEC 19-069, TMEC 18-034).

Results

Demographic characteristics of the study population

In total, 9418 individuals participated in the study, and 7034 and 8671 blood samples were collected in January and May 2019, respectively. The median (range) age of the participants was 39 (0.5–108) years. The majority of participants were aged ≥ 15 years (82.6%) and female (50.9%). Additional details are shown in Table 1.

Table 1
Demographics of participants.

Characteristics		No. of subjects (%)
Age (years)		
	0.5–6	494 (5.2)
	7–14	1146 (12.2)
	≥15	7696 (82.6)
	unknown	82 (0.9)
Sex		
	Male	4555 (48.4)
	Female	4794 (50.9)
	unknown	69 (0.7)
Occupation		
	Agriculture/livestock/lumberjack/ mining	5947 (63.1)
	Child/student/unemployed	2402 (25.5)
	Others	1069 (11.4)
District		
	Chaiya	2489 (26.4)
	Vibhavadi	2898 (30.8)
	Kirirat Nikhom	2884 (30.6)
	Phanom	1147 (12.2)
Total		9418

Prevalence of malaria infection

The prevalence of malaria infection (all species) was 0.45% (32 7034) in January and 0.61% (53 8671) in May 2019 (Table 2). All but two infections (both due to *P. falciparum*) had an 18S copy number that indicated parasitaemia <50 parasites/μl (Supplementary Table S2), which is the approximate limit of detection by field microscopy (Coleman et al., 2002). Fifteen of 32 (47%, January) and 14 of 53 (26%, May) participants were positive for *P. falciparum*. Eleven (34%, January) and 18 (34%, May) were positive for *P. vivax*. Unexpectedly, *P. knowlesi* was detected in one (3%, January) and two (4%, May) blood samples by nested PCR (Supplementary Figure S1). These three *P. knowlesi* infections were found in three separate districts. The remaining infections, five (16%) in January and 19 (36%) in May, were negative in the *P. falciparum* and *P. vivax*-specific qPCR assays, as well as nested PCR, presumably because of the low parasite density. Two (6%) and one (2%) participants with malaria infection had fever at the time of blood collection or within the previous 2 days in January and May, respectively; all three were infected with *P. vivax*.

Table 2
Malaria infections by species.

	Malaria infections found in	
Plasmodium species	January 2019	May 2019
P. falciparum	15 (47%)	14 (26%)
P. vivax	11 (34%)	18 (34%)
P. knowlesi	1 (3.1%)	2 (4%)
Unknown	5 (16%)	19 (36%)
Total	32	53

Risk factors for malaria infection

Staying outdoors during night-time was the only common significant risk factor for malaria infection in both surveys (adjusted OR 3.15 and 2.26, 95 % CI 1.57–6.32 and 1.30–3.93, $p = 0.001$ and $p = 0.004$, in January and May, respectively) (Tables 3a and 3b). Being male was a significant risk factor in January (adjusted OR 2.26, 95% CI 1.06–4.78, $p = 0.03$) and was nearly significant in May (adjusted OR 1.72, 95% CI 0.98–3.01, $p = 0.06$). In the May survey, participants living in Kirirat Nikhom District had higher risk (adjusted OR 2.61, 95 % CI 1.29–5.28, $p = 0.02$), while no significant difference was found among the four districts in January. Age group, occupation, fever in previous 2 days, bed net use, indoor residual spraying (IRS) in the previous 4 months, and self-protection against mosquitos were not associated with malaria infection in either survey. Among the 6282 participants who participated in both surveys, 34 were infected in May but not in January. The risk factors for these new malaria infections in May were being male (adjusted OR 2.06, 95% CI 1.01–4.18, $p = 0.046$) and staying outdoor at night-time (adjusted OR 2.10, 95% CI 1.05–4.18, $p = 0.04$) (Table 4). No infected participant reported to have had a malaria episode in the previous 4 months in either survey.

Potential long-term malaria infection

Among the 6282 participants who participated in both surveys, 25 had infection in January (12 *P. falciparum*, eight *P. vivax*, one *P. knowlesi*, and four unknown *Plasmodium* spp). Of these 25 infected participants, one reported to have become sick with malaria between the January and May surveys. Of the rest, 17 (68%) became negative for infection in May, but the remaining seven (five *P. falciparum* and two *P. vivax*) were still positive in May, mostly with the same parasite species (Table 5).

Discussion

This study conducted cross-sectional surveys in four districts of Surat Thani, a southern province of Thailand. It is believed that this is the first report of large-scale malaria surveys in this area. Using qPCR and nested PCR, malaria prevalence was determined in the dry (January) and rainy (May) seasons of 2019. Despite the sensitive molecular diagnostics that were used, the prevalence of malaria infection was low at 0.45% in January and slightly increased at 0.61% in May. Notably, almost all infections were asymptomatic, defined herein as *Plasmodium* infection without fever within the past 48 h. Although *P. falciparum* was the predominant parasite species in Surat Thani, based on clinical incidence in 2013–2018 (Ministry of Public Health, 2019), it was found that the number of *P. falciparum* infections was slightly higher than that of *P. vivax* infections in January (15 *P. falciparum* vs 11 *P. vivax*) and lower than that of *P. vivax* in May (14 *P. falciparum* vs 18 *P. vivax*). This *P. falciparum*-to-*P. vivax* ratio was unexpected but nonetheless consistent with the case numbers reported in Thailand's national database in the first half (January–June) of 2019 when the *P. vivax* cases outnumbered *P. falciparum* cases (Ministry of Public Health, 2019). Thus, the species distribution of asymptomatic infections was broadly consistent with that of the clinical cases, suggesting that *P. vivax* may soon become the major parasite species in the area.

A few malaria-infected participants presented fever at the time of blood collection or in the preceding 2 days. Additionally, a regression analysis revealed that fever at the time of the surveys or in the previous 2 days was not associated with malaria infection. Thus, the vast majority of infections were asymptomatic, which was consistent with previous reports in Southeast Asia (Chaumeau et al., 2019; Imwong et al., 2015a; Jiram et al., 2019; Liu et al., 2019; Nguitragool et al., 2017; Nguyen et al., 2018; Pongvongsa et al., 2018b; Sattabongkot et al., 2018; Zaw et al., 2017). Interestingly, seven individuals were found to be asymptomatically infected in both surveys. Because the parasite species in these people remained unchanged, they may represent long-lasting (i.e. ≥4 months) infections. Unfortunately, the DNA quantities that were obtained were too small for molecular genotyping to confirm the genetic identity of the parasites in January and May. That asymptomatic infection can last several months is in line with a previous cohort study (White et al., 2018) as well as a study conducted in Vietnam (Nguyen et al., 2018).

Table 3a
Risk factors for malaria infections in January 2019.

Variables		No. infected/total (%)	Crude OR	95% CI	*p*-Value	Adjusted OR[¶]	95% CI	*p*-Value
Sex	Male	22/3333 (0.66)	2.45	1.16–5.19	0.02	2.26	1.06–4.78	0.03
	Female	10/3665 (0.27)	Ref			Ref		
Education	Primary school or lower	24/4405 (0.54)	1.80	0.81–4.00	0.15	1.81	0.81–4.05	0.15
	Secondary school or higher	8/2629 (0.30)	Ref			Ref		
Keeping animals	Yes	27/6397 (0.42)	Ref					
	No	5/637 (0.78)	1.87	0.72–4.86	0.20			
Malaria episode in previous 4 months	Yes	0/130 (0.00)	0.00		[#]			
	No	32/6904 (0.46)	Ref					
Fever in previous 2 days	Yes	2/236 (0.85)	1.93	0.46–8.12	0.37			
	No	30/6799 (0.44)	Ref					
Outdoor during night-time in previous 4 months	Yes	16/1642 (0.97)	3.31	1.65–6.63	0.001	3.15	1.57–6.32	0.001
	No	16/5392 (0.30)	Ref			Ref		
District	Chaiya	0/1755 (0.00)	0.00		[#]			
	Kirirat Nikhom	15/2190 (0.68)	1.35	0.62–2.95				
	Phanom	6/920 (0.65)	1.29	0.48–3.49				
	Vibhavadi	11/2169 (0.51)	Ref					
Total		32/7034 (0.45)						

[#] *p*-Value could not be determined because of the zero event.
[¶] Multiple logistic regression analysis was carried out using the variables with $p < 0.2$ in univariate logistic regression.

Table 3b
Risk factors for malaria infections in May 2019.

Variables		No. infected/total (%)	Crude OR	95% CI	*p*-Value	Adjusted OR[¶]	95% CI	*p*-Value
Sex	Male	34/4144 (0.82)	1.81	1.04–3.16	0.04	1.72	0.98–3.01	0.06
	Female	20/4462 (0.45)	Ref			Ref		
Education	Primary school or lower	29/4953 (0.59)	0.91	0.53–1.56	0.72			
	Secondary school or higher	24/3718 (0.65)	Ref					
Keeping animals	Yes	44/7918 (0.56)	Ref			Ref		
	No	9/753 (1.20)	2.17	1.05–4.45	0.04	1.98	0.96–4.10	0.07
Malaria episode in previous 4 months	Yes	0/63 (0.00)	0.00		[#]			
	No	53/8608 (0.62)	Ref					
Fever in previous 2 days	Yes	1/141 (0.71)	1.17	0.16–8.52	0.88			
	No	53/8531 (0.62)	Ref					
Outdoor during nighttime in previous 4 months	Yes	28/2747 (1.02)	2.43	1.41–4.18	0.001	2.26	1.30–3.93	0.004
	No	25/5924 (0.42)	Ref			Ref		
District	Chaiya	7/2267 (0.31)	0.76	0.29–1.95	0.01	0.96	0.37–2.52	0.02
	Kirirat Nikhom	27/2689 (1.00)	2.48	1.23–5.00		2.61	1.29–5.28	
	Phanom	8/1019 (0.79)	1.93	0.78–4.82		2.01	0.80–5.02	
	Vibhavadi	11/2696 (0.41)	Ref			Ref		
Total		53/8671 (0.61)						

[#] *p*-Value could not be determined because of the zero event.
[¶] Multiple logistic regression analysis was carried out using the variables with $p < 0.2$ in univariate logistic regression.

Table 4
Risk factors for new malaria infections between January and May 2019.

Variables		No. infected/total (%)	Crude OR	95% CI	*p*-Value	Adjusted OR[¶]	95% CI	*p*-Value
Sex	Male	22/2918 (0.75)	2.12	1.05–4.30	0.04	2.06	1.01–4.18	0.046
	Female	12/3347 (0.36)	Ref			Ref		
Education	Primary school or lower	17/3777 (0.45)	0.66	0.34–1.30	0.23			
	Secondary school or higher	17/2505 (0.68)	Ref					
Keeping animals	Yes	28/5711 (0.49)	Ref			Ref		
	No	6/571 (1.05)	2.16	0.89–5.23	0.09	2.03	0.83–4.97	0.12
Malaria episode in previous 4 months	Yes	0/47 (0.00)	0.00		[#]			
	No	33/6140 (0.54)	Ref					
Fever in previous 2 days	Yes	0/97 (0.00)	0.00		[#]			
	No	33/6125 (0.54)	Ref					
Outdoor during nighttime in previous 4 months	Yes	18/2169 (0.83)	2.14	1.09–4.21	0.03	2.10	1.05–4.18	0.04
	No	16/4113 (0.39)	Ref			Ref		
District	Chaiya	6/1533 (0.39)	1.29	0.42–4.02	0.12	1.58	0.50–4.98	0.15
	Kirirat Nikhom	15/1981 (0.76)	2.51	0.97–6.49		2.58	1.00–6.68	
	Phanom	7/787 (0.89)	2.95	0.99–8.82		3.05	1.02–9.15	
	Vibhavadi	6/1981 (0.30)	Ref			Ref		
Total		34/6282 (0.54)						

[#] *p*-Value could not be determined because of the zero event.
[¶] Multiple logistic regression analysis was carried out using the variables with $p < 0.2$ in univariate logistic regression.

A male-biased risk was detected in this study, which was similar to elsewhere in Greater Mekong Subregion (Imwong et al., 2015a; Nguitragool et al., 2017; Zhao et al., 2018). This is presumably due to behaviours that expose males to more vector bites. Staying outdoors at night-time was also a risk factor, but neither bed net use nor IRS was associated with protection, which was in contrast with a previous study in Western Thailand where IRS was linked to reduced risk of infection (Nguitragool et al., 2019). Thus, it appears that transmission in Surat Thani may be primarily outdoors. An on-going study using human landing collection has found that the most common *Anopheles* mosquito species in the study area are *Anopheles minimus* and *Anopheles dirus* (K. Kobylinsky, manuscript in prep). Outdoor transmission is therefore consistent with the exophagic behaviour of these mosquitoes (Chinh et al., 2019; Pimnon and Bhumiratana, 2018;

Table 5
Characteristics of participants with malaria infection in both surveys.

ID	Sex	Age (years)	Occupation	Education level	Malaria episode[*]	Fever in Jan.	Fever in May	Malaria species in Jan.[**]	Malaria species in May[**]
1	Male	42	Agriculture	Primary school	No	No	No	Pv	Pv
2	Male	36	Agriculture	Secondary school	No	No	No	Pv	Pv
3	Female	11	Child/student	Primary school	No	No	No	Pf	Pf
4	Female	12	Child/student	Primary school	No	No	No	Pf	Pf
5	Female	45	Agriculture	Primary school	No	No	No	Pf	unknown
6	Female	52	Labourer/contractor	Secondary school	No	No	No	Pf	Pf
7	Male	71	Agriculture	Primary school	No	No	No	Pf	Pf

[*] Had clinical malaria between the period September 2018–May 2019.
[**] Pf, *Plasmodium falciparum*; Pv, *Plasmodium vivax*; unknown, unidentified *Plasmodium* infection.

Table 6
Characteristics of *Plasmodium knowlesi* infected participants.

No.	Sex	Age (years)	District	Occupation	Education level	Animals in household	Malaria episode*	Fever in January	Fever in May	Outdoor during night-time	Malaria species in January**	Malaria species in May**
1	Male	38	Vibhavadi	Agriculture	University	No	No	No	No	No	Pk	ND
2	Female	46	Chaiya	Agriculture	Primary school	Dog/cat/bird	No	No	No	No	ND	Pk
3	Male	20	Kirirat Nikhom	Agriculture	Primary school	No	No	No	No	Yes	ND	Pk

* Had clinical malaria between the period September 2018–May 2019.
** Pk, *Plasmodium knowlesi*; ND, not detected.

Sungvornyothin et al., 2006; Tananchai et al., 2012; Tisgratog et al., 2012; Trung et al., 2005).

It is believed that there has only been one reported case of *P. knowlesi* in Surat Thani. The case, recorded in the national malaria database (Ministry of Public Health, 2019), was in 2018. Three *P. knowlesi* infections were observed in the current study: one in January and two in May 2019 (Table 6). All three were asymptomatic. Because asymptomatic malaria infection is often believed to depend on clinical immunity acquired through repeated exposures to the parasites (Bousema et al., 2014; Roper et al., 1996), it is perplexing how the immunity to *P. knowlesi* could arise at such a low endemicity for the parasite. Nonetheless, this finding confirms recent reports from mainland Southeast Asia, Malaysian Saba and the Philippines that asymptomatic *P. knowlesi* can occur in humans (Fornace et al., 2018; Imwong et al., 2019; Pongvongsa et al., 2018a).

Several limitations are acknowledged. Asymptomatic malaria was defined as afebrile participants with malaria infection. It remains possible that some asymptomatic infections were in fact pre-symptomatic. Inversely, even though some participants with malaria infection had fever, the cause of fever could have been due to another cause such as dengue fever, which was also present in the study area. In addition, no blood smear examination was performed, precluding direct comparison between qPCR and light microscopic observation. Finally, the fingerprick PCR method used in this study cannot detect all malaria infections. A previous report suggested that fingerprick PCR may miss approximately half of all malaria infections (Imwong et al., 2015b); thus, the true prevalence is likely higher than reported here.

Conclusion

This study revealed that most malaria infections in Surat Thani, a near-elimination setting in Southern Thailand, were asymptomatic. Although the majority of malaria infection spontaneously disappeared within 4 months, some infection appeared to last longer. Outdoor mosquito biting at night-time is likely an important mode of transmission in the province, and targeting it may be an effective means of accelerating malaria elimination. Lastly, the presence of *P. knowlesi* in Surat Thani was confirmed in three different districts.

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Competing interests

None.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ijid.2020.05.022.

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