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# Highly heterogeneous residual malaria risk in western Thailand

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5

1 **Abstract**

2 Over the past decades, the malaria burden in Thailand has substantially declined. Most infections  
3 now originate from the national border regions. In these areas, the prevalence of asymptomatic  
4 infections is still substantial and poses a challenge for the national malaria elimination program. To  
5 determine epidemiological parameters as well as risk factors for malaria infection in western  
6 Thailand, we carried out a cohort study in Kanchanaburi and Ratchaburi provinces on the Thailand-  
7 Myanmar border. Blood samples from 999 local participants were examined for malaria infection  
8 every 4 weeks between May 2013 and Jun 2014. Prevalence of *Plasmodium falciparum* and  
9 *Plasmodium vivax* was determined by quantitative PCR (qPCR) and showed a seasonal variation  
10 with values fluctuating from 1.7 to 4.2% for *P. vivax* and 0 to 1.3% for *P. falciparum*. Ninety  
11 percent of infections were asymptomatic. The annual molecular force of blood-stage infection  
12 ( $_{\text{mol}}\text{FOB}$ ) was estimated by microsatellite genotyping to be 0.24 new infections per person-year for  
13 *P. vivax* and 0.02 new infections per person-year for *P. falciparum*. The distribution of infections  
14 was heterogenous, that is, the vast majority of infections (>80%) were found in a small number of  
15 individuals (<8% of the study population) who tested positive at multiple timepoints. Significant  
16 risk factors were detected for *P. vivax* infections, including previous clinical malaria, occupation in  
17 agriculture and travel to Myanmar. In contrast, indoor residual spraying was associated with a  
18 protection from infection. These findings provide a recent landscape of malaria epidemiology and  
19 emphasize the importance of novel strategies to target asymptomatic and imported infections.

20

21 *Keywords:* Malaria, Epidemiology, *Plasmodium vivax*, *Plasmodium falciparum*, Force of infection,  
22 Prevalence, Incidence, Thailand

## 1 **1. Introduction**

2 In recent years, significant progress has been made in controlling malaria worldwide. The  
3 malaria incidence and mortality rates of Southeast Asia declined by approximately 50% between  
4 2000 and 2015 (WHO, 2015). In the same period, the number of malaria cases in Thailand was  
5 reduced from 150,000 to 24,850 (ThaiDDC, 2016). The incidence rate in most areas is now below 1  
6 case per 1,000 person-years at risk. As part of the global malaria elimination effort, the country is  
7 aiming to achieve malaria elimination before 2025 (WPRO, 2015), a commendable goal which will  
8 require commitment from all parties involved.

9 Notwithstanding the recent success in reducing the malaria burden, a significant proportion  
10 of the population living in endemic areas of Thailand is still at risk of *Plasmodium* spp. infections.  
11 Historically, *Plasmodium falciparum* was the predominant parasite species in Thailand, but  
12 *Plasmodium vivax* has recently taken over as the primary parasite whereas *Plasmodium malariae*,  
13 *Plasmodium ovale* and *Plasmodium knowlesi* are only found sporadically (ThaiMoPH, 2017).  
14 Malaria infections in Thailand are seasonal, the peak season lasting from May to September  
15 (Phimpraphi et al., 2008). Transmission is concentrated along the western Myanmar border and the  
16 eastern Cambodian border. Cross border movements are thought to contribute significantly to  
17 maintenance of the disease (Sriwichai et al., 2017). *Anopheles dirus*, *Anopheles minimus* and  
18 *Anopheles maculatus* are the most important local malaria vectors in these parts of the country  
19 (Tananchai et al., 2012; Sriwichai et al., 2016). Recently, an increasing number of clinical  
20 infections were also reported from the southern provinces (ThaiMoPH, 2017) which is likely due to  
21 reduced surveillance and control activities as a result of political unrest in the area.

22 Several studies have reported the presence of a large number of asymptomatic infections in  
23 Thailand (Li et al., 2014; Baum et al., 2015, 2016; Parker et al., 2015; Nguitragool et al., 2017). As  
24 most asymptomatic carriers do not seek treatment, they are a neglected reservoir that may help  
25 sustain disease transmission (Kiattibutr et al., 2017). To accelerate progress towards elimination, it  
26 is important for the national malaria program to have an appropriate strategy to manage these

1 infections. A better understanding of which individuals are at the greatest risk of harboring  
2 infections, symptomatic or asymptomatic, is thus of fundamental importance, especially when  
3 resources for malaria control are limited. At present, the Thai national malaria control program  
4 relies mostly on clinical cases detected at health facilities as the main indicator of malaria incidence  
5 to coordinate its control efforts. Data on asymptomatic infection rates in Thailand are thus restricted  
6 to cross-sectional surveys conducted in a few areas (Kritsiriwuthinan and Ngrenngamlert, 2011; Li  
7 et al., 2014; Baum et al., 2015, 2016; Parker et al., 2015).

8 In this study, we sought to better understand the dynamics of malaria infection and its risk  
9 factors in populations along the Thailand-Myanmar border, an area where malaria infection rates  
10 are still substantially higher than in other parts of Thailand. We conducted a longitudinal cohort  
11 study in two villages between May 2013 and Jun 2014 in which finger-prick blood samples were  
12 obtained from participants every 4 weeks. Malaria parasites were detected by qPCR and parasite  
13 genotypes were assessed by amplification of length polymorphic markers. Parasite positivity and  
14 molecular force of blood-stage infection ( $_{\text{mol}}\text{FOB}$ , the rate of acquisition of new blood-stage clones)  
15 (Mueller et al., 2012; Koepfli et al., 2013) were used to quantify the risk of infection as well as the  
16 underlying risk factors.

17

## 18 **2. Materials and methods**

### 19 *2.1. Study sites*

20 This study was conducted in two villages, one in the Bong Ti subdistrict of Kanchanaburi  
21 province and the other in the Suan Phueng subdistrict of Ratchaburi Province of Thailand (Fig. 1).  
22 These two sites are located within a few km of the Thailand-Myanmar border. The main ethnic  
23 groups in both sites were Thai and Karen. Agriculture and farming were the main occupations.  
24 Forest foraging was a frequent activity for residents in these areas. The village of Bong Ti is located  
25 approximately 65 km west of the main district of Kanchanaburi province in a hilly terrain. Most  
26 houses were accessible by paved roads. A cross-sectional survey of malaria infection by qPCR

1 (Nguitragool et al., 2017) indicated 3.8% *Plasmodium vivax* and 1.4% *P. falciparum* prevalence in  
2 August-September of 2012. The study village in Suan Phueng subdistrict is located approximately  
3 160 km from Bangkok. The village is located in a mountainous area on the Tanaosri mountain  
4 range. Our survey in September-October of 2012 indicated a prevalence of 3.1% for *P. vivax* and  
5 1.2% for *P. falciparum* at this site (Nguitragool et al., 2017).

## 6 7 2.2. Study design

8 A total of 999 participants were enrolled for a 1 year prospective cohort study. No treatment  
9 was given at the baseline. A total of 14 visits to each study participant were made between May  
10 2013 and June 2014, with time between consecutive visits being approximately 4 weeks. A finger  
11 prick blood sample was collected from each participant at each visit for malaria parasite detection,  
12 species identification, and genotyping by length polymorphic markers for *mol*FOB determination.  
13 Additional finger prick blood samples were also obtained through passive case detection, when  
14 participants visited malaria clinics in the study areas during the time between study visits.

15 At each visit, participants were asked to provide information regarding factors relevant for  
16 malaria control including access to and usage of insecticide-treated nets, travel history, clinical  
17 malaria history, and treatment taken since the last visit. Before taking the blood sample, the body  
18 temperature of each participant was measured using an infrared thermometer. Participants with  
19 fever (body temperature  $\geq 37.5^{\circ}\text{C}$ ) were tested for malaria infection on-site using a rapid diagnostic  
20 test (SD BIOLINE Malaria Ag P.f./Pan, Standard Diagnostics, Republic of Korea) and referred to a  
21 nearby malaria clinic for treatment if the test was positive. The study was approved by the Ethics  
22 Committee of the Faculty of Tropical Medicine, Mahidol University, Thailand (EC approval  
23 number TMEC 13-020).

## 24 25 2.3. Molecular analysis

1 Finger prick whole blood (200  $\mu$ l) was collected from each participant into an EDTA-  
2 containing microtainer. After plasma removal, blood pellets were stored at -20°C until DNA  
3 extraction. DNA was extracted using FavorPrep 96-well Genomic DNA Extraction Kits (Favorgen,  
4 Taiwan), following the standard protocol provided by the manufacturer. The final purified DNA  
5 was eluted into 100  $\mu$ l of elution buffer. A volume of 4  $\mu$ l of purified DNA (equivalent to 8  $\mu$ l of  
6 whole blood) was used as the template for malaria parasite detection by a genus-specific qPCR  
7 assay targeting the *Plasmodium* 18S rRNA genes (Wampfler et al., 2013). For samples positive by  
8 the genus-specific assay, *P. vivax*- and *P. falciparum*-specific assays (Rosanas-Urgell et al., 2010)  
9 were used to determine the parasite species. *Plasmodium vivax* genotypes were determined using  
10 PvMS2 and Pvmsp1F3 markers as previously described (Schoepflin et al., 2009). The combined  
11 expected heterozygosity,  $H_e$ , of the two markers was 0.89. Capillary electrophoresis for  
12 microsatellite genotyping was performed on an ABI3730XL instrument (Macrogen, Republic of  
13 Korea).

#### 14 15 2.4. Determination of the molecular force of blood infection ( $_{mol}FOB$ )

16 The molecular force of blood infection ( $_{mol}FOB$ ) was determined as described previously  
17 (Koepfli et al., 2013). Briefly, the  $_{mol}FOB$  is the observed number of new blood-stage infections, as  
18 identified by individual parasite genotypes, divided by the time-at-risk (i.e., the incidence of new  
19 blood-stage infections). An observed infection, as identified by a specific genotype, was considered  
20 to be 'new' if the same genotype had not been seen in the two previous active or passive case  
21 detection visits. As such, the  $_{mol}FOB$  can be determined for each study participant or as a sum over  
22 the entire study population or subpopulations.

#### 23 24 2.5. Statistical analysis

25 Factors influencing i) the overall rate of acquisition of new blood infections ( $_{mol}FOB$ ); ii)  
26 being parasite-positive for *P. vivax* and/or *P. falciparum* and iii) being subject to a clinical episode,

1 defined by fever + parasitemia detectable by light microscopy (Robinson et al., 2015) were  
2 investigated using statistical models. Travel to Myanmar was quantified as ‘trips per year’ based on  
3 answers to questions about travel history at each visit. Since the  $_{mol}FOB$  is a count variable  
4 measured per individual over a specific exposure time (time at risk), and is overdispersed, a  
5 negative binomial regression model was chosen in which the exposure time at risk is used as the  
6 offset ( $\mu^*_j = \exp(\beta x_j + \text{offset}_j + v_j)$ ), where  $\text{offset}_j = \log(\text{exposure time})$  and  $e^{v_j}$  follows a gamma  
7 distribution. Because using the collapsed data to model the  $_{mol}FOB$  for each individual does not  
8 allow for the analysis of time-changing covariates, factors influencing the frequency of parasite  
9 positivity and frequency of clinical episodes within the study period were explored using multiple  
10 failure time models allowing for time-changing covariates (Therneau and Grambsch, 2000). In these  
11 models, parasite positivity and clinical episodes were equivalent to a ‘failed’ outcome, respectively.  
12 In addition to the statistical models presented in the main manuscript, univariate analyses  
13 (Supplementary Tables S1, S3, S5, S7) and multivariate analyses with backward selection  
14 (Supplementary Tables S2, S4, S6) are provided.

15 To test whether infections were spatially correlated, the  $_{mol}FOB$  was tested for spatial  
16 autocorrelation using the Moran’s I test statistic, which takes into account the value of the  $_{mol}FOB$   
17 for each location and the distance between all locations. A Moran’s I >0 indicates spatial clustering  
18 of high/high and/or low/low  $_{mol}FOB$  values. This analysis was done once for each individual,  
19 accepting that the distance between individuals in the same household is 0, and once for each  
20 household by calculating the average  $_{mol}FOB$  per household (i.e., the total number of new infections  
21 divided by the total time at risk per household).

22

### 23 **3. Results**

#### 24 *3.1. Characteristics of the study population*

25 In total, 999 participants were enrolled into this study in May 2013. Eight hundred and  
26 twelve participants were from Bong Ti, Kanchanaburi and 187 participants were from Suan Phueng,

1 Ratchaburi. Fig. 1 shows the maps of these two study sites where the household locations of the  
2 study participants were marked.

3 Of the 999 participants, the large majority ( $n = 799$ , 80%) were seen 13 or more times  
4 (interquartile range (IQR): 13-14; range: 1-14) during the study period resulting in 12,559 blood  
5 samples from active case detection (ACD). In addition, 281 blood samples were obtained through  
6 passive case detection (PCD) when participants presented at local malaria clinics due to illness. The  
7 median time that the participants remained in the study was 368 days (IQR: 361-379; range: 0-378).  
8 An overview of the characteristics of the study population is given in Table 1.

9

### 10 3.2. Parasite prevalence and *mol*FOB

11 From samples collected at both ACD and PCD visits, the genus-specific PCR assay detected  
12 735 infections in the study participants, of which 512 (70.0%) were successfully genotyped for the  
13 *mol*FOB (i.e. the incidence of genetically distinct blood-staged infections) analysis. *Plasmodium*  
14 *vivax* monoinfections constituted 84.6% ( $n = 433/512$ ), *P. falciparum* monoinfections 9.2% ( $n =$   
15  $47/512$ ) and mixed species infections 6.3% ( $n = 32/512$ ). As such, mixed species infections were  
16 over-represented by a factor of approximately 10 (Fisher's exact test,  $P < 0.001$ ) indicating a strong  
17 clustering of infection. A proportion of 8.5% (37/433) of *P. vivax* infections and 21.3% (10/47) of  
18 the *P. falciparum* infections were classified as clinical, i.e., detectable parasitemia was accompanied  
19 by a measured or reported fever in either PCD or ACD visits. The difference is statistically  
20 significant (analysis of proportions, chi-square test,  $P = 0.006$ ) and suggests that *P. falciparum* was  
21 more likely to cause clinical symptoms. Notably, none of the 32 mixed infections was classified as a  
22 clinical episode.

23 The prevalence of *P. falciparum* and *P. vivax* showed seasonal variation with prevalence  
24 varying from 4.2% to 1.7% for *P. vivax* and from 1.3% to 0% for *P. falciparum* (Fig. 2). The peak  
25 between June and September coincided with the rainy season.

1           During the study period, an estimated total of 226 new (i.e. genetically distinct) *P. vivax* and  
2 18 new *P. falciparum* blood-stage infections were detected, resulting in an average molFOB of 0.24  
3 new infections per person-year for *P. vivax* and 0.02 new infections per person-year for *P.*  
4 *falciparum*. The seasonal variation of the molFOB resembled that of parasite prevalence for both  
5 species, with the peak in the wet season.

### 7 3.3. Clustering of new infections

8           A number of study participants experienced a disproportionately high molFOB (Fig. 3). The  
9 excess of high molFOB values compared with the homogeneous distribution (Poisson) is statistically  
10 significant for *P. vivax* (goodness of fit test for Poisson distribution  $P < 10^{-6}$ ), suggesting either a  
11 non-uniform risk of acquiring new infection through mosquito bites or relapses of parasites from the  
12 liver. Spatial analysis suggests that the *P. vivax* molFOB in Bong Ti, Kanchanaburi was clustered at  
13 both the individual level (global Moran's I = 0.12,  $P < 0.0001$ ) and, more loosely, at the household  
14 level (global Moran's I = 0.03,  $P = 0.02$ ). The low number of infections precluded an in-depth  
15 analysis of heterogeneities in the risk of *P. falciparum* infection.

### 17 3.4. Factors associated with *P. vivax* infection and illness

18           The age profile of the molFOB for *P. vivax* revealed a higher number of new blood-stage  
19 clones in adolescents and adults compared with children (Fig. 4). In addition to age, analysis of risk  
20 factors revealed that increased frequency of travel to Myanmar, previous malaria episodes, and  
21 employment in agriculture were significantly associated with increased risk of new *P. vivax* blood-  
22 stage infections as measured by the molFOB (Table 2).

23           In order to assess time changing covariates, risk factors associated with *P. vivax* positivity  
24 were also determined using a multiple failure time model (Table 3). In general, predictors of *P.*  
25 *vivax* molFOB were predictors of *P. vivax* positivity. While similar effect sizes were observed, the

1 analysis of *P. vivax* positivity additionally detected indoor residual spraying (IRS) as a significant  
2 factor associated with reduced risk of infection.

3 Several predictors of *P. vivax* infection including season, age, occupation, and reported  
4 previous clinical malaria, were also significantly associated with clinical episodes of malaria (Table  
5 4). Notably, while adult age was a predictor of increased risk of infection (Tables 2 and 3), it was  
6 associated with a reduced risk of clinical episodes (Table 4). This indicates stronger protective  
7 immunity in adults compared with young children. Although travel to Myanmar was a predictor of  
8 infection and IRS was associated with a reduced risk of infection, these two factors were not  
9 significantly associated with clinical episodes. This is consistent with their expected relationship  
10 with parasite exposure but not clinical outcomes. Interestingly, despite the lack of association of  
11 bednet usage with *P. vivax* molFOB and positivity, bednet usage was significantly associated with a  
12 small reduction in clinical episodes. The reason for this is unclear and may deserve further  
13 investigation. None of the mixed species infections (*P. falciparum*/*P. vivax*,  $n = 32$ ) corresponded to  
14 a clinical episode, possibly reflecting parasite-parasite interaction or co-development of host  
15 protective immunity against these sympatric species.

16

### 17 3.5. Factors associated with *P. falciparum* infection

18 For *P. falciparum*, risk factors for increased molFOB were not determined due to the small  
19 number of observations ( $n = 18$  new *P. falciparum* infections out of 12,559 samples). Predictors of  
20 infection were determined only from positivity data. In the univariate analysis, only travel  
21 frequency to Myanmar was found to be a predictor of parasite positivity (Supplementary Table S7).  
22 The small number of detected *P. falciparum* infections did not allow detection of other risk factors.  
23 Based on the results presented in Table 5, however, season, age, male gender and IRS may also  
24 show association with a higher infection rate in a larger, more highly powered, study.

25

## 26 4. Discussion

1           This study has confirmed the high degree of heterogeneity of malaria risk in Thai  
2 populations living along the Thailand – Myanmar boarder and identified important risk factors for  
3 *P. vivax* infection and disease. One key epidemiological parameter determined in this study is the  
4  $\text{molFOB}$  which reflects the rate of acquiring a new blood-stage clone of the parasite. Two genetic  
5 markers (PvMS2 and Pvmsp1F3) were used to genotype *P. vivax*. Because the combined  $H_e$  of the  
6 two loci was 0.89, approximately 10% of all new clones are expected to have been missed. This,  
7 together with the limited sensitivity of molecular genotyping (69.5% of samples were PvMS2-  
8 positive; 78.9% of samples were Pvmsp1F3-positive), would lead to an underestimation of the true  
9  $\text{molFOB}$ . These limitations are shared by our previous studies using the same methodology (Mueller  
10 et al., 2012; Koepfli et al., 2013; Hofmann et al., 2017). The analyses of risk factors, however,  
11 depends only on the relative values of the  $\text{molFOB}$ .

12           Overall the prevalences of *P. falciparum* (0-1.3%) and *P. vivax* (1.7-4.2%) were similar to  
13 the 4.9-5.7% *Plasmodium* prevalence previously reported in Kanchanaburi and Ratchaburi in 2012  
14 (Patel et al., 2014; Nguitragool et al., 2017). The majority of infections detected at each visit were  
15 asymptomatic and resolved naturally without developing into clinical malaria. Using the same  
16 dataset, we recently estimated the duration of blood-stage infection detectable by molecular  
17 genotyping to be 29 days for *P. vivax* and 135 days for *P. falciparum* (White et al., 2018).

18           The overall prevalence as well as the predominance of *P. vivax* infections reflect the current  
19 trend in Thailand and the Greater Mekong Subregion where *P. vivax* has become the predominant  
20 malaria species (Baum et al., 2015; Imwong et al., 2015; Nguitragool et al., 2017). The *P. vivax* pre-  
21 dominance is less pronounced among the clinical/febrile cases (Pv: 79%) than asymptomatic  
22 infections (Pv: 91.5%). The predominance was most striking in the number of genetically distinct  
23 blood-stage infections acquired during follow-up ( $\text{molFOB}$ ) with only 7.4% of all newly acquired  
24 infections due to *P. falciparum*, resulting in an average  $\text{molFOB}$  of 0.02 and 0.24 new infections per  
25 person-year for *P. falciparum* and *P. vivax*, respectively. The proportion of *P. vivax* infection  
26 relative to *P. falciparum* was much higher in our cohort than among clinical cases recorded by the

1 Thai health system (ThaiDDC, 2016; WHO, 2016), indicating that the true burden of (mostly  
2 asymptomatic) *P. vivax* infections in Thailand may be substantially higher than reported.

3 The risk of *P. vivax* infection was highly heterogeneous in our study sites, which are fairly  
4 typical of areas along the Thailand-Myanmar border where the landscape is mountainous,  
5 agriculture and forest harvesting are the means of living, and cross-border migrant workers are  
6 common. Only 15% of the 999 study participants tested positive during the study, and people who  
7 were infected at multiple visits accounted for >80% of all infections. The observed concentration of  
8 infections in a small sub-population suggests that appropriate interventions targeted at high-risk  
9 individuals may be effective in accelerating malaria elimination.

10 In order to target malaria interventions more efficiently, it is essential to better understand  
11 the geographical, demographic, and behavioral risk factors for *Plasmodium* spp. infections in these  
12 Thai border communities. For *P. vivax*, the risk factors for higher parasite prevalence and molFOB  
13 were very similar and included rainy season, aged 7-60 years, male gender, past experience of  
14 clinical malaria, being employed in agriculture, and travel to Myanmar. As areas with perennial  
15 transmission, our study sites received deltamethrin IRS twice each year according to the policy of  
16 the Thai Ministry of Public Health. Our analysis found that this intervention was associated with  
17 reduced infection risk. Thus, even though malaria infection risk was generally related to  
18 occupational exposure (as exemplified by male adults working in agriculture or in the forest and  
19 people visiting areas of higher transmission in Myanmar), the high risk in children 7-17 years of age  
20 and the association of IRS with protection indicate ongoing local *P. vivax* transmission, including in  
21 or near people's houses. While the high risk in male adults and those regularly crossing the  
22 Myanmar border has been well established (Nguitrageol et al., 2017), the association between IRS  
23 and reduced *P. vivax* positivity was a surprise in light of the predominantly outdoor biting  
24 preference of the malaria vectors in western Thailand (Muenworn et al., 2009; Tananchai et al.,  
25 2012; Tisgratog et al., 2012; Sriwichai et al., 2017). It provides a strong argument for continued  
26 support for the national IRS program in country.

1 The risk of suffering a clinical *P. vivax* episode was highest in children <7 years of age and  
2 decreased strongly with age, indicating that residents with higher risks of *P. vivax* infections did  
3 acquire substantial clinical immunity. Although it is well established that immunity to *P. vivax* is  
4 acquired more rapidly than to *P. falciparum* (Longley et al., 2016), it cannot be ruled out that at  
5 least part of the immunity observed in older age groups was acquired during earlier periods when  
6 transmission intensity was substantially higher (Phimpraphi et al., 2008). Although they are less  
7 infectious to mosquitoes than symptomatic infections (Kiattibutr et al., 2017), asymptomatic *P.*  
8 *vivax* infections are much more prevalent and may pose a particular challenge to malaria  
9 elimination in Thailand. Overall, the observed risk patterns indicate that residual transmission of *P.*  
10 *vivax* persists in our study areas.

11 There was only a small number of *P. falciparum*-positive cases detected during the study  
12 period. Due to this, for *P. falciparum* the risk factor analysis was limited to qPCR positivity. Only  
13 frequency of travel to Myanmar was found to be a statistically significant determinant for risk of *P.*  
14 *falciparum* infection. Although not statistically significant due to the profound lack of power, the  
15 multivariate analysis does indicate that the risk of *P. falciparum* was highest in male (adjusted  
16 hazard ratio (aHR) = 2.9) adults (aHR = 10.0) with an excess risk also during the rainy seasons and  
17 in areas where IRS had been conducted. The higher risk in people who traveled frequently across  
18 the border is a common feature of the residual burden both in this (Nguitragool et al., 2017) and  
19 other border regions of Thailand (Kitvatanachai et al., 2003; Bhumiratana et al., 2013). As found in  
20 neighboring Tak province, where *P. falciparum* malaria was four times more likely in recent  
21 migrants compared with Thai patients and correlated with *Anopheline* vector capture rates  
22 (Sriwichai et al., 2017), this indicates that importation of *P. falciparum* to Ratchaburi and  
23 Kanchanaburi contributed significantly to prevalence.

24 To our knowledge, this study is the first from a hypoendemic area to determine the molFOB.  
25 Previous studies of *Plasmodium* molFOB were restricted to Papua New Guinea where transmission  
26 intensity was much higher with molFOB in the range of 5-14 new infections per person-year

1 (Mueller et al., 2012; Koepfli et al., 2013; Hofmann et al., 2017). These previous studies  
2 demonstrated that in such a setting, acquisition of new *P. falciparum* clones was a major factor of  
3 clinical malaria in children (Mueller et al., 2012) and that high molFOB likely contributed to rapid  
4 acquisition of immunity against *P. vivax* malaria (Koepfli et al., 2013). The molFOB for both *P.*  
5 *falciparum* and *P. vivax* were at least 50-fold lower in the current study. This study thus represents a  
6 scenario at the opposite end of the malaria transmission spectrum.

7        Compared with parasite positivity, the molFOB is a more direct measure of *P. falciparum*  
8 transmission. For *P. vivax*, the molFOB is a combined measure of blood-stage infections arising from  
9 both new mosquito bites and relapses. Without the knowledge about the relative contributions of  
10 mosquito bites and relapses, it is difficult to estimate how much the molFOB reflects transmission  
11 intensity. In this study, we found that the seasonal variation of parasite positivity closely followed  
12 that of the molFOB (Fig. 2) for both parasite species. Risk factor analyses of the molFOB and  
13 positivity of *P. vivax* also yielded similar results; all significant risk factors apparent in the molFOB  
14 analysis were apparent in the analysis that used positivity. The close association between *P. vivax*  
15 prevalence and the molFOB is consistent with the short duration (29 days) estimated for blood-stage  
16 infection in Thailand (White et al., 2018).

17        In summary, our study highlights the different challenges posed by *P. falciparum* and *P.*  
18 *vivax* to Thailand's declared goal of eliminating local malaria transmission by 2025. In line with our  
19 earlier studies in Tak Province (Baum et al., 2015, 2016; Parker et al., 2015; Sriwichai et al., 2017),  
20 we have confirmed the continued presence of local *P. vivax* transmission in Thai villages along the  
21 western border. Most of the *P. vivax* infections were asymptomatic and often of very low density  
22 and would not have been detected by the standard surveillance methods based on microscopy and  
23 centered around passive case detection. Efficient elimination of *P. vivax* from Thailand will thus  
24 require additional novel strategies targeting this asymptomatic reservoir, including the silent  
25 hypnozoite reservoir (Robinson et al., 2015). On the other hand, *P. falciparum* infections were rare.  
26 With a large proportion of infection linked to recent travel to Myanmar, it will be important to

1 strengthen malaria control measures on both sides of the border and to raise awareness of the travel-  
2 associated risk.

3

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17

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45

1 **Legends to Figures**

2

3 **Fig. 1.** Map of the two study sites in Bong Ti, Kanchanaburi and Suan Phueng, Ratchaburi on the  
4 Thailand-Myanmar border. Study households are shown (●) as well as households in which  
5 infections with *Plasmodium falciparum* or *Plasmodium vivax* were detected over the course of the  
6 study (★).

7

8 **Fig. 2.** Prevalence and molecular force of blood-stage infection ( $_{\text{mol}}\text{FOB}$ ) of *Plasmodium vivax* (A)  
9 and *Plasmodium falciparum* (B) at each study site by month of study visit. Error bars (prevalence)  
10 represent the upper limit of the 95% confidence interval; shaded area ( $_{\text{mol}}\text{FOB}$ ) represents S.E.M.  
11 Note that  $_{\text{mol}}\text{FOB}$  could not be determined for visits one and two as it depends on the observations  
12 from two previous study visits.

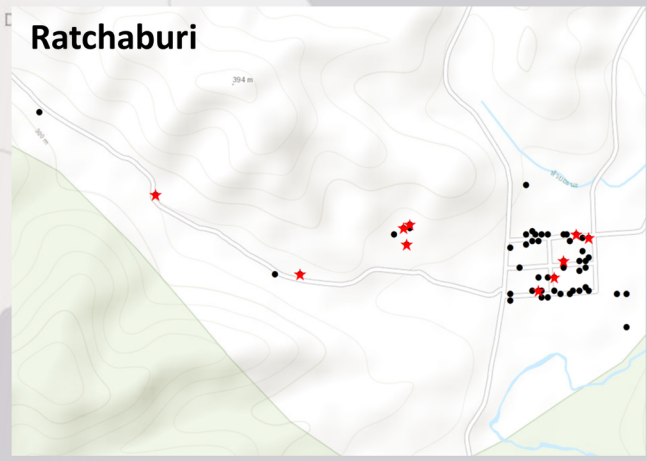
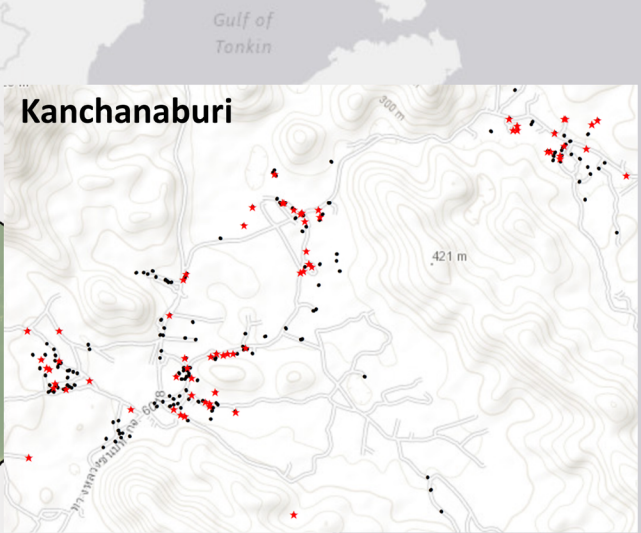
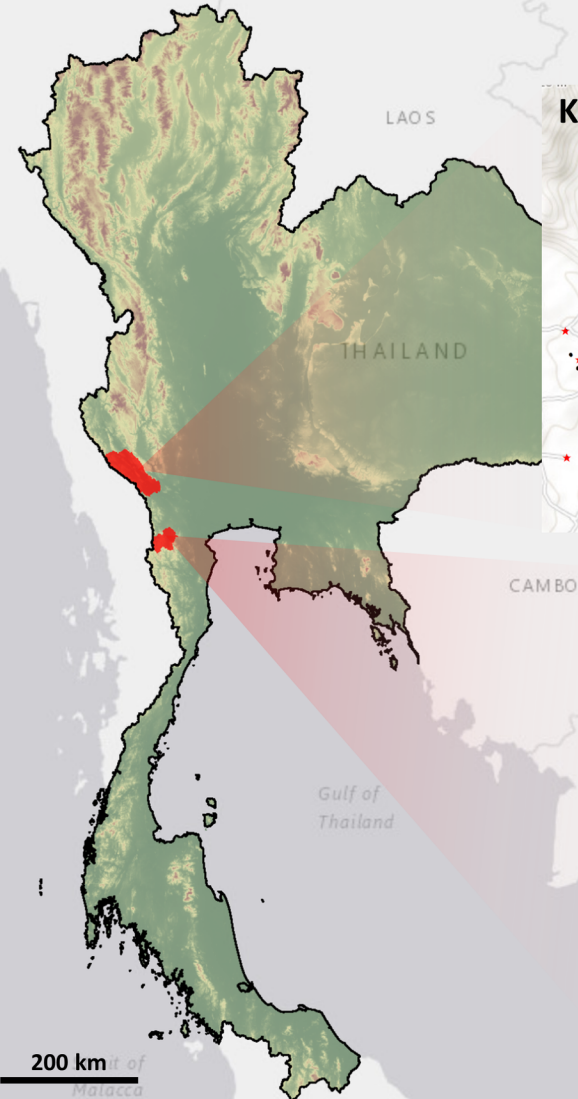
13

14 **Fig. 3.** Distributions of the molecular force of blood-stage infection ( $_{\text{mol}}\text{FOB}$ ). The proportion of  
15 study participants at each level of  $_{\text{mol}}\text{FOB}$  is shown for *Plasmodium vivax* (A) and *Plasmodium*  
16 *falciparum* (B). Red: normalized distributions of observed  $_{\text{mol}}\text{FOB}$  in the study participants. Grey:  
17 Poisson distributions computed based on the mean  $_{\text{mol}}\text{FOB}$ . The Poisson distributions are overlaid  
18 on top of the observed distributions.

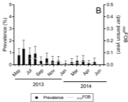
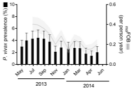
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20 **Fig. 4.** The molecular force of blood-stage infection ( $_{\text{mol}}\text{FOB}$ ) as a function of age in the study  
21 population. (Aa) The average *Plasmodium vivax*  $_{\text{mol}}\text{FOB}$  in each age group. (Ab) The proportion of  
22 individuals with *P. vivax*  $_{\text{mol}}\text{FOB}>0$  in each age group. (Ba) The average *Plasmodium falciparum*  
23  $_{\text{mol}}\text{FOB}$  in each age group. (Bb) The proportion of individuals with *P. falciparum*  $_{\text{mol}}\text{FOB}>0$  in each  
24 age group. Error bars indicate the S.E.M. (Aa, Ba) or the upper limit of the 95% confidence interval  
25 (Ab, Bb).

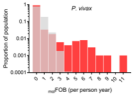
26



- ★ Household with infections
- Surveyed household with no infected cases



*P. vivax*



*P. falciparum*

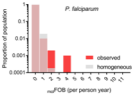




Table 1. Characteristics of the western Thailand study population

<b>Characteristic</b>	<b><i>n</i> (%) or median (range)</b>
Age <sup>a</sup> (yr)	23 (1-83)
Male / Female	452 (46.2) / 527 (53.8)
Kanchanaburi / Ratchaburi	812 (81.3) / 187 (18.7)
Exposure (days)	368 (22-378)
Infection status at first visit	
<i>Plasmodium vivax</i>	34 (3.4)
<i>Plasmodium falciparum</i>	7 (0.8)
Mixed <i>P. vivax/P. falciparum</i>	3 (0.3)
Insecticide treated net possession/usage*	
Never	62 (6.3)
< 6 months	11 (1.1)
6 months - 1 year	12 (1.2)
1 year -2 years	34 (3.5)
> 2 years	860 (87.8)
Indoor residual spraying <sup>a</sup>	719 (73%)
Window screens <sup>a</sup>	20 (0.2%)

<sup>a</sup> At first visit (May 2013).

Table 2. Factors associated with *Plasmodium vivax* molFOB (negative binomial model) in western Thailand

<b>Risk factor</b>	<b>aIRR</b>	<b>95% CI</b>	<b>P</b>
Kanchanaburi	1.83	0.64-5.23	0.257
Age group (years; ref: 0-6)			
7-12	22.79	3.34-155.6	<0.001
13-17	17.10	2.15-135.84	
18-60	15.67	2.15-114.15	
>60	3.85	0.44-33.31	
Male	1.86	1.1-3.14	0.02
Frequency of travel to Myanmar (per visit/year)	1.15	1.05-1.24	0.001
Previous clinical malaria <sup>a</sup>	2.43	1.2-4.91	0.014
House treated with IRS <sup>a</sup>	0.56	0.3-1.04	0.067
Reported bednet possession <sup>a</sup>	0.98	0.39-2.43	0.957
Work in agriculture <sup>a</sup>	2.25	1.04-4.86	0.039

<sup>a</sup>Status at enrolment.

aIRR, adjusted incidence risk ratio; CI, confidence interval; IRS, indoor residual spray

Table 3. Risk factors associated with western Thailand *Plasmodium vivax* positivity (multiple failure time model)

Risk factor	aHR	P
Kanchanaburi	2.22 (1.06-4.76)	0.03
Season (May-September)	3.41 (2.19-5.31)	<0.001
Age group (years; ref: 0-6)		
7-12	3.64 (0.94-14.02)	
13-17	4.99 (1.22-20.34)	0.02
18-60	3.69 (1.03-13.21)	
>60	0.99 (0.22-4.48)	
Male	2.32 (1.36-3.96)	<0.001
Work in agriculture <sup>a</sup>	2.01 (1.14-3.55)	0.02
Frequency of travel to Myanmar <sup>b</sup>	1.06 (1.01-1.11)	0.01
Average bednet usage <sup>b,c</sup>	1 (0.95-1.05)	0.92
House treated with IRS <sup>a</sup>	0.47 (0.28-0.79)	<0.001
Previous clinical malaria <sup>a</sup>	1.87 (1.13-3.1)	0.02

<sup>a</sup>Status at enrolment.

<sup>b</sup>As a time-changing covariate (average observed at time of outcome).

<sup>c</sup>Average bednet usage was defined as the proportion of times a person had answered 'yes' to the question: 'Did you sleep under a bednet last night?' during active case detection.

aHR, adjusted hazard ratio; IRS, indoor residual spray.

Table 4: Risk factors associated with western Thailand *Plasmodium vivax* clinical episodes (multiple failure time model)

<b>Risk factor</b>	<b>aHR</b>	<b>P</b>
Kanchanaburi	1.01 (0.3-3.33)	0.99
Season (May-September)	19.47 (1.82-208.79)	0.01
Age group (ref: 0-12) <sup>b</sup>		
13-17	0.75 (0.19-3.05)	
18-60	0.24 (0.1-0.6)	<0.001
>60	0.35 (0.09-1.29)	
Male	1.66 (0.63-4.36)	0.31
Work in agriculture <sup>c</sup>	4.64 (2.18-9.86)	<0.001
Travel frequency to Myanmar <sup>a</sup>	1.04 (0.94-1.15)	0.50
Average bednet usage <sup>a,d</sup>	0.89 (0.83-0.96)	<0.001
IRS <sup>c</sup>	1.64 (0.61-4.45)	0.33
Previous clinical malaria <sup>c</sup>	2.21 (0.98-4.97)	0.06

<sup>a</sup>As a time-changing covariate (floating average at time of outcome).

<sup>b</sup>The age groups were further aggregated when compared with Table 3, as some age groups did not contain any positive outcomes.

<sup>c</sup>Status at enrolment.

<sup>d</sup>Average bednet usage was defined as the proportion of times a person had answered 'yes' to the question: 'Did you sleep under a bednet last night?'.  
aHR, adjusted hazard ratio; IRS, indoor residual spray.

Table 5. Risk factors associated with western Thailand *Plasmodium falciparum* positivity (multiple failure time model)

<b>Risk factor</b>	<b>aHR</b>	<b>P</b>
Kanchanaburi	1.69 (0.54-5.26)	0.36
Season (May-September)	2.12 (1.03-4.36)	0.04
Age group (years; ref: 0-6)		
7-12	4.15 (0.38-45.23)	
13-17	4.54 (0.45-46.25)	0.12
≥18	10 (1.1-91.05)	
Male	2.9 (0.73-11.52)	0.13
Work in agriculture <sup>a</sup>	0.36 (0.05-2.63)	0.32
Travel frequency to Myanmar <sup>b</sup>	1.08 (0.96-1.22)	0.18
Average bednet usage <sup>b,c</sup>	1.04 (0.83-1.3)	0.76
IRS <sup>a</sup>	2.12 (0.77-5.82)	0.15
Previous clinical malaria <sup>a</sup>	0.96 (0.28-3.24)	0.95

<sup>a</sup>Status at enrolment.

<sup>b</sup>As a time-changing covariate (floating average at time of outcome).

<sup>c</sup>Average bednet usage was defined as the proportion of times a person had answered 'yes' to the question: 'Did you sleep under a bednet last night?'

aHR, adjusted hazard ratio; IRS, indoor residual spray.

