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Contribution to Malaria Transmission of Symptomatic and Asymptomatic Parasite Carriers in Cambodia

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Background. Eliminating falciparum malaria in Cambodia is a top priority, requiring the implementation of novel tools and strategies to interrupt its transmission. To date, few data are available regarding the contributions to malaria transmission of symptomatic and asymptomatic carriers.

Methods. Direct-membrane and skin feeding assays (DMFAs, SFAs) were performed, using *Anopheles minimus* and *Anopheles dirus*, to determine infectivity of symptomatic falciparum-infected patients and malaria asymptomatic carriers; a subset of the latter were followed up for 2 months to assess their transmission potential.

Results. By microscopy and real-time polymerase chain reaction, *Plasmodium falciparum* gametocyte prevalence rates were, respectively, 19.3% (n = 21/109) and 44% (n = 47/109) on day (D) 0 and 17.9% (n = 5/28) and 89.3% (n = 25/28) in recrudescent patients (Drec) (RT-PCR Drec vs D0 $P = .002$). Falciparum malaria patient infectivity was low on D0 (6.2%; n = 3/48) and in Drec (8.3%; n = 1/12). Direct-membrane feeding assays and SFAs gave similar results. None of the falciparum (n = 0/19) and 3 of 28 *Plasmodium vivax* asymptomatic carriers were infectious to mosquitoes, including those that were followed up for 2 months. Overall, *P. falciparum* gametocytemias were low except in a few symptomatic carriers.

Conclusions. Only symptomatic falciparum malaria patients were infectious to mosquito vectors at baseline and recrudescence, highlighting the need to detect promptly and treat effectively *P. falciparum* patients.

Keywords. *Plasmodium falciparum*; *Plasmodium vivax*; infectivity; *Anopheles*; low-transmission setting.

Over the last decade, the global malaria burden has decreased markedly due to concerted international efforts to improve malaria control. However, this disease remains a major public health challenge with an estimated 212 million cases recorded in 2015 [1]. In Southeast Asia, the burden of *Plasmodium falciparum* malaria has followed the same trend, despite the emergence of artemisinin- and partner drug resistance, a major threat that could reverse the current global achievement in malaria control [2–5]. Consequently, eliminating *P. falciparum* malaria is a top priority for the Cambodian government and international organizations. However, moving toward malaria elimination requires implementation of novel tools and strategies that specifically aim at interrupting malaria transmission, such as the introduction of single low-dose primaquine, advocated by the World Health Organization (WHO) in 2012 [6]. Development of such effective tools relies on understanding the nature of malaria transmission in Cambodia; knowing the who, when, and where of parasite transmission to mosquito vectors; and assessing the contribution to malaria transmission of symptomatic malaria cases and asymptomatic parasite carriers.

Human-to-mosquito transmission is mediated by gametocytes, which are ingested by mosquitoes taking a blood meal from malaria-infected individuals. To assess malaria parasite carrier infectivity, mosquito feeding assays are considered the gold standard [7, 8]. Gametocytemia is strongly correlated to infectivity in a nonlinear relationship, but this association may vary according to seasonality and the epidemiological context [9, 10]. For instance, individuals with high gametocytemia do not always have high mosquito infection rates, whereas individuals with submicroscopic gametocytemia may infect mosquitoes [11–13].

To date, few studies have investigated the infectivity of malaria parasite carriers in Southeast Asia, and human-to-mosquito transmission from symptomatic or asymptomatic *Plasmodium*-infected individuals remains unclear. For vivax malaria infections, it has been shown that symptomatic carriers are highly infectious, with 58%–84% of patients infecting mosquito vectors [14–16], but this falls to 0%–13.5% for asymptomatic vivax carriers [11, 16]. For *P. falciparum*, infectivity

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appears much lower, both in symptomatic Cambodian (5.9% [17]) and Thai patients (>25% [15]) and in asymptomatic Thai carriers (10% [11]).

Limited data suggest that the major source of malaria transmission in Southeast Asia is *P. falciparum* symptomatic carriers who have patent gametocytemia (96% of all mosquito infections [17]). However, data on the infectivity of submicroscopic carriers over time are lacking, and no comprehensive studies comparing symptomatic and asymptomatic malaria parasite carriers of both main species have been carried out in Cambodia. Therefore, we assessed the infectivity of patients with uncomplicated falciparum malaria seeking treatment and asymptomatic carriers in the dry and the rainy seasons.

METHODS

Symptomatic Patients Study

An open-label randomized control trial assessing the tolerability and the safety of single low-dose primaquine (SLDPQ) was carried out in Banlung, Rattanakiri province in 2015–2016 (Clinical trials registration: NCT02434952). Rattanakiri province is located in the northeast of Cambodia and is sparsely populated. Residents generally live in villages of 20–60 families. The 2 main malaria vector species are *Anopheles minimus* and *Anopheles dirus* [18, 19].

Briefly, nonpregnant, nonbreastfeeding patients aged ≥1 year with acute uncomplicated falciparum malaria (≥1 asexual parasite/500 white blood cells) and a hemoglobin concentration ≥6 g/dL were recruited to receive either dihydroartemisinin-piperaquine (DHA-PP, Duo-Cotecxin, DHA40 mg and PP320 mg, Zhejiang Holley Nanhu Pharamaceutical Co Ltd, Jiaxing, Zhejiang Province, China) alone or combined with SLDPQ (0.25 mg/kg given with the first dose of DHA-PP, Thai Government Pharmaceutical Organization, 15-mg base primaquine tablet). Clinical and laboratory assessments were performed on days (D) 0, 1, 2, 3, 7, 14, 21 and 28. *Anopheles minimus* mosquitoes were fed on D0 blood samples collected from a subset of patients and on the first day of recurrent (Drec) falciparum parasitemia (defined by the WHO as late treatment failure) depending on mosquito availability using direct-membrane feeding assays (DMFAs) (Figure 1).

An additional cohort of symptomatic *P. falciparum* monoinfection patients were recruited later in 2016 from villages or the local health centers in the same province. Both *A. minimus* and *A. dirus* mosquitoes were fed directly on patient's skin (skin-feeding assays [SFAs]) and on blood samples collected on D0 before artesunate-mefloquine treatment (DMFAs) (Figure 1).

Asymptomatic Malaria Infection Studies

Follow-up in the Dry Season

In January 2016, participants were screened in Kam village (Laork commune, Ohchum district, Rattanakiri province) (Supplementary Figure 1). All nonpregnant, nonbreastfeeding persons aged >5 year who had not received a malaria treatment

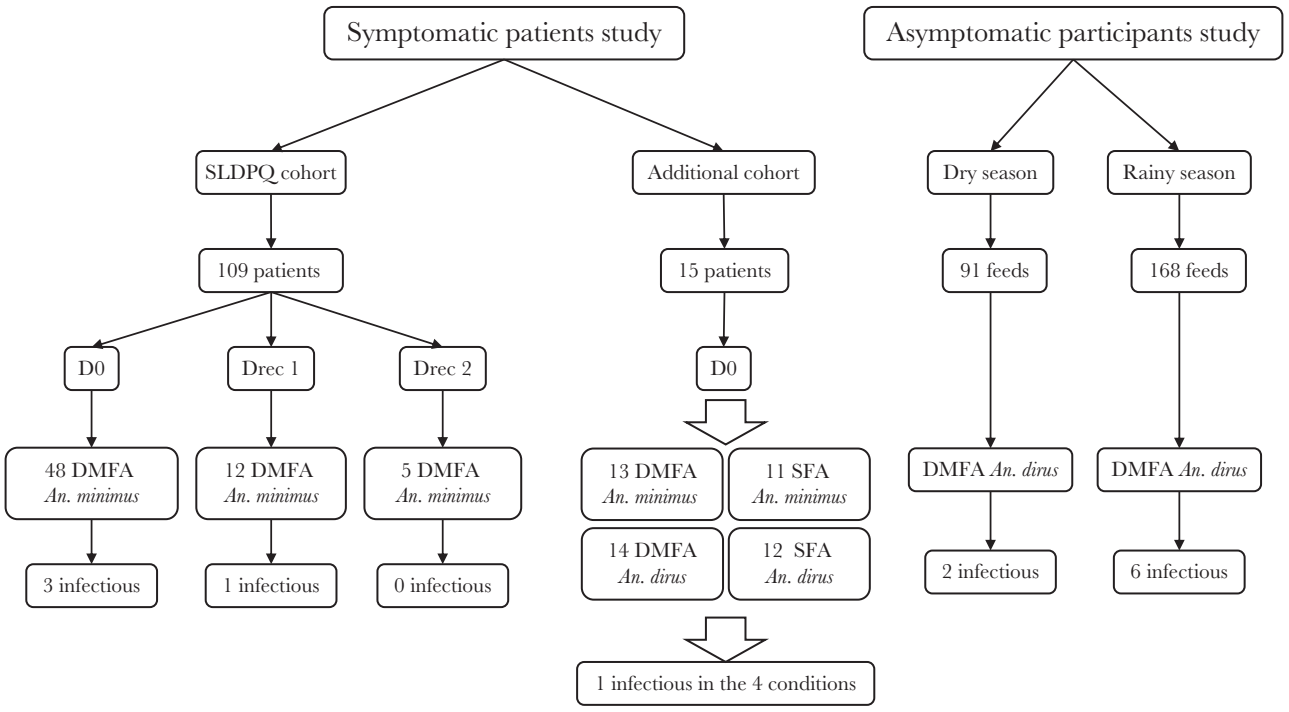

Figure 1. Schematic design of the symptomatic and asymptomatic studies. Abbreviations: D0, day 0; Drec1, day 0 of the first recrudescence; Drec2, day 0 of the second recrudescence; DMFA, direct-membrane feeding assay; SFA, skin feeding assay; SLDPQ, single low-dose primaquine.

in the last 4 weeks were invited to participate in the study. *Plasmodium* species were detected by real-time polymerase chain reaction (RT-PCR) on filter paper blood spots, and symptomatic malaria-positive participants were treated according to national guidelines. All asymptomatic malaria carriers (temperature <37.5°C) and 3 malaria-free individuals were enrolled for DMFAs (*A. dirus*) and were screened for malaria by RT-PCR on whole blood sample during the first week (Figure 1). Among them, 17 asymptomatic participants (all *P. falciparum* and mixed *P. falciparum*/*P. vivax* carriers and 1 randomly selected *P. vivax* carrier participants from week 0) were enrolled for an additional 7 weeks of follow-up. Direct-membrane feeding assays and gametocyte detection were carried out weekly in the first month and then every 2 weeks in the second month. All participants who became symptomatic were immediately treated and excluded from further follow-up.

Follow-up in the Rainy Season

In August 2016, volunteer participants from the same village (Kam village) and 3 additional villages (Ohlang, Kres, and Tangach villages, Poy commune) (Supplementary Figure 1) were screened. A total of 26 asymptomatic (all *P. falciparum,* mixed *P. falciparum*/*P. vivax,* and *P. malariae* cases and 15 randomly selected *P. vivax* participants from week 0) were included and followed up for 2 months with the same protocol.

Entomological Investigations

Direct-membrane feeding assays were carried out to assess individual malaria infectivity. Briefly, 6–8-day-old *A. dirus* or 3–5-day-old *A. minimus* females were fed through membranes on patient's blood. Mosquitoes were starved for 24 hours before being provided a blood meal. Venous blood samples were collected in heparinized tubes, and 400 µL of blood was made available in membrane feeders maintained at 37°C. Females were fed only once on freshly drawn blood. Skin feeding assays were carried out by allowing females to feed for 20 minutes on patient skin using the forearm. After feeding, unfed females were discarded, and fed females were kept in cages (20 × 20 × 20 cm) with constant access to a 10% sucrose solution. Patient infectivity was determined by assessing infection prevalence (ie, proportion of infected mosquitoes) and infection intensity (ie, number of *Plasmodium sp.* oocysts in infected females). Midguts were dissected in a 1% Mercurochrome stain, and the presence and the number of oocysts were determined under a microscope (20× magnification). Dissections were performed 7 days after blood meal. A median number of 50 females was dissected in all assays (DMFAs and SFAs).

Biological Investigations

Sexual and asexual parasite counts (D0 and follow-up days) were determined on Giemsa-stained thick films and recorded as the number of parasites per 500 white blood cells (assuming a white blood cell count of 8000/µL). Two qualified microscopists read the slides, and parasite densities were recorded as the average of these 2 counts. A third reading was conducted if the discrepancy between parasite densities exceeded 30%.

Parasite DNA was extracted from filter paper blood spots or 200 µL of whole blood with Instagene Matrix (Bio-Rad, Courtaboeuf, France) or DNA Blood Mini kit (Qiagen, Germany), according to the manufacturer's instructions. Parasite RNA was extracted from the Trizol (Life Technologies Holdings Pte Ltd, Singapore) conserved whole blood samples using QIAamp RNA Blood Mini Kit (Qiagen, Germany), following the manufacturer's protocol.

A 2-step semiquantitative RT-PCR was performed to detect malaria parasites, as previously described [20]. Following PCR amplification, falciparum-positive samples were analyzed for the presence of gametocytes by a Taqman RT-PCR, using primers spanning an exon-exon junction and targeting the *Plasmodium falciparum* meiotic recombination protein DMC1-like protein gene (AF356553), as in Lawaly and colleagues [21]. Gametocyte dilution series based on in vitro cultured local strain were used to estimate gametocyte blood concentrations.

Plasmodium falciparum–positive samples were screened for mutations in the K13-propeller domain gene, which is associated with artemisinin resistance [22]. Polymerase chain reaction products were sequenced by Macrogen (Seoul, Korea), and electropherograms were analyzed on both strands, using PF3D7_1343700 as the reference sequence. *Plasmodium falciparum* barcoding assays were performed as per Daniels and colleagues with minor modifications [23]. Polymorphisms in 12 single nucleotide polymorphisms were assessed by nested PCR approach. Polymerase chain reaction–ligase detection reaction–fluorescence microspheres assay reactions were used to define a molecular barcode for each isolate [24].

The capillary electrophoresis (MINICAP syste, Sebia, France) and the quantitative determination of G6PD activity (Trinity Biotech quantitative G6PD assay, Ref 345-UV, Trinity Biotech) were carried out on blood collected from symptomatic patients. Hemoglobin disorders were classified as in Khim and colleagues [25].

Statistical Analyses

Groups were compared using the χ^2 test, Fisher's exact test, McNemar test, or Wilcoxon test, as appropriate. For data from D0 samples, binomial general linear models were fitted to investigate gametocyte prevalence estimated by RT-PCR. In these models, sex, G6PD phenotype, hemoglobinopathies, age, seasonality (dry vs rainy season), K13 mutation (mutant vs wild-type alleles), and clinical outcome (cured vs PCR-corrected recrudescence) were coded as fixed factors. Infection prevalence determined with DMFAs only was assessed using binomial generalized linear mixed models to compare symptomatic and asymptomatic carriers. In this model, clinical status (asymptomatic vs symptomatic) was coded as a fixed factor, individual was coded as a random factor to account for repeated measures

on the same parasite carrier, and an observation-level random effect was added to account for overdispersion. Infection prevalence was also compared on the subset of *P. falciparum* symptomatic and asymptomatic carriers only, using a similar generalized linear mixed model. For model selection, we used the stepwise removal of terms, followed by likelihood ratio tests. Term removals that significantly reduced explanatory power ($P < .05$) were retained in the minimal adequate model [26]. All analyses were performed in R v.3.0.3.

Ethical Statement

Ethical approvals were obtained from the Cambodian National Ethics Committee for Health Research (0370NECHR, 197NECHR, 319NECHR). The protocols conformed to the Helsinki Declaration on ethical principles for medical research involving human subjects (version 2002), and informed written consent was obtained for all volunteers.

RESULTS

Contribution to Malaria Transmission of *Plasmodium falciparum*

Symptomatic Patients

One hundred nine patients with uncomplicated falciparum malaria were enrolled in the study and treated with standard 3-day DHA-PP alone (48%; n = 53/109) or DHA-PP plus SLDPQ (52%; n = 56/109). Seven patients were lost to follow-up (6%). Among patients who completed the follow-up, 27.4% (n = 28/102) experienced *P. falciparum* PCR-corrected recrudescence. Of the 28 recrudescent patients, 10 were retreated with the standard 3-day DHA-PP and the remainder with quinine or quinine plus tetracycline; 6 of the 10 patients retreated with DHA-PP experienced a second recrudescence.

At D0, gametocytes were detected in 21 of 109 patients (19.3%; median, 39 gametocytes/μL; range, 16–1432 gametocytes/μL) by microscopy and in 47 of 109 patients (44%) by RT-PCR. Blood samples from 48 patients were fed to mosquitoes, and 3 patients with patent gametocytes (6.2%) were infectious to mosquitoes (Table 1, Supplementary Table 1). Gametocyte prevalence by RT-PCR was not significantly different between the patients enrolled versus those not enrolled in the feeding assays ($P = .92$) (Table 1).

The proportion of gametocytes in isolates collected from recrudescent patients (Drec) were 17.9% by microscopy (n = 5/28; median, 40 gametocytes/μL; range, 8–1823 gametocytes/μL) and 89.3% by RT-PCR (n = 25/28). Blood samples from 12 of 28 recrudescent patients were tested for mosquito infection, and only 1 patient (8.3%) was infectious. For this patient (PFPQD_002), infection prevalence and intensity were lower at Drec compared with D0 (prevalence 10 vs 41.7%; 2.6 vs 4.7 oocysts (Supplementary Table 1). Among the 6 patients who experienced a second recrudescence, the proportion of gametocytes in isolates was 20% by microscopy (n = 1/5; gametocyte density = 80 gametocytes/μL) and 80% by RT-PCR (n = 4/5). Blood samples from 5 of 6 recrudescent patients were tested for mosquito infection, and none were infectious to mosquitoes (Table 1, Supplementary Table 1).

Out of the 109 enrolled patients, the proportion of gametocyte carriers detected by RT-PCR on D0 samples was not significantly affected by the studied factors of sex ($P = .98$), G6PD phenotype ($P = .40$), age ($P = .45$), seasonality ($P = .07$), hemoglobinopathies ($P = .11$), K13 alleles (44.1% for wild-type vs 42.5% for mutant-type; $P = .87$), and clinical outcome (cured vs recrudescent; $P = .83$). However, among the 28 recrudescent patients, the proportion of gametocyte carriers detected by RT-PCR increased significantly between D0 and Drec samples (46.4% vs 89.3%; $P = .002$).

Additional Symptomatic Cohort

Fifteen additional patients with uncomplicated falciparum malaria were enrolled in 2016. *Plasmodium falciparum* gametocytes were detected in 2 of 15 patients (13.3%; 5 and 15 205 gametocytes/μL) by microscopy and in 7 of 15 patients (47%) by RT-PCR. Among the 14 patients enrolled in the feeding

Table 1. Characteristics of the symptomatic *Plasmodium falciparum* patients enrolled in Rattanakiri province, Cambodia, 2015–2016

Study	Case	No.	Mean age, y (SD)	No. (%) of gametocyte-positive slides	Mean gametocytemia/μL (SD, range)	No. (%) of positive gametocytes by RT-PCR	Infectivity
SLDPQ							
	D0 (feeds)	48	26 (15)	8 (16.7)	71 (254, 0–1432)	20 (41.7)	6.2%
	D0 (no feed)	61	24 (14)	13 (21)	36 (142, 0–800)	26 (43)	NA
	Drec1 (feeds)	12	24 (14)	2 (17)	161 (524, 0–1883)	10 (83)	8.3%
	Drec1 (no feed)	16	19 (10)	2 (12.5)	3 (10, 0–40)	15 (94)	NA
	Drec2 (feeds)	5	20 (15)	1 (20)	16 (36, 0–80)	3 (75)	0
	Drec2 (no feed)	1	10	NA	NA	NA	NA
Additional cohort							
	D0 (feeds)	14	20 (10)	2 (14.3)	1086 (4063, 0–15 205)	7 (50)	7.1%
	D0 (no feed)	1	54	0	0	1	NA

Single low-dose primaquine (SLDPQ) study represents the individuals enrolled in the open-label randomized control trial assessing the tolerability and the safety of SLDPQ, whereas the second study represents the additional symptomatic cohort from 2016. Drec1 and Drec2 correspond to day 0 sampling of the first and second recrudescences, respectively.

Abbreviations: *A. dirus*, *Anopheles dirus*; *A. minimus*, *Anopheles minimus*; NA, not applicable; RT-PCR, real-time polymerase chain reaction; SD, standard deviation; SLDPQ, single low-dose primaquine.

assays, only the patient with the highest gametocytemia (7.1%; n = 1/14) was found to be infectious to mosquitoes in both DMFAs and SFAs. The overall parasite prevalence in mosquitoes (DMFAs and SFAs) was higher for *A. minimus* (94%; n = 89/94) compared with *A. dirus* (71%; n = 71/100), whereas the mean number of oocysts was lower in *A. minimus* compared with *A. dirus* (184 vs. 210, respectively) (Supplementary Figure 2). These differences were still observed regardless of the feeding system: 94% (mean oocysts, 171) for *A. minimus* versus 60% (mean oocysts, 106) for *A. dirus* using DMFAs and 95.5% (mean oocysts, 198) for *A. minimus* versus 82% (mean oocysts, 286) for *A. dirus* using SFAs.

Contribution to Malaria Transmission of Asymptomatic Parasite Carriers

Follow-up During the Dry Season

The proportion of *Plasmodium* infections detected by RT-PCR was 12.2% (n = 43/352) and did not differ significantly between febrile patients (16%; n = 12/75) and asymptomatic individuals (11.2%; n = 31/277; $P = .35$). The distribution of the *Plasmodium* species was similar between the 2 groups (febrile group: 3 *P. falciparum*, 7 *P. vivax,* and 2 mixed *P. falciparum/P. vivax*; asymptomatic group: 13 *P. falciparum*, 13 *P. vivax*, 3 mixed *P. falciparum/P. vivax,* and 2 *P. malariae*).

Direct-membrane feeding assays and PCR analyses were carried out 1 week later from 3 malaria-free individuals (confirmed by RT-PCR) and 29 asymptomatic *Plasmodium* sp. carriers (all *P. falciparum*, *P. vivax,* and mixed *P. falciparum/P. vivax*). None were infectious to mosquitoes, except 1 *P. vivax* participant (58%; 3 ± 0.45 oocysts) (Table 2, Supplementary Table 2).

Among the 17 participants (13 *P. falciparum*, 1 *P.* vivax, 3 mixed *P. falciparum/P. vivax*) followed up weekly for DMFAs and PCR analyses until week 8 (Suppplementary Table 3), 5 became symptomatic: 2 in week 1, 2 in week 2, and 1 in week 3. Only 1 mixed *P. falciparum/P. vivax* participant who became symptomatic in week 2 was infectious to mosquitoes (n = 1/50 fed mosquitoes; 2% ± 4%; 1 oocyst (Supplementary Table 3). One participant was lost to follow-up at week 5. Of the 11 participants who completed the 2 months follow-up (all were *P. falciparum* positive on week 0), none was infectious to mosquitoes over time. The highest *P. falciparum* gametocyte density found by RT-PCR was 50 gametocytes/µL, regardless of the collection date (Supplementary Table 3).

Follow-up During the Rainy Season

Among the 706 villagers screened for *Plasmodium* infections, 36 (5.1%) were febrile (>37.5°C), and 2 (0.28%) of them were RT-PCR positive for *P. vivax* and mixed *P. falciparum/P. vivax.* Among the 670 asymptomatic participants, 44 (6.6%) were found positive by RT-PCR (6 *P. falciparum*, 33 *P. vivax*, 4 mixed *P. falciparum/P. vivax*, 1 *P. malariae*). The proportion of asymptomatic carriers was significantly lower during the rainy season compared with the dry season (6.6% vs 11.2%; $P = .02$).

After screening, 3 malaria-free individuals and 26 *Plasmodium* sp. carriers (6 *P. falciparum*, 15 *P. vivax*, 4 mixed *P. falciparum/P. vivax*, 1 *P. malariae* from week 0) were enrolled for serial DMFAs and RT-PCR (Supplementary Table 3). One *P. falciparum* asymptomatic participant became symptomatic in week 1 without infecting mosquitoes. The 3 malaria-free individuals became positive during the follow-up. Except for 2 *P. vivax* asymptomatic participants, all individuals were not infectious to mosquitoes.

The first *P. vivax* participant was infectious in week 1 (88% ± 9%; 81.8 ± 5.8 oocysts), week 4 (88% ± 9%; 16 ± 1.25 oocysts), and week 6 (78% ± 11%; 21.3 ± 2.55 oocysts). Although positive for a *P. falciparum/P. vivax* infection in week 1, the *P. falciparum* gametocyte RT-PCR was negative, supporting only *P. vivax* transmission. The second *P. vivax* asymptomatic participant was infectious in week 1 (76% ± 12%; 11.1 ± 1.4 oocysts), week 3 (6% ± 6.6%; 1 ± 0 oocyst), and week 6 (31.6% ± 13%; 1.8 ± 0.3 oocysts). The highest *P. falciparum* gametocyte density found by RT-PCR was 5 gametocytes/µL (Supplementary Table 3).

Comparing the Infectivity of Asymptomatic and Symptomatic Parasite Carriers

Of the 8 asymptomatic carriers infectious to mosquitoes (n = 8/192; n = 0/52 *P. falciparum*, 2/56 *P. falciparum/ P. vivax*, 6/84 *P. vivax*), 2.25% ± 0.3% (n = 213/9458) mosquitoes became infected, whereas 2.7% ± 0.5% (n = 107/3933) mosquitoes fed on symptomatic carriers (n = 5/79) became infected ($P = .80$). The number of mosquitoes infected by *P. falciparum* asymptomatic carriers (0%; n = 0/2565) and *P. falciparum* symptomatic carriers (2.7% ± 0.5%; n = 107/3933) was not significantly different ($P = .56$).

DISCUSSION

Our study set out to characterize infectivity in patients attending local health centers and asymptomatic individuals in the community using both DFMA and SFA techniques and the 2 most important mosquito vectors in Cambodia, *A. minimus and A. dirus*. We found very low infectivity in the

Table 2. Characteristics of the asymptomatic participants in follow-ups carried out in the dry and the rainy season in Rattanakiri province, Cambodia 2016

Season	No. of participants	No. of feeds	No. of falciparum-positive gametocytes by RT-PCR	Infectivity
Dry	32	91	71.7% (43/60)	2.2% (2/91)
Rainy	29	168	49 % (27/55)	3.6% (6/168)

Abbreviation: RT-PCR, real-time polymerase chain reaction.

falciparum-infected patients at baseline (~6%) and on the day of treatment failure (~8%) and a complete absence of infectivity in *P. falciparum* asymptomatic carriers despite 2 months of follow-up in the wet and dry seasons. Direct-membrane feeding assays are usually strongly correlated with SFA techniques [7]; however, their precision in carriers with low gametocytemias has been questioned [13]. On a subset of patients, both assays gave similar results, reconfirming their good correlation [7] as well as a low infectivity rate of falciparum carriers and excluding a technical limitation in our hands.

Our low infectivity in patients is consistent with the work from Oddar Meanchey province (northwest Cambodia) in which an almost identical figure was observed: 5.9% of symptomatic carriers were infectious [17]. In contrast with *P. falciparum*, 2 *P. vivax* asymptomatic carriers were infectious to *A. dirus* mosquitoes, with the infectivity persisting over several weeks. Real-time polymerase chain reaction–quantified gametocytemia in the *P. falciparum* asymptomatic carriers was low, and this is probably the main cause of the low infectivity.

Although recent studies conducted in Pailin province (western Cambodia) observed that 16% of the population (n = 1447) was malaria positive by using a high blood volume–based molecular assay, the geometric mean parasite density was low, approximately 5 parasites/μL with an upper interquartile range of 5.5/μL; densities of ≥10 000 μL were observed in the 90th percentile and above [27, 28]. We also found low densities of *P. falciparum* gametocytes in asymptomatic carriers, but our highest gametocytemia was only 50 gametocytes/μL, which did not increase over time. Our finding a tight distribution of gametocyte densities in the asymptomatic carriers is more concordant with data from low-transmission areas of Ethiopia [29] compared with the low transmission areas of Pailin province, Thailand, and Vietnam [27, 28].

Surprisingly we observed a significantly higher proportion of asymptomatic carriers in the dry season (~11%) compared with the rainy season (~7%). One hypothesis could be that *P. falciparum* parasites in low-transmission areas have been selected to limit their commitment toward reproductive stages during the dry season when few vectors are present, and asexual stages are capable of surviving at low densities in the human host. By contrast, the rainy season offers increased transmission opportunities, so multiplication rates of asexual stages may increase, leading to increased gametocytogenesis. Previous reports have shown that *P. falciparum* strains are able to modulate their transmission strategy according to the environmental context [30–34].

We also observed the long-term carriage of monoclonal infection in most asymptomatic carriers (Supplementary Table 4), suggesting that the ability of the host immune system to control falciparum infections at very low densities might be even further selected by the low genetic diversity [35, 36]. However, when immunity fails, breakthrough clinical episodes occur, which we observed for 5 asymptomatic carriers during the dry season. More research is needed in low transmission areas to decipher the capacity of *P. falciparum* parasites to adapt their transmission strategy and to determine which internal and external factors might trigger it (seasonality, waning immunity, vector presence, new infecting strains, etc).

Our findings highlight the need to consider carefully optimal elimination strategies in our setting, where the majority of malaria cases occur in young adults who work in the forests. Our study and that of Lin and colleagues [17] show that infectivity is greater in symptomatic patients. Accordingly, the prompt detection and treatment of *P. falciparum* patients with an effective antimalarial drug against asexual parasites and the rapid implementation of adding SLDPQ without the need to screen for G6PD deficiency are essential elements of an elimination strategy for Cambodia. We assessed infectivity in 55 asymptomatic carriers and 6 malaria-free individuals, among whom only 46 participants were followed over time. However, the very low infectivity of these carriers hampered our ability to statistically assess their contribution to malaria transmission. Consequently, more data in this important group in other low transmission areas of Southeast Asia are needed, especially near transmission hotspots like forests.

To conclude, we have demonstrated low infectivity in both symptomatic patients and asymptomatic carriers but were under powered to show a statistically significant difference between the 2 groups. We reconfirm the high failure rate of DHA-PP and its associated high gametocyte prevalence. More infectivity data would support optimal elimination strategies between symptomatic and asymptomatic malaria reservoirs, but in the interim SLDPQ should be deployed to support malaria elimination efforts in patients.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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