

FOREST MALARIA IN VIETNAM: A CHALLENGE FOR CONTROL

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Abstract. Forest malaria is a complex but common phenomenon occurring in southeast Asia. We studied its epidemiology through a prospective community-based study in central Vietnam. A total of 585 individuals were followed for two years by active case detection and biannual cross-sectional surveys. The prevalence of antibodies to *Plasmodium falciparum* was constantly about 20% across surveys and the incidence rate of clinical episodes of *P. falciparum* malaria was 0.11/person-year. Multivariate analysis showed that regular forest activity was the main risk factor for clinical malaria and malaria infections. Untreated bed nets had a significant protective effect (60%), except for people regularly sleeping in the forest. The population-attributable fraction for regular forest activity was estimated to be 53%. Our results confirm the major role played by forest activity on the malaria burden in this area and provide the basis for targeting control activities to forest workers. New interventions based on insecticide-treated materials need to be urgently evaluated.

INTRODUCTION

After 10 years of intensive control efforts, malaria morbidity and mortality in Vietnam have decreased by 60% and 97%, respectively.^{1–3} Although malaria has disappeared in many areas,⁴ it is still endemic in the central and southern provinces of the Tay Nguyen highlands. These forested mountainous areas, where agriculture and woodcutting are highly profitable, attract many migrant workers from non-endemic provinces and from border areas of Laos and Cambodia where malaria is endemic.^{5,6} Such population movements contribute to maintain malaria transmission in central Vietnam and to the re-introduction of parasites in places where they had disappeared. This is a highly variable situation where target groups are not well defined and consequently where malaria control measures are difficult to implement. Moreover, the main vector, *Anopheles dirus A*, is highly exophagic and exophilic and interventions based on residual indoor spraying or insecticide treated bed nets have had a limited impact.^{7,8} In this context, it is extremely important to identify and quantify the effects of factors involved in malaria transmission. We carried out a longitudinal study in four sentinel sites aiming at describing the epidemiology and the transmission dynamics of malaria in relation to local entomologic and ecologic parameters.⁹ We report here the epidemiologic results of a two-year follow-up in one sentinel site located in a forested area of central southern Vietnam. This is one of the few community-based studies on malaria morbidity in Vietnam.¹⁰

MATERIALS AND METHODS

Study population. The study was carried out between September 1999 and August 2001 in Village 3, Suoi Kiet commune, a hilly and forested area situated in Binh Thuan province (in the southern part of the central coast of Vietnam). The dry season is from January to April, with the coldest months in January and February and the rainy season is from May to December. The annual average temperature is 26.4°C and the average humidity is 82%. Malaria transmission is perennial with two peaks: June and October. *Anopheles dirus A* is the main vector and several potential secondary vectors such as *An. aconitus*, *An. maculatus*, and *An. sinensis* are

present.^{7,8} Village 3 has approximately 1,000 inhabitants, all belonging to the Kinh ethnic group. Most people are farmers growing cashew nut trees, manioc, and maize. The soil is dry and poor, and people have established fields in the forest directly surrounding the village (hut farmers). The most profitable but illegal activity is woodcutting. All the 104 households (total = 541 individuals), situated in the eastern half of the village where the mosquito collections were carried out were selected for the study. This section of the village was situated between the district road and the river and was representative of the different kind of house structures found in the entire village (bamboo/wooden or brick walls; palm leaf/wooden or tiled roofs; mud or concrete floors). The forest was situated beyond the river approximately 1 km from the nearest houses. Only permanent residents were included in the study and a unique code number was given to each of them. Informed consent was obtained from all study participants, the village leaders, and the People's Committee. Ethical approval for the study was obtained from the Ministry of Health of Vietnam and the National Institute for Malariology, Parasitology and Entomology in Hanoi.

Data collection. A census of the study population was done at the start of the study and information on age, sex, occupation, forest activity, bed net availability, and previous vector control measures was collected. Forest activity was categorized into four groups: never, occasionally (less than once a week), regularly working (on average every week), and regularly working and sleeping in the forest (same as previous but also regularly sleeping in the forest). Bed net availability is an estimate of the average number of individuals sleeping under a net in each house (number of individuals sleeping in the house divided by the number of bed nets in use). This variable was arbitrarily categorized into two groups: inadequate = no bed nets or more than three persons sleeping under one net and adequate = 1–3 persons/net. In our study we consider bed nets as non-treated with insecticide since in this commune such activities ended in 1998, one year before the study started.

Active case detection (ACD) started in September 1999 after the census and the first cross-sectional survey, and consisted in weekly home visits. Information on malaria symptoms and treatments between visits, as well as body (axillary)

temperature, was collected and reported on pre-coded standardized questionnaires. A blood slide (thick and thin films) was taken whenever the body temperature was $\geq 37.5^{\circ}\text{C}$ and/or there was a history of fever within the past 48 hours. Suspected malaria cases were treated presumptively by the hamlet health workers with a full course of artesunate. Treatment compliance was not checked. Reasons for any absence of more than one week were recorded and an updated census with population movements was kept. If an individual could not be followed-up for a total period of 12 weeks or more during the two-year study, he or she was considered not to be representative of the population at risk and was excluded from the analysis of ACD. Malaria attacks detected during the first week of follow-up were also excluded from the analysis because infection occurred before the follow-up.

All five cross-sectional surveys concerned all individuals living in the 104 households included in follow-up study. The first survey was done in September 1999; four additional surveys were carried out twice a year at the end of each transmission period. Each participant was administered a questionnaire on malaria symptoms and treatments and clinical examination measured body temperature and spleen size. Blood samples were taken for microscopic examination (thick and thin films), hematocrit (micro-capillary tubes), and identification of antibodies to malaria parasites (filter papers). Suspected malaria cases were treated presumptively.

Laboratory methods. *Microscopic examination.* Blood slides were stained with a 3% Giemsa solution for 45 minutes. The number of asexual forms per 200 white blood cells (WBCs) was counted and parasite densities were computed assuming a mean WBC count of $8,000/\mu\text{L}$. A slide was defined as negative if no asexual forms were found after counting 1,000 WBCs. Slide reading was blinded and an external quality control done at the Institute of Tropical Medicine (Antwerp, Belgium) (all positive slides and 10% of the negative slides) found a 99% agreement at first reading, then 100% after re-reading by the two laboratories.

Hematocrit. Blood in micro-capillary tubes was centrifuged and the packed cell volume was determined according to the principles of the Hawksley micro-hematocrit reader method, as recommended by the World Health Organization.

Indirect fluorescent antibody test (IFAT). Filter papers (No. 3; Whatman, Kent, United Kingdom) were stored at -20°C . Total immunoglobulin titers for *P. falciparum* were measured by an IFAT that has been previously described.¹¹ *Plasmodium falciparum* antigen was prepared from *in vitro* cultures of an isolate from a patient in southern Vietnam. Negative control serum was obtained by pooling the sera of five malaria-free individuals; positive control serum was obtained from five patients that had several malaria episodes. The serum dilutions studied ranged from 1:40 to 1:640. Slide reading was blinded and double reading for all samples found an agreement of more than 98% between two independent readers.

Case definition. A clinical case of malaria was defined as fever (body temperature $\geq 37.5^{\circ}\text{C}$) and/or a history of fever in the past 48 hours, with a blood slide positive for *Plasmodium* asexual forms. The terms cases or episodes will be equally used in this paper. Recrudescence was defined as clinical *P. falciparum* malaria occurring within 28 days following the first episode. All recrudescences were excluded from the final analysis of ACD. A positive IFAT result for either cur-

rent or recent infection with *P. falciparum* was defined as an IFAT titer $\geq 1:80$. New infections determined by an IFAT (five surveys) were defined as all individuals whose titers became positive or, if previously positive, increased two-fold or remained $\geq 1:320$ between two consecutive surveys.

Statistical analysis. Data were analyzed with STATA 7.0 software (Stata Corp., College Station, TX). Incidence rates of *P. falciparum* malaria attacks were calculated for each group at risk in the cohort study. Person-years at risk were calculated by defining as one week at risk for malaria each individual record in which the subject was present and not currently under anti-malarial treatment. To assess the effect of different risk factors on new clinical episodes, we restricted the analysis to *P. falciparum* (excluding recrudescence) because it was impossible to distinguish new infections from relapses with *P. vivax*.

For cross-sectional surveys, *P. falciparum* seroprevalence, which is a cumulative prevalence of all recent and current infections, was calculated for each group at risk and was the outcome measure for the analysis of the overall risk of *P. falciparum* infections. Since repeated measurements on individuals were done both in longitudinal and cross-sectional studies, clustering was addressed by applying generalized estimating equations as previously described.¹² A binomial family and its default link and exchangeable working correlation structure were used. The time variable (t) taken was either the week number (longitudinal study) or the time of survey (cross-sectional studies). Individuals were taken as the cluster units. Univariate and multivariate-adjusted odds ratios (ORs) (adjusting for age, sex, forest activity, and bed net availability) were calculated for the risk of new clinical episodes (ACD) and for the overall risk of *P. falciparum* infections. Within household clustering effect was investigated as well as all possible interactions of up to order two. The population-attributable fraction (PAF) for forest activity represents the proportion of *P. falciparum* infections in the population that would be avoided if the exposure (forest activity) were removed.¹³

RESULTS

A total of 573 individuals were included in the main analysis of the cohort study (ACD), (Figure 1). Two hundred eleven malaria clinical episodes were detected in 109 (19%) subjects, while 464 (81%) remained symptom-free during the two-year follow-up. The study population was very young, 50% of the individuals were less than 20 years old, and ethnically homogeneous (all but two were Kinh) (Table 1). Half of the active population (175 of 338) regularly worked in the forest, either farming or woodcutting, although many people had both activities. The population was stable because more than 80% were permanent residents. Emigrations were evenly distributed during the follow-up period. A total of 956.7 person-years at risk were investigated with a median follow-up of 98 weeks. Ten percent of the individuals were lost to follow-up due to emigration and 15 (25%) were regular forest workers.

Risk factors for *P. falciparum* malaria attacks determined by ACD. Among the 211 malaria clinical episodes, 123 (58.3%) were due to *P. falciparum*, 85 (40.3%) to *P. vivax*, and 3 (1.4%) to *P. malariae* (single and/or mixed infections).

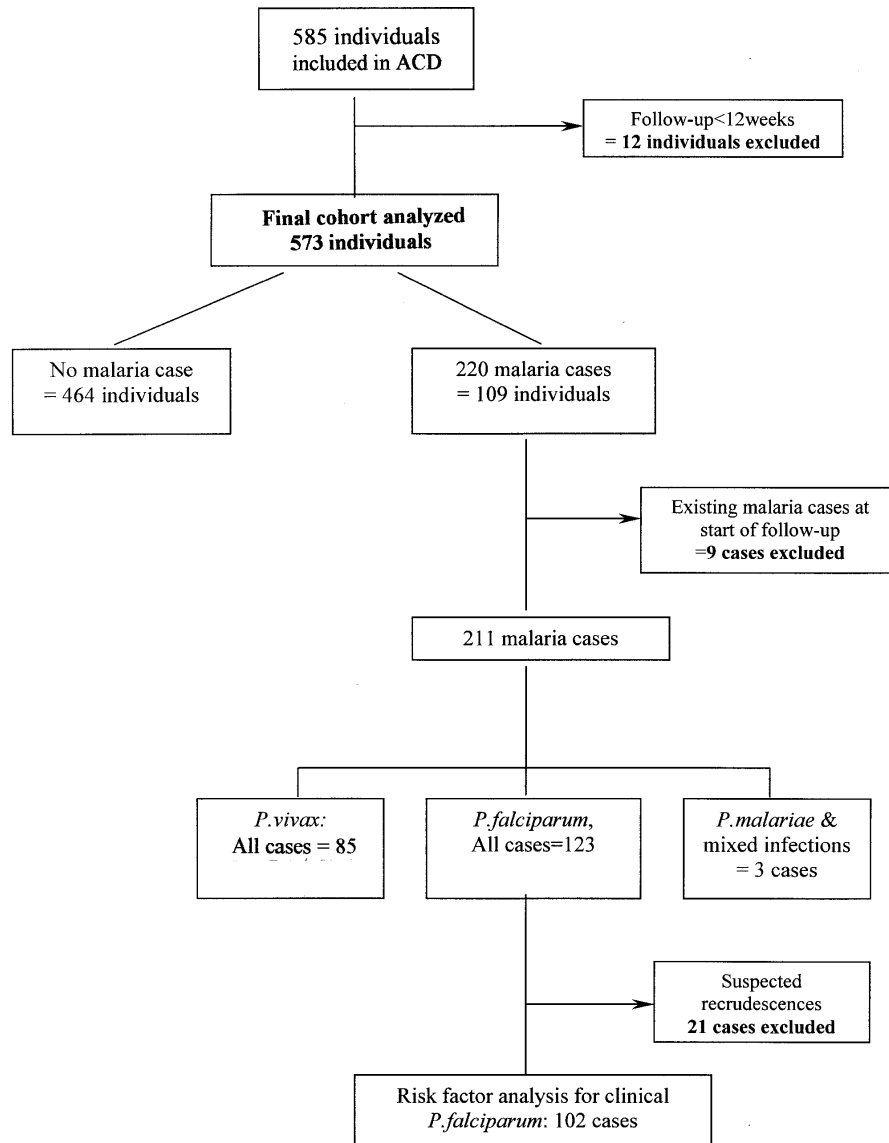


FIGURE 1. Cohort selection procedure and exclusion criteria. ACD = active case detection.

The overall incidence rate of malaria cases was 0.22/person-year; the incidence rate of new clinical cases of *P. falciparum* malaria (excluding 21 recrudescences) was 0.11/person-year (0.13/person-year when including the 21 recrudescences). The incidence rate of clinical cases of *P. falciparum* malaria among individuals lost to follow-up was similar to that in the cohort (0.13/person-years). The results of the univariate analysis show that sex, age, profession, regular forest activity, bed net availability, and season were significant risk factors for new episodes of *P. falciparum* malaria (Table 2). Working regularly in the forest (especially woodcutting) was a significant risk factor (OR = 5.20, $P < 0.001$), although it was not different from that of people sleeping regularly in the forest (OR = 4.63, $P < 0.001$). Males and young adults (less than 40 years old) were significantly more at risk than females and children less than nine years old. Untreated bed nets had a significant protective effect (OR = 0.40, $P < 0.001$). The incidence rate among people lost to follow-up was 0.13/person-years.

Profession and forest activity were strongly correlated;

thus, both of them could not be kept in the final model. Forest activity was chosen as the primary exposure in the multivariate analysis. Multivariate-adjusted ORs for effects of risk factors on clinical episodes of *P. falciparum* malaria are shown in Table 3. There was a significant interaction between bed net availability and regular work and sleeping in the forest. If people had bed nets at home, sleeping in the forest increased significantly the risk of clinical malaria attacks (OR = 8.09, $P < 0.001$), whereas such a risk did not change for those without nets (OR = 1.72, $P = 0.25$). People with regular forest activity, but who did not usually sleep in the forest, were also at higher risk for clinical malaria (OR = 3.85, $P < 0.001$) but this was independent of bed net availability at home (no interaction). Conversely, untreated nets had a significant protective effect, except for people sleeping in the forest. Such risk factors with corresponding ORs and significance levels remained similar after including the 21 clinical attacks classified as recrudescences. The monthly incidence rate of clinical cases of *P. falciparum* malaria are shown in

TABLE 1
Baseline characteristics of the study population

Total number of individuals	573
Total person-years	956.7
Median follow-up time, weeks (range)	98 (12–99)
Median date of entry, week (range)	1 (1–82)
Median date of exit, week (range)	100 (14–100)
Males/females (ratio)	285/288 (1.0)
Median age at entry, years (range)	19 (0–78)
Age (years) categories, no. (%)	
<1	16 (2.8)
1–9	154 (26.9)
10–19	117 (20.4)
20–39	190 (33.2)
40–59	69 (12.0)
≥60	27 (4.7)
Ethnic group, no. (%)	
Kinh	571 (99.7)
Chinese	2 (0.3)
Bed net availability,* no. (%)	
No	186 (32.4)
Yes	370 (64.6)
Missing data	17 (3.0)
Forest activity, no. (%)	
Never	342 (59.7)
Occasionally	56 (9.8)
Regular work (not sleeping in forest)	129 (22.5)
Regular work and sleeping in forest	46 (8.0)
Professional categories, no. (%)	
None (children, students)	235 (41.0)
Woodcutters	119 (20.8)
Farmers	53 (9.2)
Housewives, retired	78 (13.6)
Others (business, officer, driver, worker, etc)	88 (15.4)
Status, no. (%)	
Residents	469 (81.9)
Emigrants	58 (10.1)
Immigrants	28 (4.9)
Several movements (immigrate, emigrate)	18 (3.1)

* No = no bed nets or more than three persons per net; yes = 1–3 persons/net.

Figure 2; the two years were combined because there was no significant difference between them ($P = 0.27$). The overall incidence rate shows that malaria transmission is perennial with two small peaks in June and October. After adjusting for regular forest activity, the monthly incidence rate was much higher in individuals with regular forest activity and the seasonal effect much more pronounced than in people without regular forest activity.

Risk factors for overall *P. falciparum* infections determined by IFAT (five surveys). Parasite and spleen rates, particularly in children 2–9 years old, were constantly less than 10% and decreased with time. No severe cases of anemia were detected (Table 4). The prevalence of clinical malaria was very low, ranging from 0.5% to 1.8% across surveys, and most (42 of 60) of the infections detected by microscopy were asymptomatic. The prevalence of antibodies to *P. falciparum* was approximately 20%, ranging from 18% to 24% across surveys. The incidence rate of new *P. falciparum* infections determined by the IFAT, was approximately two times higher (0.22/person-year) than that estimated by ACD. Most of the clinical episodes missed by ACD occurred in people with regular forest activity. The risk factors for malaria infection identified by serology were the same (except for season) than those identified by ACD (Table 5). Multivariate-adjusted ORs are shown in Table 6. The two former categories of regular forest activity (sleeping and not sleeping in the forest)

were merged into one (ORs and 95% confidence intervals were identical) and the effect of regular forest activity was compared with the baseline of never going to the forest. A significant interaction was present between regular forest activity and sex because the former was significantly associated with the risk of infection in men (OR = 10.76, $P < 0.001$), but not in women (OR = 1.56, $P = 0.25$). Conversely, sex was an important risk factor because women were significantly less at risk of malaria infections than men (OR = 0.18, $P < 0.001$), but only in those with regular forest activity. The summary OR for the effect of occasional forest activity was 2.85 ($P = 0.006$) regardless of sex (no interaction).

Age, bed net availability, and survey time remained significant risk factors for malaria infections after adjusting for the combined effect of forest activity and sex. The odds of infection increased significantly with age and there was a significant protective effect of untreated nets (OR = 0.38, $P < 0.001$). In the risk factor analysis for all *P. falciparum* infections, the estimated PAF was 53% for regular forest activity and 60% when considering any forest activities (including occasional).

DISCUSSION

Malaria and forest activity is a well-known association in southeast Asia,^{14,15} and many studies carried out in this part of the world have reported forest activity as a strong risk factor for malaria.^{16–22} We report here one of the few prospective community-based studies on malaria morbidity in Vietnam. The combination of ACD and regular cross-sectional surveys gives a dynamic picture of malaria transmission and an estimation of the importance of several risk factors, such as forest activity, for both clinical malaria and malaria infection. According to the malarionometric indices estimated by the five surveys, this area can be classified as hypoendemic because parasite and spleen rates in children 2–9 years old were consistently less than 10%. Malaria transmission within the village is low according to the malaria incidence rates in people without regular forest activity. This observation is consistent with the estimated entomologic inoculation rate of approximately one infective bite/person-year due to the main malaria vector (*An. dirus A*).⁷

We arbitrarily defined clinical attacks occurring within 28 days after treatment as recrudescences because we wanted to specifically estimate risk factors for new malaria infections. A total of 21 recrudescences were identified, indicating an artesunate failure rate of 17%. If one considers the duration of the treatment and the fact that it was not supervised, this failure rate might be due to low compliance.

Regular forest activity (woodcutting and agriculture), a common activity in Village 3, was a strong and significant risk factor for malaria and increased by 4-fold and 10-fold the odds of disease and infection, respectively. Our estimation is unlikely to be biased because the information on forest activity was collected independently of malaria symptoms and diagnosis (microscopic examination and IFAT reading) was blind to the subject's identity and exposure. Moreover, only 10% of the people under surveillance were lost to follow-up, and the malaria incidence rate in this group was similar to the one estimated in the whole cohort.

In the cohort study, regular forest activity (without sleeping in the forest) was a significant risk factor for clinical malaria

TABLE 2

Risk factors for *Plasmodium falciparum* clinical episodes determined in active case detection by univariate analysis*

Risk factors	Rate	Cases/ person-year	OR	P	95% CI
Overall	0.11	102/956.7			
Sex					
Male	0.15	71/466.8	1		
Female	0.06	31/489.9	0.42	0.001	(0.25–0.69)
Age, years					
0–9	0.04	10/251.3	1		
10–19	0.13	27/212.4	2.89	0.01	(1.28–6.53)
20–39	0.16	51/318	3.74	0.002	(1.65–8.48)
≥40	0.08	14/175.1	1.93	0.21	(0.69–5.42)
Profession					
None	0.06	23/400.0	1		
Woodcutters	0.29	56/192.2	5.05	<0.001	(2.70–9.44)
Hut farmers	0.14	13/95.7	2.42	0.07	(0.92–6.38)
Housewives, retired	0.04	5/123.5	0.75	0.58	(0.27–2.07)
Others	0.03	5/145.3	0.63	0.36	(0.23–1.71)
Forest activity					
Never	0.05	27/578.4	1		
Occasionally	0.07	6/85.7	1.56	0.33	(0.63–3.88)
Regular work (not sleeping in forest)	0.24	51/211.4	5.20	<0.001	(2.81–9.64)
Regular work and sleeping in forest	0.22	18/81.2	4.63	<0.001	(2.46–8.72)
Bed net availability					
No	0.18	52/295.6	1		
Yes	0.07	44/636.9	0.40	<0.001	(0.24–0.66)
Missing data	0.25	6/24.2			
Season					
Dry: Jan–Apr	0.06	21/331.0	1		
Rainy: May–Aug	0.14	45/323.6	2.18	0.006	(1.25–3.81)
Sep–Dec	0.12	36/302.0	1.88	0.020	(1.11–3.19)

* OR = odds ratio; CI = confidence interval.

and this effect was independent of bed net availability at home. Nevertheless, regularly sleeping in the forest increased eight times the risk of clinical malaria, but only if people had bed nets at home. Such a finding can be explained only by admitting the protective efficacy of untreated nets and consequently a non-negligible (even if low) level of malaria transmission within the village. People not having bed nets are exposed to the risk of clinical malaria in the village and sleeping in the forest does not increase significantly such a risk. Conversely, people with bed nets are protected when sleeping at home but are exposed to infection when sleeping in the

forest where they usually do not use nets. Thus, bed nets are not adequate for preventing malaria in forest workers.

The ACD was based on weekly home visits and might have missed some clinical cases. Antibody titers to *P. falciparum* measured from samples collected during the five surveys can estimate the cumulative frequency of all infections. The comparison between these two methods can estimate the amount of malaria cases missed by ACD. The incidence rate estimated by the evolution of antibody titers is approximately double that estimated by ACD. Since most of the missed infections occurred in people with regular forest activity, they

TABLE 3

Risk factors for clinical *Plasmodium falciparum* cased determined by multivariate-adjusted analysis*

Risk factors	OR	P	95% CI
Forest activity			
Never/occasional	1		
Regular work (not sleeping in forest)	3.85	<0.001	(2.15–6.89)
Effect of regular work and sleeping in forest*†			
In people not using bed nets	1.72	0.25	(0.68–4.39)
In people using bed nets	8.09	<0.001	(3.96–16.54)
Effect of bed nets according to forest activity‡			
Never/occasional	0.31	0.004	(0.14–0.68)
Regular work, not sleeping in forest	0.38	0.025	(0.16–0.89)
Regular work and sleeping in forest	1.47	0.39	(0.61–3.53)
Season			
Dry	1		
Rainy	2.01	0.009	(1.19–3.39)

* using generalized estimating equation. OR = odds ratio; CI = confidence interval. An interaction term was included between regular work and sleep in forest and bed net protection. For the interaction term, OR = 4.70, P = 0.01, by Wald test.

† OR for regular work and sleep in forest (compared with never/occasional) according to bed net availability.

‡ OR for bed net availability (compared with non-availability) according to forest activity.

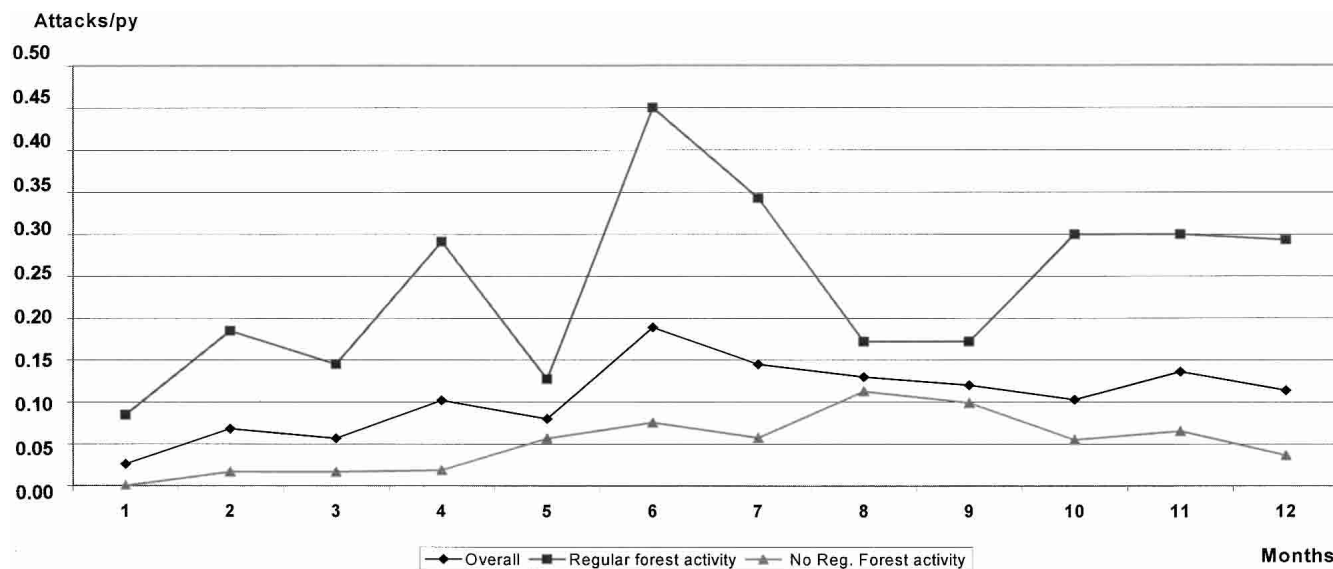


FIGURE 2. Monthly incidence of cases of *Plasmodium falciparum* malaria. Months 1, 2, etc. refer to January, February, etc. The two years of follow-up were combined. py = person-year; Reg. = regular.

might have some level of immunity resulting in asymptomatic or mild clinical episodes that would be self-treated and not reported to the community health workers. Self-treatment is a current practice in Vietnam, and anti-malarial drugs can be bought in any shop. If one considers the efforts and the resources involved in the set up of a performing ACD system, serologic surveys might be more cost-effective for the surveillance of malaria morbidity in Vietnam or in any other place with low transmission. Other studies carried out in areas of low endemicity, such as The Philippines, also suggested the usefulness of serology for the surveillance of malaria morbidity and the identification of focal areas at risk.¹⁸

The multivariate analysis carried out with the serologic results of the five surveys specifically examined risk factors for malaria infections, and confirmed the strong and significant effect of both forest activity and bed net protection. Moreover, there was a significant interaction between regular forest activity and sex. Regular forest activity was a significant risk factor for men, but not for women. This could be explained by different exposure to mosquito bites because

women, even if they performed regular forest activity, do not go into the forest as often as men and do not stay as long because they also perform additional domestic tasks. Women also remain well covered while men usually work stripped to the waist and in shorts, making them more exposed to mosquito bites. Among people who never worked in the forest, no sex difference was found.

In the analysis of risk factors for *P. falciparum* infections, the PAF for regular forest activity estimated that half of the malaria infections in the village could be avoided by removing this factor. However, even if this estimate needs to be interpreted with caution, given the underlying assumptions in the PAF calculation,¹³ it shows the contribution of forest activity in the overall malaria burden.

Our study shows that untreated nets have a significant protective effect on the risk of clinical malaria and malaria infection. The ORs estimated by ACD and cross-sectional surveys were similar and highly significant, showing a protective effect of approximately 60%. The protective effect of insecticide-treated bed nets has been frequently reported,²³⁻²⁵ but

TABLE 4
Participation rates and malariometric indices in the five cross-sectional surveys*

Parameters	Survey 1 Sep 1999	Survey 2 Jan 2000	Survey 3 Aug 2000	Survey 4 Feb 2001	Survey 5 Aug 2001
Number of participants	338	429	398	412	389
Survey coverage, %	62.5	82.1	77.0	81.1	76.4
Mean PCV. (SD)	ND	40.5 (4.6)	39.6 (4.0)	40.9 (4.1)	40.9 (4.1)
Spleen rate, % (n)	5.3 (18)	2.1 (9)	2.5 (10)	3.4 (14)	0.8 (3)
In those 2-9 years old, % (n)	6.0 (6)	0	2.7 (3)	0.9 (1)	0
Parasite rate, % (n)	5.0 (17)	3.5 (15)	3.8 (15)	1.5 (6)	1.8 (7)
<i>P. falciparum</i> , no.	13	9	4	1	1
<i>P. vivax</i> , no.	4	6	11	5	5
<i>P. malariae</i> , no.	0	0	0	0	1
In those 2-9 years old, % (n)	7.0 (7)	2.9 (3)	1.8 (2)	0	1.0 (1)
Symptomatic malaria, % (n)	1.8 (6)	0.7 (3)	0.5 (2)	0.5 (2)	1.3 (5)
Seroprevalence, % (n/N†)	21.0 (66/314)	24.3 (103/424)	22.0 (87/395)	18.0 (70/390)	20.7 (79/381)

* PCV = packed cell volume; ND = not done.

† N = total number of individuals with an indirect fluorescent antibody test sample taken.

TABLE 5

Risk factors for all *Plasmodium falciparum* infections (n = 405) determined by IFAT in five surveys by univariate analysis*

Risk factors	Prevalence (%)	Cases n/N	OR	95% CI	P
Survey					
1	21.02	(66/314)	1.30	(1.02–1.67)	0.033
2	24.29	(103/424)	1.54	(1.24–1.91)	<0.001
3	22.03	(87/395)	1.33	(1.07–1.66)	0.009
4	17.95	(70/390)	1		
5	20.73	(79/381)	1.28	(1.04–1.57)	0.017
Sex					
Males	30.25	(258/853)	1		
Females	13.99	(147/1051)	0.36	(0.25–0.52)	<0.001
Age (years)					
0–9	1.88	(10/532)	1		
10–19	12.04	(55/457)	1.99	(0.80–4.94)	0.14
20–39	33.45	(193/577)	10.25	(5.31–19.79)	<0.001
≥40	43.49	(147/338)	16.54	(8.34–32.83)	<0.001
Forest activity					
Never	8.21	(75/913)	1		
Occasionally	24.00	(36/150)	2.83	(1.44–5.56)	0.003
Regular (not sleeping)	46.89	(151/322)	9.28	(5.88–14.65)	<0.001
Regular work and sleeping in forest	46.38	(64/138)	9.73	(5.39–17.56)	<0.001
Profession					
None (children, students)	3.71	32/862	1		
Woodcutters	51.84	197/380	30.92	(17.43–54.87)	<0.001
Hut farmers	43.32	81/187	20.27	(10.54–39.00)	<0.001
Housewives, retired	25.21	61/242	8.13	(4.06–16.27)	<0.001
Others	21.46	50/233	7.39	(3.75–14.56)	<0.001
Bed net availability					
No	28.45	(173/608)	1		
Yes	17.64	(220/1247)	0.58	(0.40–0.84)	0.005
Missing data	24.49	(12/49)			

* IFAT = indirect fluorescent antibody test; OR = odds ratio; CI = confidence interval.

there are few reports on the impact of untreated bed nets.^{26,27} We did not measure individual bed net use in our study; instead we used the bed net coverage per household as a proxy measure assuming that people are more likely to use

them if they are available in their house. This is likely to underestimate the true individual protection as a result of an effect dilution among all people sleeping in the household (under and not under net). At the same time, unmeasured

TABLE 6

Risk factors for *P. falciparum* infections (n = 405) determined by IFAT in five surveys by multivariate-adjusted analysis*

Risk factors	OR	95% CI	P
Forest activity			
Never	1		
Occasionally	2.85	(1.36–5.99)	0.006
Effect of regular forest activity according to sex†			
Males	10.76	(5.41–21.41)	<0.001
Females	1.56	(0.73–3.36)	0.25
Effect of sex according to forest activity‡			
Never	1.21	(0.60–2.42)	0.59
Occasionally	0.50	(0.10–2.45)	0.39
Regular	0.18	(0.08–0.36)	<0.001
Effect of age (years)			
0–19	1		
20–39	3.59	(2.12–6.08)	<0.001
≥40	9.18	(5.10–16.50)	<0.001
Bed net availability			
No	1		
Yes	0.38	(0.23–0.58)	<0.001
Effect of surveys			
Survey 1, 2, and 3 (first year)	1		
Surveys 4 and 5 (second year)	0.55	(0.41–0.73)	<0.001

* Using generalized estimating equation. An interaction was included between forest activity and sex. IFAT = indirect fluorescent antibody test; OR = odds ratio; CI = confidence interval.

† OR for the effect of regular forest activity (compared to never) separately in males and in females.

‡ OR for the effect sex (females compared to males) separately for different forest activity. For the interaction term, OR = 0.15, P < 0.001, by Wald test.

confounding factors such as socioeconomic status might have overestimated such protection. However, the magnitude of the protection was consistent between the different measurement methods (ACD and cross-sectional surveys). Therefore, our estimation of the protective effect of untreated bed nets is probably close to the true value.

Age in itself was not a significant risk factor for clinical malaria after adjusting for forest activity and bed net availability. Nevertheless, the odds of having antibodies to *P. falciparum* (i.e., the odds of infection) significantly increased by age groups even after adjusting for all the other risk factors. This suggests that outdoor night activities could be an important risk factor for malaria infection inside the village since the main vector, *An. dirus* A, is highly exophagic and exophilic. Human landing catches in Village 3 were found to be four times more frequent outdoors than indoors.⁷ Children go to sleep earlier and usually under bed nets when available, while adults stay outside and often sleep in hammocks in front of the house during the hot season.

In conclusion, most of the malaria burden in Village 3 is attributable to forest activity. Transmission inside the village is low but nevertheless high enough to observe a significant protective effect of untreated bed nets. Moreover, transmission occurs also outdoors because the main vector is highly exophagic and exophilic. The role of the numerous secondary vectors collected in Village 3 has yet to be defined. In this situation, control measures should focus on the protection of forest workers who constitute an important reservoir of parasites, and the impact of new interventions such as insecticide-treated materials (hammocks, sheets, etc.) specifically targeted to them should be tested in field trials. Ecologic and sociologic factors, movements within and towards the forests, and migration are important features in the dynamics of forest malaria transmission resulting in an epidemiologic mosaic with large site-specific variations. In this context, epidemiologic surveillance based on serologic follow-up can be a cost-effective tool for monitoring malaria morbidity, identifying site-specific risk factors, and evaluating new interventions to control forest malaria.

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