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High Prevalence of Asymptomatic *Plasmodium falciparum* Infections in a Highland Area of Western Kenya: A Cohort Study

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Background.   Transmission of malaria in an area of hypoendemicity in the highlands of western Kenya is not expected to lead to rapid acquisition of immunity to malaria. However, the subpopulation of individuals with asymptomatic *Plasmodium falciparum* infection may play a significant role as an infection reservoir and should be considered in malaria-control programs. Determination of the spatiotemporal dynamics of asymptomatic sub-populations provides an opportunity to estimate the epidemiological importance of this group to malaria transmission.

Methods.   Monthly parasitological surveys were undertaken for a cohort of 246 schoolchildren over 12 months. The prevalence of *P. falciparum* infection among 2611 blood samples was analyzed by both microscopy and polymerase chain reaction, and infection durations were determined.

Results.   Infection prevalence and duration (range, 1–12 months) decreased with age and altitude. The prevalence was high among pooled blood samples recovered from children aged 5–9 years (34.4%) and from those aged 10–14 years (34.1%) but was significantly lower among blood samples obtained from older children (9.1%). The prevalence decreased from 52.4% among pooled blood samples from children living at an altitude of ∼1430 m to 23.3% among pooled samples from children living at an altitude of 1580 m.

Conclusions.   The prevalence of asymptomatic *P. falciparum* infection was high, with polymerase chain reaction analysis detecting a significantly greater number of infections, compared with microscopy. Our results are consistent with gradual acquisition of immunity with increasing age upon repeated infection, and they also show that the risk of malaria transmission is highly heterogeneous in the highland area. The results provide strong support for targeted malaria-control interventions.

Plasmodium falciparum infection affects approximately half a billion people annually, with disease manifestations ranging from asymptomatic to severe clinical illness. Africa has the largest share (∼90%) of malaria-attributable deaths [1]. During the past 2 decades, malaria epidemics have increased in frequency and intensity in the East African highlands in populations with little or no previous exposure to plasmodial infections [2]. Although the prevalence of symptomatic and asymptomatic infections in the malaria-endemic lowlands is well documented, there is a paucity of data from the highlands. Many asymptomatic infections go undetected and untreated while causing little or no clinical manifestation. The extent of the prevalence of asymptomatic parasitemia in a given population is inversely related to the population's susceptibility to clinical disease [3, 4], but more importantly, asymptomatic people are major reservoirs of infection [5, 6]. Parasites from asymptomatic people are more infectious to mosquitoes than are parasites from symptomatic individuals [7]. Furthermore, field surveys of malaria that use microscopy may fail to detect very low-level parasitemia densities that are common among asymptomatic

people. The advent of polymerase chain reaction (PCR) analysis [8] has improved the ability to detect cases of asymptomatic parasitemia, leading to better estimation of their potential role in the transmission of malaria in human populations [6].

In the highlands, small differences in altitude may lead to contrasting suitability and availability of vector -breeding habitats and, consequently, to divergent risks of malaria transmission and malaria prevalences [9, 10]. In Tanzania, for example, altitude alone accurately predicted the presence or absence of splenomegaly in 593 (73%) of 811 households analyzed [11]. The success of malaria control in the complex highland eco-epidemiological systems will depend on a systematic understanding of the microgeographic risk of malaria transmission that would enable identification of high-risk spots. The identification, quantification, and spatiotemporal mapping of the asymptomatic subpopulation in the highlands of western Kenya provides an opportunity to estimate the epidemiological importance of this group to malaria transmission [12]. The present study sought to determine the prevalence and dynamics of asymptomatic *P. falciparum* infections among schoolchildren living at different altitudes and distances from major breeding habitats of malaria vectors in a highland area of western Kenya where the risk of malaria transmission is unstable.

SUBJECTS, MATERIALS, AND METHODS

Study area, subjects, and protocol. The study was performed in the 3 neighboring villages of Iguhu, Makhokho, and Sigalagala in the Kakamega district of western Kenya. Iguhu is located at the valley bottom (altitude, ~1430 m), Makhokho at the middle (~1500 m), and Sigalagala at the top (~1580 m) of a hill (figure 1). By road, the distance from the valley bottom to the hilltop is ~4.5 km. Other details of the study area have

been reported elsewhere [13, 14]. Rainfall data for the study area were obtained from Kakamega Weather Station. Monthly parasitological surveys (including data on age, sex, and bed net use) were undertaken for 246 schoolchildren aged 5–17 years from the 3 villages from January through December 2006 after receipt of informed assent from the participants and informed consent from their parents or guardians. Data on bed net use were recorded by means of a questionnaire. A total of 84 participating children lived in the valley bottom, 81 were from the midhill area, and 81 were from the hilltop. Table 1 shows the demographic profile of the 3 cohorts. Inclusion criteria for children were random selection for study participation, residence in the catchment area, and willingness to participate in the study, regardless of sex and socioeconomic status. Children who intended to relocate during the study period or were unwilling to participate in the study were excluded. The study was approved by the ethical review committee of the Kenya Medical Research Institute and by the institutional review board of the University of California, Irvine.

In brief, blood samples were obtained by fingerprick. A total of 2–3 drops of blood were used to prepare thick and thin smears for microscopy, and ~200 μL of blood was spotted on filter paper (Whatman) for PCR analysis. After air drying, thick smears were dipped twice in acetone, and thin smears were fixed in methanol and stained with 4% Giemsa for 30–45 min. Slides were examined by experienced microscopists at the original magnification ×1000. Readings were verified by a second reader, with discrepancies resolved by a third reader. The entire slide was carefully scanned before being declared negative for parasites. Parasites were counted against 200 leukocytes, and parasite densities were estimated on the assumption of a standard leukocyte count of 8000 leukocytes/μL, as previously re-
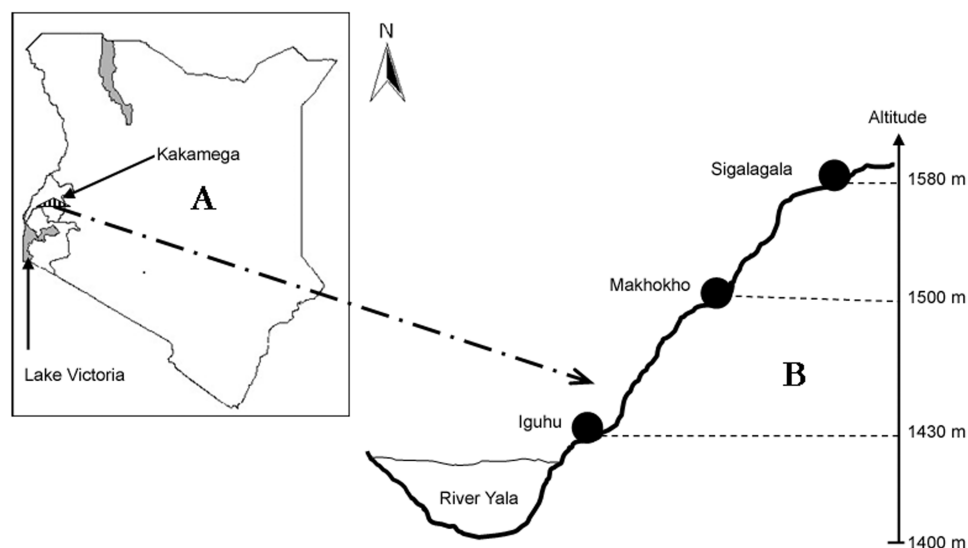
Figure 1. Map of Kenya *(A),* showing sampling sites *(B).* The Yala river is a major breeding site of malaria vectors. Maps are not drawn to scale.

Table 1. Age and sex of schoolchildren from a highland area in western Kenya, by locality.

Category	Iguhu (n = 84)			Makhokho (n = 81)			Sigalagala (n = 81)		
	Children, no.	Children per locality, %	Children per category, %	Children, no.	Children per locality, %	Children per category, %	Children, no.	Children per locality, %	Children per category, %
Age[a]									
5–9 years (n = 88)	31	37	35	40	49	56	17	21	19
10–14 years (n = 153)	52	62	34	41	51	27	60	74	39
15–17 years (n = 5)	1	1	20	0	0	0	4	5	80
Sex									
Male (n = 95)	33	39	35	25	31	26	37	54	39
Female (n = 151)	51	61	34	56	69	37	44	46	29

NOTE. Iguhu is located at the valley bottom (altitude, 1430 m), Makhokho at the middle (1500 m), and Sigalagala at the top (1580 m) of a hill in the Kakamega district of western Kenya.

[a] The median age of participating children was 10 years.

ported [13]. Parasite DNA was extracted from filter papers by the Saponin/Chelex method [15]. While investigators were blinded to microscopy findings, they diagnosed infections by nested PCR amplification of the species-specific small-subunit ribosomal RNA gene, using primers and conditions previously reported [16]. DNA from *P. falciparum* strain 3D7 (MR4) and sterile water (Mediatech) were used as positive and negative controls, respectively, for PCR. Amplicons were resolved in ethidium bromide–stained 2% agarose and visualized by ultraviolet translumination.

Microscopy findings were immediately reported to the study subjects and their parents or guardians. However, because the guidelines of Kenyan Ministry of Health did not advise treatment of asymptomatic people, individuals who had asymptomatic infection were advised to seek antimalarial treatment at the nearby Iguhu Health Center if they subsequently developed any clinical signs of malaria, such as fever, chills, severe malaise, headache, or vomiting.

Definition of P. falciparum infection episodes. A blood sample was considered infected when parasitemia was detected either by microscopy or PCR during the monthly surveys [17, 18]. One or more consecutive *P. falciparum*–positive samples defined a single episode of infection, whereas a parasite-negative intervening sample differentiated between infection episodes [19]. The duration of a single episode was calculated as the number of months between the first and final consecutive samples positive for *P. falciparum* [17].

Data analysis. Concordance between microscopy and PCR findings was estimated using Cohen's κ. Parasite densities and mean numbers of infections, stratified by village and season, were compared by analysis of variance, whereas the overall proportions of infections diagnosed using each test method were compared by means of the Fisher exact test. Parasite densities for samples positive for parasites only by PCR were designated as <40 parasites/μL because microscopic examination

would not detect a parasite density that is less than this threshold [17]. Densities were log-transformed and comparisons done using the Tukey honestly significant differences test with repeated measures. Alternating logistic regression analysis was used to test the effects of living locality, bed net use, age, and sex on infection [20]. Analysis of the duration of episodes was performed using the Kaplan-Meier survival estimator. Comparisons were based on the longest episode that an individual experienced during the study period. Duration estimates of episodes for individuals who were positive for *P. falciparum* at the start or end of the study period were censored. Data analyses were performed using SAS 9.2 (SAS Institute), JMP 5.1 (SAS Institute), and GraphPad (GraphPad Software).

RESULTS

Comparison of microscopy-based and PCR-based diagnoses of asymptomatic **P. falciparum** *infections.* Nineteen subjects (7.7%) were lost to follow-up over the first 11 months of the study, with an additional 170 subjects (69.1%) lost to follow-up during the final month, which coincided with the long holiday season. Infection was detected by microscopy and/or PCR analysis in 880 of 2611 blood samples obtained from children across the 3 villages during the 12-month study period. Microscopy detected only 330 infections. PCR detected 869 infections—a significant, ∼3-fold increase over the number detected by microscopy ($P < .001$) (table 2). Despite repeated DNA extraction and reamplification, PCR missed 11 (3.3%) of 330 of cases detected by microscopy, whereas microscopy missed 550 (63.3%) of 869 cases detected by PCR. Compared with PCR, microscopy had a low sensitivity (36.7%) but a high specificity (99.4%) and positive predictive value (96.7%). When microscopy was considered the gold standard, PCR showed a high sensitivity (96.7%) and negative predictive value (99.4%). Overall, there was moderate concordance between microscopy

Table 2. Comparison of microscopic and polymerase chain reaction (PCR) diagnosis of asymptomatic *Plasmodium falciparum* infections in schoolchildren from a highland area in western Kenya.

	Finding, blood samples, no. (%)		Diagnostic measure,[a] % (95% CI)			
Test	Positive for *P. falciparum*	Negative for *P. falciparum*	Sensitivity	Specificity	PPV	NPV
Microscopy	330 (12.6)	2281	36.7 (33.5–39.9)	99.4 (99.0–99.7)	96.7 (94.7–98.6)	75.9 (74.1–77.6)
PCR	869 (33.3[b])	1742	96.7 (94.7–98.6)	75.9 (74.1–77.6)	36.7 (33.5–39.9)	99.4 (99.0–99.7)

NOTE. A total of 11 samples tested positive by microscopy and negative by PCR, 550 samples tested negative by microscopy and positive by PCR, and 1731 tested negative by both microscopy and PCR. CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value.

[a] Values for microscopy were calculated using PCR findings as the gold standard, whereas values for PCR were calculated using microscopy findings as the gold standard.

[b] Significant difference between microscopy and PCR findings ($P < .001$, by a 2-tailed Fisher test).

and PCR findings ($\kappa = 0.427$; 95% confidence interval, 0.385–0.469). However, during some months, such as October for Makhokho and Sigalagala and December for all sites, infections were detected only by PCR.

Prevalence of asymptomatic* P. falciparum *infection, by age. To assess the overall prevalence of asymptomatic *P. falciparum* parasitemia by age, data from the 3 sampling sites were pooled by age group [19]. The prevalence of infection detected in pooled blood samples by microscopy and/or PCR analysis was similar among subjects aged 5–9 years (34.4% [321 of 933 samples]) and those aged 10–14 years (34.1% [553 of 1623]). On the other hand, the prevalence among pooled blood samples from children aged >14 years (9.1% [5 of 55 samples] by microscopy and/or PCR analysis) was significantly less than that among pooled samples from younger children.

Number of infected samples per child and parasite densities. The number of infected blood samples collected from individual children over the study period ranged from 1 to 12. Village altitude had a significant effect on the number of infected samples ($F_{2,223} = 19.47$; $P < .001$): the mean number ($\pm$ SE) of infected samples recovered per child in Iguhu (5.66 ± 0.41) was twice that observed in Makhokho (2.87 ± 0.40) and in Sigalagala (2.65 ± 0.33). Post hoc Bonferroni tests showed that the mean number of infected samples per child in Iguhu was significantly greater than that in each of the higher-altitude villages ($P < .001$). However, there was no significant difference in the mean number of infected samples per child between Makhokho and Sigalagala ($P > .05$).

Parasite densities in blood decreased with age, ranging from <40 to 44,600 parasites/μL among children aged 5–9 years, from <40 to 27,840 parasites/μL among those aged 10–14 years, and from <40 to 360 parasites/μL among those aged >14 years. The geometric mean parasite density among children aged 5–9 years (649 parasites/μL) was 1.6-fold higher than that for children aged 10–14 years (400 parasites/μL). The geometric mean parasite density for children aged >14 years was not calculated because parasitemia was quantifiable for only 1 sample, in

which 360 parasites/μL were detected by microscopy. Parasite densities were significantly different among age groups ($F_{1,328} = 8.90$; $P = .003$) but not among villages ($F_{2,327} = 1.51$; $P = .223$). Gametocyte densities in blood were low in all cases, with <40 to 160 gametocytes/μL detected in subjects from the valley bottom, $\leq$40 gametocytes/μL detected in those from the midhill region, and <40 to 80 gametocytes/μL detected in those from hilltop.
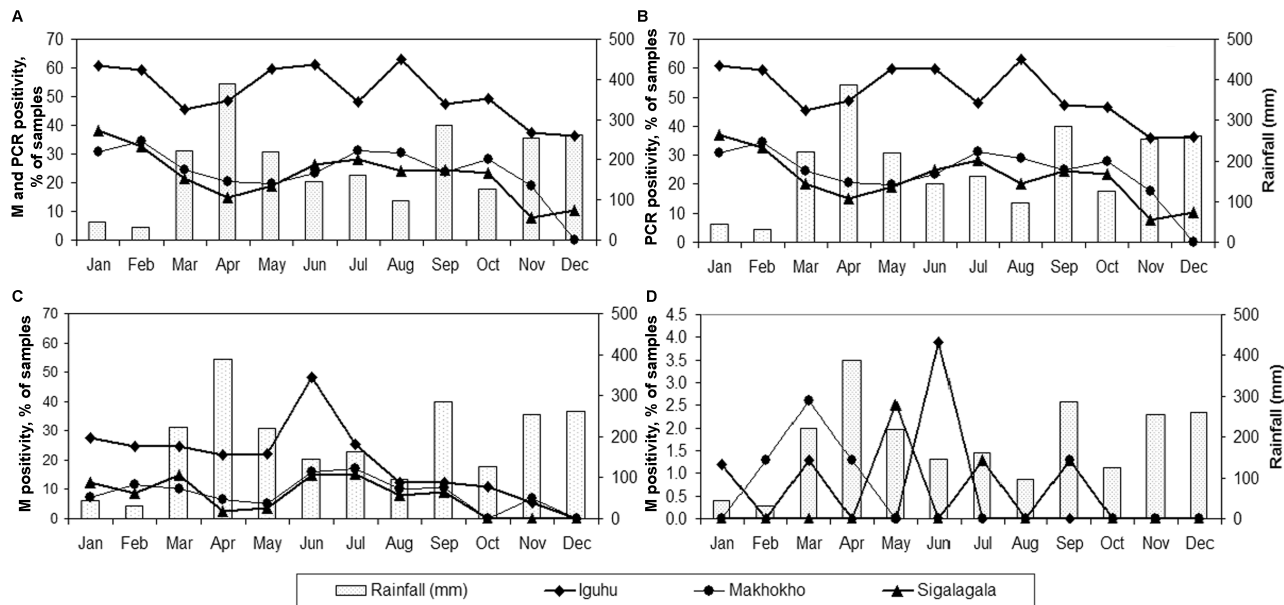
Prevalence of asymptomatic* P. falciparum *infection, by monthly rainfall levels. Monthly prevalence data showed an overdispersed distribution of infections in all villages, with no significant correlation between asymptomatic infection prevalence and rainfall intensity (figure 2). Infections were generally prevalent throughout the year. The monthly prevalence of *P. falciparum* positivity among blood samples, based on positive findings of microscopy and/or PCR, ranged from 36.4% to 63.0% in Iguhu, from 7.8% to 38.3% in Sigalagala, and from 0% to 34.6% in Makhokho (figure 2A). However, gametocyte prevalence was generally low but also decreased with altitude, ranging from 0% to 3.9% of blood samples in Iguhu, 0% to 2.6% in Makhokho, and 0% to 2.5% in Sigalagala, with the highest prevalence at all sites occurring during the long rainy season (March through June) (figure 2B).

Microgeographic* P. falciparum *infection prevalence and odds. An inverse relationship was observed between infection prevalence and altitude (table 3). The prevalence was highest in Iguhu (altitude, 1430 m), followed by Makhokho (1500 m) and Sigalagala (1580 m). The overall prevalence of *P. falciparum* infection among blood samples, based on positive microscopy and/or PCR findings, was 52.4% in Iguhu, 25.8% in Makhokho, and 23.3% in Sigalagala.

Regression analysis of blood sample data showed a significantly greater odds of infection at the valley bottom, compared with uphill sites (odds ratio, ~3.3; table 4). Use of insecticide-treated bed nets was generally infrequent (<20% of subjects in each village), with no significant difference among localities ($F_{2,484} = 2.52$; $P = .08$). Nevertheless, the odds of infection

Figure 2. Monthly correlations between rainfall level and the prevalence of asymptomatic *Plasmodium falciparum* infection (*A–C*) and gametocyte carriage (*D*) among blood samples from schoolchildren living in 3 neighboring villages of various altitudes in the western Kenya highlands. M, microscopy; PCR, polymerase chain reaction.

among bed net users was ~0.7 times that among subjects without bed nets. The odds of infection among children aged 5–9 years was ~6 times that for older children. Girls had significantly less chance of being infected, compared with boys (odds ratio, ~0.6).

Frequency of recurrent asymptomatic parasitemia and dynamics of infection episodes. Overall, 334 asymptomatic infection episodes occurred among children from the 3 villages. The majority (44.0%) of the episodes occurred in Iguhu, which had almost twice as many episodes as Makhokho (24.9%) and Sigalagala (31.1%). A large percentage of children had episodes lasting >1 month (infection in 15.0% of subjects lasted for 1 month; in 38.2%, for 2–5 months; and in 14.2%, for 6–12 months), whereas 32.5% experienced no infection episode. The longest episode (duration, 12 months) occurred at the valley bottom.

Estimates of the duration of the longest episode of infection in each age group and locality are shown as Kaplan-Meier plots (figure 3). All episodes in children aged >14 years lasted only 1 month. A trend of decreasing episode duration with age and altitude is evident. The median duration of infection was higher, albeit trivially so, among children aged 5–9 years, compared with those aged 10–14 years (5 vs. 4 months; $\chi_1^2 = 2.1$; $P = .15$; figure 3*A*). Episode durations were significantly different between microgeographic locales, with children living at the valley bottom having median durations of 6 months, compared with durations of 4 months at midhill and 3 months at the hilltop ($\chi_2^2 = 8.6$; $P = .01$; figure 3*B*). Significant differences occurred between Iguhu and both Makhokho ($\chi_1^2 =$

5.6; $P = .02$) and Sigalagala ($\chi_1^2 = 7.4$; $P = .01$) but not between Makhokho and Sigalagala ($\chi_1^2 = 0.05$; $P = .83$). Boys had marginally longer median infection durations, compared with girls (5 vs. 4 months; $\chi_1^2 = 3.4$; $P = .07$).

DISCUSSION

The present altitudinal cohort study is one of the few focusing on asymptomatic *P. falciparum* infection in African highlands [5, 21]. The study documents high levels of asymptomatic *P. falciparum* infections with microgeographic differences in prevalence within a radius of <5 km in an epidemic-prone area in western Kenya. Our findings contrast with those from studies in Kipsamoite, another highland area in western Kenya, where very low levels of asymptomatic *P. falciparum* infection were observed during the peak season (microscopy detected an infection prevalence of 7.9%, and PCR analysis detected a prevalence of 14.5%) [21]. This suggests that the risk of malaria transmission is highly heterogeneous in the Kenyan highlands, implying that results from one highland area may not be readily extrapolated to other areas.

Estimates of malaria prevalence have primarily been based on microscopy because most cases are detected in health care facilities, where microscopy remains the standard diagnostic method [22]. In our study, PCR analysis consistently detected a significantly greater number of asymptomatic *P. falciparum* infections, compared with microscopy. A number of other studies involving people with asymptomatic *P. falciparum* infection have documented similar findings [3, 17, 21, 23], suggesting

that many studies and health statistics may have grossly underestimated the true prevalence of malaria infection in various areas. The high specificity (99.4%) and positive predictive value (96.7%) of microscopy, using PCR analysis as the gold standard, attests to the quality of our readings. We cannot rule out the possibility that detection of dead parasite forms increased the sensitivity of PCR analysis. However, DNA fragments are briskly eliminated from the bloodstream, decreasing the likelihood of PCR detection of nonviable parasites [24]. The high prevalence of asymptomatic parasitemia observed here indicates a change to a higher level of malaria endemicity than would be expected for an area defined as epidemic prone with an unstable, low rate of transmission [25]. New definitions of transmission stability based on PCR findings are now required. Perhaps the significant decrease in parasitemia concentrations to submicroscopic levels may also be indicative of the parasite's biological and transmission characteristics, especially through the dry season [18], but this needs further investigation.

The consistently higher prevalence of infection (36%–63% of blood samples) and increased infection episode durations at the valley bottom (irrespective of transmission season) could be explained by the closer proximity of this region to vector-breeding sites, making residents more prone to infection and reinfection [26, 27]. In particular, Iguhu is transected by the Yala river, which has thriving, year-round breeding grounds for malaria vectors [28]. Previous studies found that most (98%) of the malaria vectors were located at the valley bottom (the midhill region and the hilltop each had 1% of the vectors), with entomological inoculation rates, defined as the number of infective bites per person per year, of 12.8 at the valley bottom, 0.05 at midhill, and 0.04 at the hilltop [9], suggesting a significantly higher risk of exposure to infected mosquitoes at the valley bottom than at the uphill sites. The valley bottom is thus a hot spot of malaria transmission [10]. That highland valleys are usually the springboard of malaria infection has also been demonstrated in Tanzania's Usambara mountains [11] and in Burundi [26].

A small percentage of children (14.2%) had long periods of persistent infection (duration, 6–12 months), whereas others (38.2%) had episodes lasting 2–5 months. This is of particular

Table 4. Findings of logistic regression analysis to determine the impact of living locality, bed net use, age, and sex on asymptomatic *Plasmodium falciparum* infection.

Variable	Coefficient[a] ± SEM	Odds ratio (95% CI)
Location		
Iguhu	1.190 ± 0.090	3.292 (2.761–3.926)
Makhokho	...	1.140 (0.908–1.430)
Sigalagala	...	0.877 (0.699–1.101)
Bed net use	−0.328 ± 0.130	0.728 (0.567–0.935)
Age		
5–9 years	1.839 ± 0.486	5.985 (2.296–15.598)
10–14 years	1.792 ± 0.483	5.842 (2.261–15.096)
>14 years	...	0.164 (0.064–0.422)
Sex		
Female	−0.437 ± 0.089	0.646 (0.542–0.769)
Male	...	1.566 (1.313–1.867)

NOTE. Iguhu is located at the valley bottom (altitude, 1430 m), Makhokho at the middle (1500 m), and Sigalagala at the top (1580 m) of a hill in the Kakamega district of western Kenya. CI, Wald confidence interval; SEM, standard error of the mean.

[a] Only coefficients with significance at an α level of 0.05 are reported.

concern, considering that the likelihood of *P. falciparum* transmission increases as the duration of asymptomatic parasitemia increases [29]. It is not uncommon for residents of areas where malaria is endemic to harbor malaria parasites for months without developing clinical symptoms. In Ghana, for example, studies found asymptomatic infections that lasted up to 16 months [17], whereas in Sudan, infections persisted for >12 months, with no evidence of reinfection [18]. The observed variation in episode duration, both in and among individuals, with a notable reduction in infection duration with increasing age across all sites, is likely due to gradual acquisition of immunity through reinfection [3, 30]. The decrease in parasite density with increasing age is consistent with findings from studies performed elsewhere and is typical of infections in malaria-endemic areas [17, 19, 31]. This is because immune responses suppress parasitemia, or even clinical disease, in proportion to immune levels, which are a function of both age and level of exposure to *P. falciparum* infections [3, 30].

The observed significantly lower infection prevalence among

Table 3. Prevalence of asymptomatic *Plasmodium falciparum* infection by microscopy and/or polymerase chain reaction (PCR) among blood samples obtained from schoolchildren from a highland area in western Kenya, by locality.

	Iguhu (n = 863)			Makhokho (n = 854)			Sigalagala (n = 894)		
Variable	By microscopy	By PCR	By both tests	By microscopy	By PCR	By both tests	By microscopy	By PCR	By both tests
P. falciparum detected, no. of samples	181	448	452	78	219	220	71	202	208
Prevalence, % of samples	21.0	51.9	52.4	9.1	25.6	25.8	7.9	22.6	23.3

NOTE. Iguhu is located at the valley bottom (altitude, 1430 m), Makhokho at the middle (1500 m), and Sigalagala at the top (1580 m) of a hill in the Kakamega district of western Kenya.
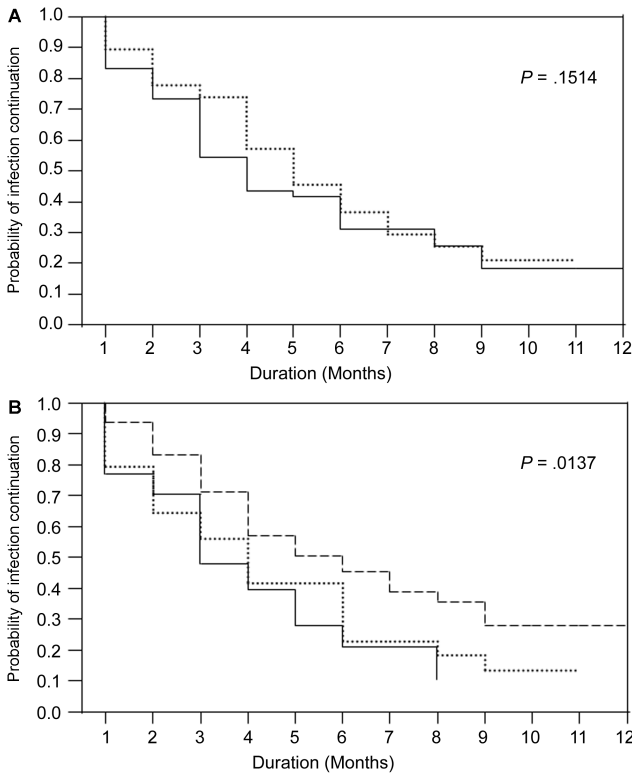
Figure 3. Kaplan-Meier survival plots of duration of episodes of asymptomatic *Plasmodium falciparum* infection by age (5–9 years [*dotted line*] and 10–14 years [*solid line*]; *A*) and location (Iguhu [altitude, ~1430 m; *broken line*], Makhokho [~1500 m; *dotted line*], and Sigalagala [~1580 m; *solid line*]; *B*). All episodes for subjects aged >14 years lasted only 1 month and were excluded from survival analysis.

blood samples from girls, compared with blood samples from boys, is consistent with findings from other studies [32]. Although the cause of these sex differences merits further investigation, including the possibility that males produce more-attractive chemicals for mosquitoes, the production of estrogens by females has been shown to augment antiplasmodial immune responses [32], whereas testosterone suppresses antiplasmodial immune responses [33].

All children were apparently healthy at the time of sampling, with only 6.7% of the infections having a parasite density of >2500 parasites/μL. Because fever was not measured, we cannot rule out the possibility of its association with some of the higher parasitemia concentrations observed. However, studies of asymptomatic people in Tanzania have found no significant association between parasite density and the risk of subsequent clinical malaria [34]. Still, a follow-up incidence study will be necessary in this highland area.

The present study may have overestimated some episode durations because it did not differentiate between recrudescent and reinfecting strains. Microsatellite and *Msp-2* genotype analysis from a few individuals ($n = 33$) showed single-genotype

persistence for up to 3 months (F. N. Baliraine, unpublished data). Nevertheless, the fact that infections were asymptomatic for months remains of epidemiological significance. Moreover, unlike numerous epidemiological studies that have relied solely on microscopy, our analysis complemented microscopy with PCR analysis, which helped detect parasites in 550 (63.0%) of 880 blood samples that would have been missed by use of microscopy alone.

A number of issues are thus raised. First, although microscopy remains the standard method for diagnosing clinical malaria [22], our results show that the technique has a substantially low sensitivity for detecting subclinical parasitemia. This raises serious doubts about the effectiveness of microscopy as the sole screening test not only for epidemiological studies of *P. falciparum*, especially in places such as our study area, but also in drug efficacy studies and malaria vaccine trials that are very sensitive to minor errors in diagnosis. The large number of false-negative results yielded by microscopy and the failure of PCR to detect some cases (albeit a small number) detected by microscopy in the present study supports the view that only the use of both methods can ensure a high validity of test results [35]. Nevertheless, although PCR analysis is useful for epidemiological purposes, great caution must be exercised when using PCR findings alone to diagnose clinical disease, especially in malaria-endemic areas where parasitemia is not necessarily associated with clinical malaria [17, 36]. The clinical implication of PCR-positive findings remains an open question that needs further investigation. Although some very-low-density parasitemias may be associated with clinical disease [37], they may reduce the risk of subsequent clinical malaria [3] and may result in the misdiagnosis of conditions such as human immunodeficiency virus or bacterial infections that produce cerebral malaria–like symptoms, resulting in poor outcomes [36].

Second, we found that the majority of potential *P. falciparum* reservoirs are in the valley bottom, where vector-breeding sites happen to be concentrated [14, 28]. This allows us to recommend that in the face of limited resources, malaria could be substantially controlled in the highland area if control efforts prioritized the valley bottom. Targeted control of malaria has been successful in the highlands of Burundi [25, 26, 38].

Presently, there are renewed calls for malaria eradication [1, 29]. The need to eradicate malaria has been high on humankind's agenda for centuries, with unrelenting attempts made over the years to achieve this objective [30]. One loophole in the attack strategies, however, has been the omission of the human reservoir of the parasites. Because mosquitoes can pick up even submicroscopic parasites [6], targeted therapy even for asymptomatic carriers may be useful as part of an integrated malaria control or eradication program. A combination of mass prophylactic antimalarial treatment with in-house DDT sprays nearly eliminated malaria in one highland area of Kenya in the

late 1940s [2]. Even when *P. falciparum* infection is asymptomatic, it remains a serious problem because parasites can impair children's brain development and academic prowess [27, 39], rework the immune system and the body's response to vaccines, and increase the infected person's vulnerability to other infections [40]. Our results therefore have implications for Kenya's malaria control program, which focuses on persons with symptomatic *P. falciparum* infection [41].

Acknowledgments

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