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Am. J. Trop. Med. Hyg., 79(4), 2008, pp. 624–635
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Malaria on the Amazonian Frontier: Transmission Dynamics, Risk Factors, Spatial Distribution, and Prospects for Control

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Abstract. Little follow-up data on malaria transmission in communities originating from frontier settlements in Amazonia are available. Here we describe a cohort study in a frontier settlement in Acre, Brazil, where 509 subjects contributed 489.7 person-years of follow-up. The association between malaria morbidity during the follow-up and individual, household, and spatial covariates was explored with mixed-effects logistic regression models and spatial analysis. Incidence rates for *Plasmodium vivax* and *Plasmodium falciparum* malaria were 30.0/100 and 16.3/100 person-years at risk, respectively. Malaria morbidity was strongly associated with land clearing and farming, and decreased after five years of residence in the area, suggesting that clinical immunity develops among subjects exposed to low malaria endemicity. Significant spatial clustering of malaria was observed in the areas of most recent occupation, indicating that the continuous influx of nonimmune settlers to forest-fringe areas perpetuates the cycle of environmental change and colonization that favors malaria transmission in rural Amazonia.

INTRODUCTION

Despite several decades of intensive control efforts, malaria remains a major cause of morbidity in Brazil.[1] Between 1970 and the mid-1980s, massive human migration to the Amazon Basin, where large-scale colonization and mining projects were started, led to a 10-fold increase in the annual incidence of malaria in the country.[2] The most recent available incidence data are for 2006, with 550,000 slide-confirmed malaria cases reported countrywide, 99.7% of which were diagnosed in Amazonia.[3]

Frontier agricultural settlements in the Amazon Basin favor malaria transmission not only by inducing massive environmental changes, such as deforestation, but also by favoring human clustering close to vector habitats.[4] Although the epidemiology of epidemic malaria in early frontier settlements in rural Amazonia was thoroughly described in the mid-1980s,[5] minimal follow-up information is available for the recent decades, during which extensive social and ecologic transformations took place.[6] To understand how malaria transmission is maintained in the more stable and organized communities originating from frontier settlements, a detailed knowledge of the transmission dynamics of malaria and associated risk factors is necessary.[7]

Here we describe the current patterns of malaria transmission in one of the largest agricultural settlements of Brazilian Amazonia, the Pedro Peixoto Settlement Project, opened in the state of Acre in the 1980s. The combined analyses of malaria morbidity data and individual and household-level risk factors for a population-based prospective cohort are presented and discussed in relation to prospects for malaria control in this and other settings with similar endemicity.

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MATERIALS AND METHODS

Study area. Granada, originally a sparsely populated rubber tapper settlement in the eastern corner of the state of Acre, northwestern Brazil (Figure 1), became part of the Pedro Peixoto Agricultural Settlement Project in 1982. With its equatorial humid climate, this area receives the most rainfall (annual average, 2198.5 mm) between December and March. The mean annual temperature is 24.5°C. Subsistence agriculture and cattle raising are the major economic activities, with coffee, bananas, and rice being the main cash crops. The majority of inhabitants are migrants from Southeast and South Brazil. The continuous influx of new settlers led more recently to the occupation of forested areas along the Iquiri River, the largest water body in the study area (Figure 1); most of the settlement has been deforested for agricultural use. Three government-run malaria diagnosis outposts provide free malaria diagnosis and treatment to the inhabitants of the study site.

This study was carried out in two adjacent localities in the Granada area, namely Ramal do Granada and Reserva da Linha 14. Ramal do Granada includes households on both sides of Linha 14, an unpaved road originating from the paved BR 364 highway, from km 14 to km 30, and also some dwellings in nearby secondary roads (Linha 27 and Linha 28); Reserva da Linha 14 consists of all households found along the 2.5 km secondary road that lies nearly perpendicular to Ramal do Granada. The Iquiri River runs parallel to Reserva da Linha 14, at an average distance of 1 km (Figure 2).

Study design. A population-based open cohort study was initiated in 2004 to identify socioeconomic, immunologic, genetic, and environmental risk factors associated with malaria morbidity in the study area. All of the area's households were visited by our field team, and 466 dwellers of all age groups (98.5% of the permanent residents in the area) were enrolled between March and April 2004. An additional 43 individuals (mostly newcomers to the area) were enrolled between September and October 2004, giving a total of 509 subjects, from < 1 month to 90 years of age and distributed into 123 households, who contributed follow-up data. Baseline data

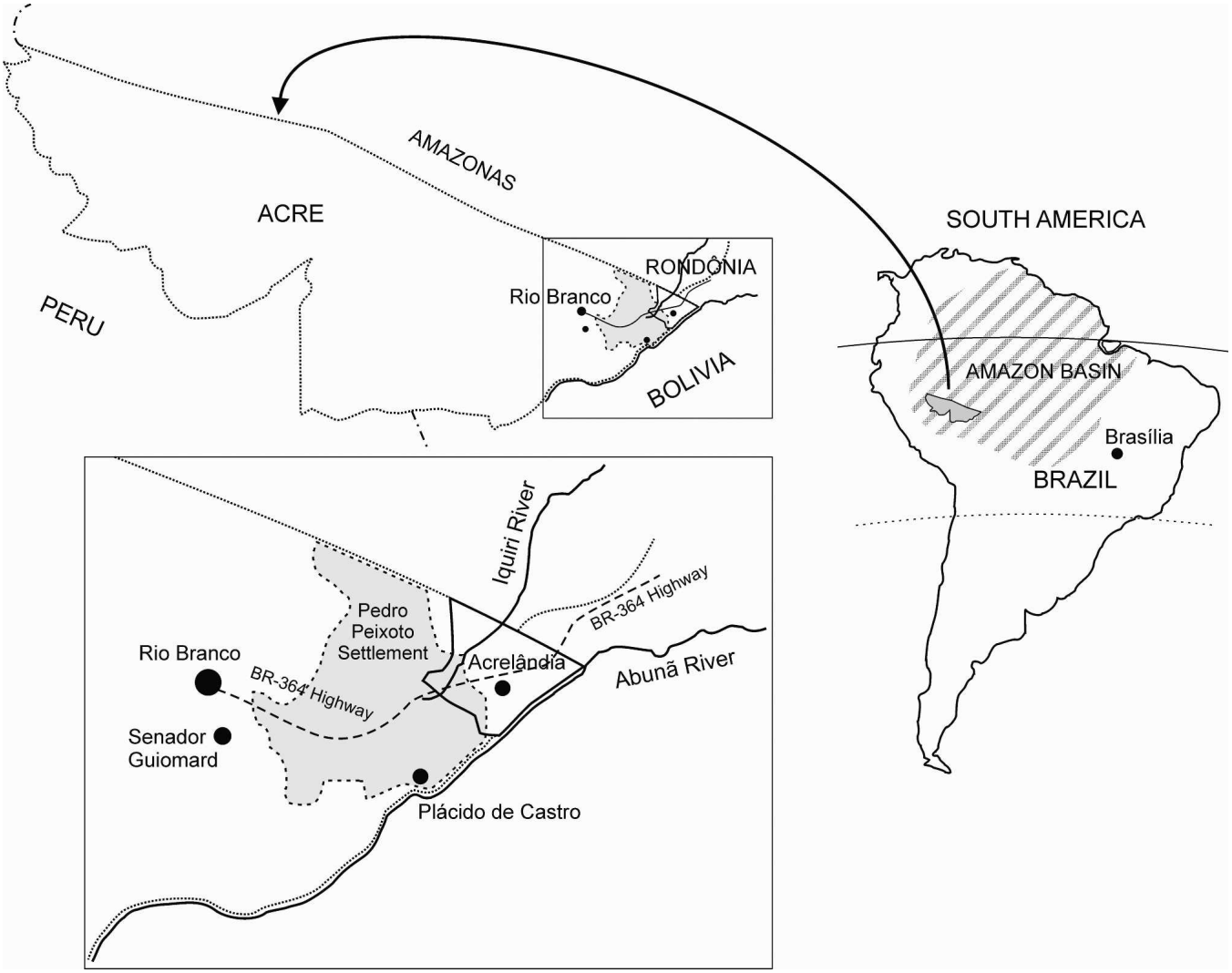

FIGURE 1. Maps showing the state of Acre, in northwestern Brazil, and the site of the cohort study carried out between 2004–2005 (Pedro Peixoto Settlement). Ramal do Granada is part of the Pedro Peixoto Agricultural Settlement, located 30–50 km northwest of the town of Acrelândia, which in turn is located 114 km east of Rio Branco, the capital of Acre.

are described elsewhere.[8] All dwellings were located using a hand-held 12-chanel global positioning system receiver (eTrex Personal Navigator, Garmin, Olathe, KS), with a positional accuracy within 15 m.

To characterize the place of residence, we created a continuous covariate, house location, which gives the linear distance in km between each dwelling and an index house located in the sector of earliest ocupation. Briefly, the location of each dwelling along Ramal do Granada and on minor secondary roads was described in relation to an index house (index house A), the first house on the left side of Ramal da Linha 14 at km 14 (Figure 2); for subjects living along Reserva da Linha 14, the place of residence was calculated as the sum of two linear distances in km: 1) the distance between the subject's house and the index house B (the last house on the left of Ramal da Linha 14 at km 30) and 2) the distance between index houses A and B. New settlers typically occupied land plots close to Iquiri River, corresponding to house locations of 12–15.5 km.

A baseline questionnaire was applied to all study participants to collect demographic, health, and socioeconomic data. Cumulative exposure to malaria was estimated using age and the duration of residence in the study area as proxies, whereas recent malaria exposure was determined from the records of slide-confirmed malaria episodes that were diagnosed in local malaria diagnosis outposts between January 2001 and March 2004.

Information on selected household assets, land ownership, type of building material, and number of inhabitants per room was used to derive a wealth index, from which socioeconomic status was estimated. We combined the following data: 1) ownership of five household assets (gas stove, coach, bicycle, motor vehicle, and cattle); 2) land tenure (yes or no); 3) type of building material (brick walls versus others); and 4) number of inhabitants per room (≤ 1 per room or > 1 per room). Principal component analysis, carried out using the XLSTAT software, version 7.5.2 (Addinsoft, New York, NY), was used to weigh each variable, as described by Filmer and Pritchett.[9] The first principal component explained 25.6% of variability and gave the greatest weights to ownership of a couch (0.670) and a motorized vehicle (car or motorcycle) (0.641), and to having a lower number of inhabitants

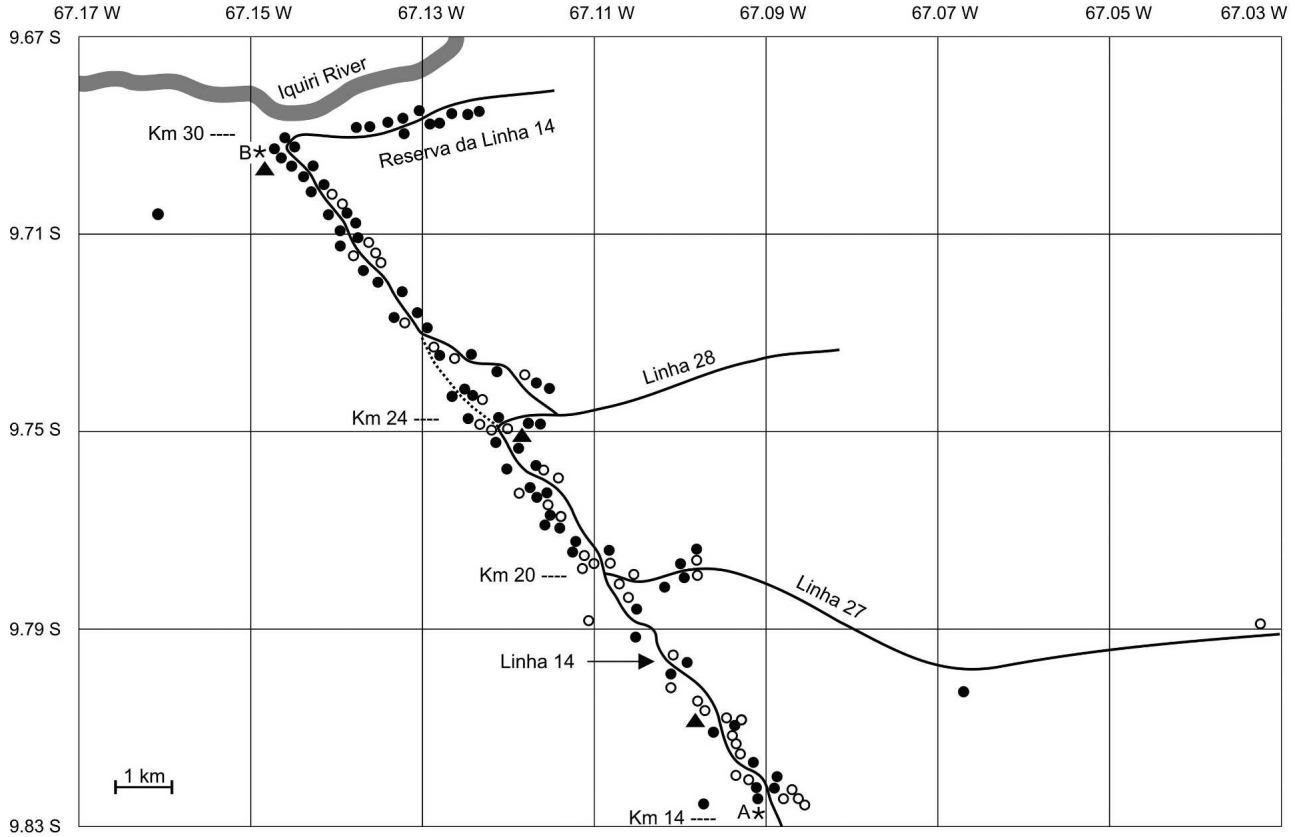

FIGURE 2. The Global Positioning System (GPS)-determined location of 122 dwellings (comprising 123 households) along Linha 14 and secondary roads in the Granada area, inhabited by the 509 study subjects. These dwellings are located in the area shaded in Figure 1, close to the town of Acrelândia, between the BR-364 highway and the Iquiri River. For analytical purposes, the location of each dwelling (represented as circles) was described in relation to an index house (index house A, black star). Dwellings with at least one case of malaria during follow-up are represented as black circles; white circles represent households without malaria during follow-up period. House location of the 388 study subjects living along Linha 14 and minor secondary roads (Linha 27 and Linha 28) was defined as the linear distance in km between the subjects' dwelling and the index house A, using GPS coordinates. For the remaining 121 study subjects who lived along Reserva da Linha 14, the place of residence was calculated as the sum of two linear distances: 1) that between the subject's house and the index house B (black star) and 2) that between index houses A and B. The three government-run malaria diagnosis outposts in the study site are represented with triangles. Note that Iquiri river, the main permanent water body in the study site, runs nearly parallel to Reserva da Linha 14.

per room (0.574). After the standardized variables were weighted, the highest scores were given to the ownership of a brick house (2.262), a couch (1.040), and a motorized vehicle (0.742). Lowest scores were given to households with no gas stove (−1.237), no land tenure (−1.054), > 1 inhabitant per room (−0.619), and no cattle (−0.614). The scores were summed to give a wealth index for each household (range, −4.871 to 5.409), and these indices were then used to stratify households into quartiles in decreasing order (first quartile, 25% richest).

Baseline malaria prevalence survey. The baseline survey participants five years of age or older were eligible for collection of 5-mL venous blood samples for malaria diagnosis and other laboratory studies, regardless of the presence of any clinical symptoms. Laboratory diagnosis of malaria was based on microscopic examination of thick smear and nested polymerase chain reaction (PCR) amplification of a species–specific segment of the 18S rRNA gene of human malaria parasites.[10,11] Thick blood smears were stained with Giemsa and examined for malaria parasites under 700× magnification (at least 100 fields) by two experienced local microscopists. Malaria infections diagnosed by onsite microscopy were treated according to the latest malaria therapy guidelines in Brazil.[12] All positive slides and the slides collected from febrile subjects that were declared negative by onsite microscopy were sent for review by an expert microscopist at the National Reference Laboratory of the Ministry of Health of Brazil, Brasília. The DNA templates for PCR amplification were isolated from 200 μL of whole blood using GFX genomic blood DNA purification kits (Amersham Pharmacia Biotech, Piscataway, NJ). Baseline blood samples were additionally used: 1) to investigate human genetic polymorphisms putatively associated with resistance to malaria (manuscript in preparation), 2) to measure hemoglobin and other iron status indicators and diagnose glucose-6-phosphate dehydrogenase deficiency,[13] 3) to measure antibodies to several arboviruses,[8] and 4) to measure antibodies to blood-stage malarial antigens.[14,15]

Malaria surveillance. Between March 2004 and May 2005, the study population was placed under clinical and laboratory surveillance for symptomatic malaria episodes, which included both active and passive case detection.[16] For this purpose, the study clinician and a health worker visited the area five times a week (Monday–Friday) and examined all study participants (irrespective of their ages) reporting current or recent fever, headache, or any other malaria symptom.

Venous or finger-prick blood samples for malaria diagnosis by microscopy and polymerase chain reaction (PCR) were obtained. As part of surveillance, all patients' files kept at the three local malaria diagnosis outposts were daily examined to detect additional malaria episodes among study participants who reported directly to these facilities. Thick blood smears were examined by local microscopists; a portion of them (all positive slides and those collected from febrile subjects that were declared negative by onsite microscopy) were reviewed by an expert microscopist at the National Reference Laboratory of the Ministry of Health of Brazil. The DNA templates for PCR amplification were obtained from venous blood samples as explained previously; those from finger-prick blood samples spotted on FTA Micro Cards were prepared as described by the manufacturer (Whatman, Clifton, NJ). As in other studies conducted in Amazonia,[17,18] our PCR often detected additional malaria species that were missed by conventional microscopy. These additional species were also considered in the analysis. A clinical case of malaria (outcome variable) was present when a symptomatic patient, enrolled through either active or passive case detection, was found to have malaria parasites on thick smear. A minimal interval of 28 days between two or more consecutive sample examinations was required to count the latter positive slide as a new malaria episode. In addition, when different species were detected in samples obtained less than 28 days apart, the subject was considered to have a single episode of mixed-species infection. On the basis of the results of onsite microscopy, the treatment was given as described previously. Two additional cross-sectional surveys of the whole study population were performed in September–October 2004 and February–March 2005 to update census data and record temporary absences from the study area that lasted more than one week. These data were considered when computing the number of person-years at risk. Subjects diagnosed with malaria were excluded from the population at risk during the 28 days after initiation of treatment.

Statistical analysis. *Exploratory analysis.* A database was created with SPSS 13.0 software (SPSS Inc., Chicago, IL). Incidence rates were expressed as the number of cases per 100 person-years or person-months at risk, and their exact Poisson 95% confidence intervals (CIs) were calculated using StatsDirect 2.6.1 software (StatsDirect, Altrincham, Cheshire, UK). Exploratory logistic regression analysis, using R software version 2.6.0,[19] examined potential risk factors and confounders, including categorical individual-level variables (gender, migration history, main occupation, recreational fishing, bednet use, recent malaria, and work-related commutation during follow-up), continuous individual-level variables (age, years of schooling, years of residence in the current house, in the study site, and in other malaria-endemic areas, age at the start of malaria-exposure, self-reported number of slide-confirmed malaria episodes, and number of slide-positive malaria episodes recorded between 2001–2004), categorical household-level variables (type of house, type of material used in roof, walls and floor, presence of gaps in the walls, presence of screens in windows and doors, presence of a separate kitchen, presence of indoor animals, type of solid and liquid waste disposal, presence of electricity on the property, wealth index [stratified into quartiles], and sharing the household with a land clearer [land clearing was hypothesized to be an occupation of high risk for malaria]), and continuous household-level variables (number of household inhabitants, years of schooling of the household head, and house location). Separate multiple logistic regression models were fitted to individual- and household-level variables using manual inclusion. Covariates were maintained in subsequent multivariate models, together with the *a priori* potential confounders (age and gender), if they were associated with the outcome, in exploratory unadjusted analysis, at a level of significance of 20%. The goodness of fit was assessed by either analysis of variance, Akaike's information criterion (AIC) values,[20] or odds ratio (OR) changes. Interaction terms were evaluated, and diagnostic tests (Cook's distance[21] for influential points and studentized residuals[22]) were applied using the package car of R 2.6.0 software. A few statistically significant interaction terms were identified but did not improve the models; therefore they were not included in the final models. Important influential points were identified for the variable "occupation," which was then re-stratified and reevaluated; as for the other variables examined, influential points were maintained because overall results did not change significantly when such influential points were excluded from the analysis. Models and outliers were reassembled if needed until the best fit was achieved. Final logistic regression models for individual variables included gender, age, occupation, years of residence in the study site, years of residence outside the study site, the occurrence of recent malaria episodes, recreational fishing, and cohabiting with a land clearer, whereas those for household-level variables included wealth index, house location in the study site, number of household inhabitants, and sharing the house with a land clearer.

Mixed-effects logistic regression. We used logistic regression models to explore the association between individual and household-level covariates and the occurrence of: 1) at least one episode of either *Plasmodium vivax* or *Plasmodium falciparum* clinical malaria during the follow-up, 2) at least one episode of clinical *P. vivax* malaria, and 3) at least one episode of clinical *P. falciparum* malaria. Malaria episodes diagnosed during the baseline survey were not considered in this analysis of incident malaria. Because of the nested structure of the data (study subjects are clustered into households), we fitted mixed-effects logistic regression models with multivariate normal random effects using restricted maximum likelihood and numerical integration via adaptive Laplace[23] with the lme4 package of R 2.6.0.[19] Clustering into households was treated as a random effect in the model (using a variable that identified to which household the subject belonged), whereas all other covariates were entered as fixed-effect variables. Only the variables associated with statistical significance at the 5% level and those that altered the parameter estimate of fixed-effects variables (odds ratio) or the unexplained residual effects at household level (random-effects estimation) by $\geq 10\%$ were retained in the final models. Because of the open-cohort design of the study, duration of follow-up (in person-years) was entered as an offset. The following variables remained in final mixed-effects logistic regression models for each outcome: gender, occupation, length of residence in the study area, occurrence of recent slide-confirmed malaria, recreational fishing, wealth index, house location, number of inhabitants in the household, and sharing the house with a land clearer. Because of missing values, only 458

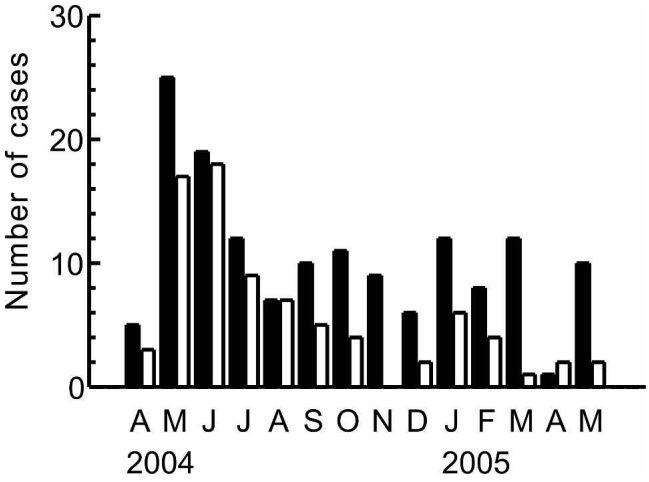

FIGURE 3. Monthly distribution of incident symptomatic infections with *Plasmodium vivax* ($N = 147$, black bars) and *Plasmodium falciparum* ($N = 80$, white bars) diagnosed in an open cohort of 509 subjects followed between March 2004 and May 2005 in rural Amazonia. The first "A" and the last "M" on the *x*-axis denote April 2004 and May 2005, respectively; no incident malaria episode was diagnosed in March 2004.

observations remained in the final adjusted models. Given the nonlinear relationship between house location and risk of *P. falciparum* malaria that was revealed by logistic additive regression (see below), a quadratic term was used to fit the corresponding parametric logistic model. The relationships between duration of residence in the study site and the expected probabilities for each outcome, derived from the final mixed-effects models, were represented graphically using a locally weighted polynomial regression.

Logistic additive models. We applied logistic additive models with integrated smoothness estimation, using penalized regression splines,[24] to explore the shape of the association between house location and the risk of clinical malaria during the follow-up. These analyses were performed with the mgcv package of R 2.6.0 software. A similar approach was used to study the association among age, years of residence inside and outside the study area, and the risk of clinical malaria during the follow-up. Because there was extensive overlap between age and time of residence in the study area for many subjects, we created a third variable (years of residence outside the study area) by subtracting the number of years of residence in the study area from the subject's age. Each time-related variable was modeled separately, and its contribution to the outcome was compared using AIC values, the percentage of deviance explained and the *P* value of the smooth term. Only years of residence in the study area was significantly associated with the risk of clinical malaria (regardless of the species) during the follow-up, whereas age and years outside the study area explained less than 1.12% of the deviance ($P > 0.05$) and were thus excluded from subsequent analysis (data not shown).

Spatial analysis. The Kulldorff spatial scan statistics was used to test whether malaria episodes were randomly distrib-

TABLE 1

Individual and household-level risk factors for having malaria of any type during the follow-up of 509 rural Amazonians between March 2004 and May 2005

Variables	*N**	*n*†	OR (95% CI)‡	*P*	aOR (95% CI)§	*P*
Gender						
Female	245	51	1		1	
Male	264	65	1.24 (0.82–1.89)	0.307	0.75 (0.37–1.51)	0.431
Main occupation						
Land clearing in Iquiri area	24	12	4.01 (1.71–9.44)	0.001	7.35 (1.88–28.65)	0.004
Land clearing elsewhere	30	9	1.72 (0.71–3.82)	0.198	6.17 (1.78–21.39)	0.004
Farming	129	30	1.21 (0.73–1.97)	0.433	2.15 (0.91–5.04)	0.078
Other (students, housekeepers)	326	65	1		1	
Years of residence in the area	507		0.95 (0.91–0.98)	0.003	0.92 (0.87–0.97)	0.003
Recent malaria¶						
No	314	37	1		1	
Yes	195	79	5.09 (3.28–8.03)	< 0.0001	2.77 (1.46–5.27)	0.001
Recreational fishing						
No	309	77	1		1	
Yes, Iquiri area	49	18	1.75 (0.91–3.27)	0.084	0.69 (0.27–1.77)	0.447
Yes, elsewhere	101	14	0.48 (0.25–0.88)	0.022	0.68 (0.30–1.58)	0.381
House location‖	509		1.22 (1.16–1.29)	< 0.0001	1.19 (1.08–1.30)	0.0001
Number of inhabitants in the house	509		1.19 (1.09–1.30)	0.00004	1.31 (1.11–1.55)	0.001
Wealth index (quartiles)**						
1 (richest)	114	12	1		1	
2	140	34	2.72 (1.37–5.78)	0.005	0.83 (0.30–2.32)	0.728
3	127	29	2.51 (1.24–5.38)	0.012	0.91 (0.31–2.67)	0.868
4 (poorest)	128	41	4.00 (2.03–8.40)	0.0001	0.50 (0.15–1.69)	0.269
Sharing the house with a land clearer						
No	325	66	1		1	
Yes	161	49	1.72 (1.11–2.64)	0.014	2.26 (1.15–4.43)	0.017

For some variables, totals do not reach 509 because of missing values. Because of missing values, only 458 observations remained in the final mixed-effects logistic regression model.

* N = number of subjects.

† n = number of subjects with the outcome.

‡ OR = odds ratio; CI = confidence interval.

§ aOR = odds ratio adjusted for all covariates listed in the table using a mixed-effects logistic regression model.

¶ Slide-confirmed symptomatic malaria diagnosed between January 2001 and March 2004.

‖ House location gives the linear distance in km between each dwelling and an index house situated in the sector of earliest human occupation in the study area.

** The wealth index was derived from information on ownership of selected household assets, land tenure, type of housing material, and number of inhabitants per room, and used as a proxy of socioeconomic status.

TABLE 2

Individual and household-level risk factors for having *Plasmodium vivax* and *Plasmodium falciparum* malaria during follow-up of 509 rural Amazonians between March 2004 and May 2005

	Plasmodium vivax malaria				*Plasmodium falciparum* malaria				
	*n**	OR (95% CI)†	*P*	aOR (95% CI)‡	*P*	*n**	OR (95% CI)	*P*	aOR (95% CI)	*P*
Gender										
Female	41	1		1		29	1		1	
Male	46	1.04 (0.66–1.67)	0.836	0.97 (0.46–2.05)	0.940	38	1.25 (0.74–2.11)	0.395	0.54 (0.21–1.35)	0.190
Main occupation										
Land clearing in Iquiri area	7	2.02 (0.75–4.95)	0.135	2.70 (0.63–11.47)	0.176	8	4.29 (1.64–10.55)	0.002	10.74 (2.25–51.27)	0.002
Land clearing elsewhere	6	1.23 (0.44–2.98)	0.664	3.24 (0.82–12.70)	0.091	5	1.72 (0.55–4.46)	0.300	7.83 (1.57–39.08)	0.012
Farming	19	0.85 (0.47–1.48)	0.577	1.09 (0.43–2.79)	0.843	34	1.57 (0.86–2.83)	0.133	3.30 (1.13–9.63)	0.028
Other (students, housekeepers)	55	1		1		20	1			
Years of residence in the area	87	0.94 (0.89–0.97)	0.001	0.93 (0.87–0.99)	0.040	67	0.96 (0.92–1.00)	0.080	0.95 (0.88–1.02)	0.175
Recent malaria§										
No	27	1		1		16	1			
Yes	60	4.72 (2.90–7.88)	< 0.0001	2.57 (1.26–5.25)	0.009	51	6.59 (3.71–12.31)	< 0.0001	2.55 (1.10–5.89)	0.027
Recreational fishing										
No	61	1		1		46	1			
Yes, Iquiri area	9	0.91 (0.40–1.91)	0.821	0.43 (0.14–1.26)	0.125	15	2.52 (1.25–4.93)	0.008	1.01 (0.35–2.90)	0.977
Yes, elsewhere	11	0.49 (0.24–0.95)	0.045	1.05 (0.42–2.61)	0.909	3	0.17 (0.04–0.49)	0.004	0.17 (0.04–0.76)	0.020
House distance¶	87	1.23 (1.16–1.31)	< 0.0001	1.18 (1.06–1.31)	0.001	67	1.29 (1.20–1.40)	< 0.0001	1.01 (1.00–1.02)	< 0.0001
Number of inhabitants in the house	87	1.19 (1.08–1.30)	0.0002	1.25 (1.05–1.49)	0.009	67	1.21 (1.10–1.33)	0.0001	1.24 (1.02–1.50)	0.028
Wealth index (quartiles)‖										
1 (richest)	5	1		1		8	1			
2	28	5.45 (2.20–16.50)	0.0007	1.97 (0.53–7.32)	0.306	16	1.71 (0.72–4.36)	0.236	0.37 (0.10–1.35)	0.134
3	22	4.57 (1.79–14.03)	0.003	1.99 (0.51–7.70)	0.315	16	1.91 (0.80–4.88)	0.153	0.31 (0.08–1.27)	0.105
4 (poorest)	32	7.27 (2.95–21.91)	0.00007	1.42 (0.33–6.10)	0.633	27	3.54 (1.60–8.68)	0.002	0.22 (0.04–1.06)	0.059
Sharing the house with a land clearer										
No	45	1		1		43	1			
Yes	41	2.12 (1.32–3.42)	0.001	2.58 (1.24–5.36)	0.010	24	1.15 (0.66–1.95)	0.614	1.73 (0.75–3.94)	0.192

Because of missing values, only 458 observations remained in the final mixed-effects logistic regression models.

* *n* = number of subjects with the outcome

† OR = odds ratio; CI = confidence interval.

‡ aOR = odds ratio adjusted for all covariates listed in the table, using mixed-effects logistic regression models.

§ Slide-confirmed symptomatic malaria diagnosed between January 2001 and March 2004.

¶ House location gives the linear distance in km between each dwelling and an index house situated in the sector of earliest human occupation in the study area. Given the nonlinear relationship between house location and risk of *Plasmodium falciparum* malaria revealed by generalized additive modeling, a quadratic term was used to fit the corresponding logistic model.

‖ The wealth index was derived from information on ownership of selected household assets, land tenure, type of housing material, and number of inhabitants per room, and used as a proxy of socioeconomic status.

uted within the study area and to identify significant spatial clusters if present.[25] Analysis was made using the Bernoulli model implemented in the version 4.0.3 of the SaTScan software.[26] This program creates circular windows that are moved systematically throughout the geographic space to identify significant clusters of infections. The windows are centered on each of the households; with the maximum window size set to include 50% of the households (i.e., the largest possible cluster would encompass 50% of the households). For each location and size of the scanning window, SaTScan performs a likelihood ratio test to evaluate whether infections are more prevalent within that specific circular window as compared with the outside. Separate analyses were made for: 1) at least one infection with any malaria parasite during the follow-up, 2) at least one infection with *P. falciparum*, and 3) at least one infection with *P. vivax.* Only subjects who stayed in the study site during the whole follow-up period (between March–April 2004 and May 2005) were included in the spatial analysis. The *P* values were determined by 100,000 Monte Carlo replications of the data set; and a level of significance of 5% was adopted.

Ethical considerations. The study protocol was approved by the Ethical Review Board of the Institute of Biomedical Sciences of the University of São Paulo, Brazil (318/2002 and 538/2004), and written informed consent was obtained from each adult participant and from the parent or legal guardian of every minor.

RESULTS

Baseline malaria prevalence. Of 405 subjects five years of age or older living in the study area, 388 (95.8%) had baseline blood samples examined for malaria parasites by thick smear microscopy and 386 (95.3%) by nested PCR. Conventional microscopy revealed 20 malarial infections at baseline (overall prevalence, 5.1%), with 14 *P. vivax* infections (prevalence, 3.6%) and 6 *P. falciparum* infections (prevalence, 1.5%). All but one slide-positive subject (infected with *P. falciparum*) had fever or other malaria-related symptoms at the time of blood sampling or within 60 days before or after the baseline survey. No mixed-species infection was found by microscopy. Nested PCR, however, revealed 60 baseline malaria infections (overall prevalence, 15.5%), with *P. falciparum* found in 20, *P. vivax* in 15, and both species in 15 subjects. The overall prevalence rates of PCR-diagnosed *P. falciparum* and *P. vivax* infections at baseline were 9.1% and 7.8%, respectively; 31 of 50 (62.0%) PCR-detected infections were asymptomatic. No *P. malariae* infections were diagnosed at the baseline by either microscopy or PCR.

Malaria morbidity during the follow-up. The study cohort comprised 264 males and 245 females (male:female ratio, 1.08:1) followed for an average of 11.5 months (10 days to 14.5 months), and thus contributing 489.7 person-years of follow-up. A total of 393 study participants (77.2%) remained free of malaria diagnosis during the follow-up. Of the remaining subjects, 71 had a single symptomatic malaria episode, 26 had two episodes, and 19 had between 3 and 6 episodes confirmed by microscopy. No episodes of severe or complicated malaria were diagnosed. Of 195 laboratory-confirmed malaria infections diagnosed during the follow-up, *P. vivax* accounted for 114 (58.5%), *P. falciparum* for 48 (24.6%), *P. falciparum* and *P. vivax* for 32 (16.4%), and *P. vivax* and *P. malariae* for 1 (0.5%). Overall, *P. vivax* (alone or in mixed-species infections) was found in 147 malaria episodes (incidence, 30/100 person-years at risk, 95% confidence interval [CI] of 25.4–35.3/100 person-years at risk), and *P. falciparum* (alone or in mixed-species infections) was found in 80 malaria episodes (incidence, 16.3/100 person-years at risk, 95% CI of 12.9–20.3/100 person-years at risk), with a 1.8:1 species ratio. The proportions of study participants who remained free of *P. vivax* and of *P. falciparum* malaria during the follow-up were similar (82.9% and 86.8%, respectively), although the proportions of subjects with two or more consecutive episodes due to each species differed substantially (6.7% and 2.2%, respectively), possibly because of relatively high rates of *P. vivax* relapses in this area.[27] The temporal distribution of malaria episodes according to species is shown in Figure 3; the monthly incidence of *P. vivax* malaria ranged between 0.24 (April 2005) and 6.25 (May 2004) episodes/100 person-months at risk, whereas that of *P. falciparum* malaria ranged between 0 (November 2004) and 4.24 (June 2004) episodes/100 person-months at risk.

Risk factors for malaria morbidity. Mixed-effects logistic regression analysis showed that being a land clearer or sharing a house with one, living in a crowded household, and having had one or more recent slide-confirmed malaria episodes were independent predictors of the risk of having at least one malaria episode of any type during the follow-up (Table 1). The risk of malaria morbidity was highest for newcomers to the study area as the following findings suggest: 1) prolonged residence in the study area, but not subjects' age, was associated with reduced risk and 2) living in the more recently occupied sectors of the study area (increased "house location") correlated with increased risk (Table 1). When we analyzed risk factors for *P. vivax* and *P. falciparum* separately, a few differences emerged (Table 2). For example, 1) occupation was a stronger predictor of risk of *P. falciparum* than *P. vivax* malaria, 2) sharing the house with a land-clearer was a stronger predictor of *P. vivax* than *P. falciparum* malaria, and 3) recreational fishing in different water bodies was

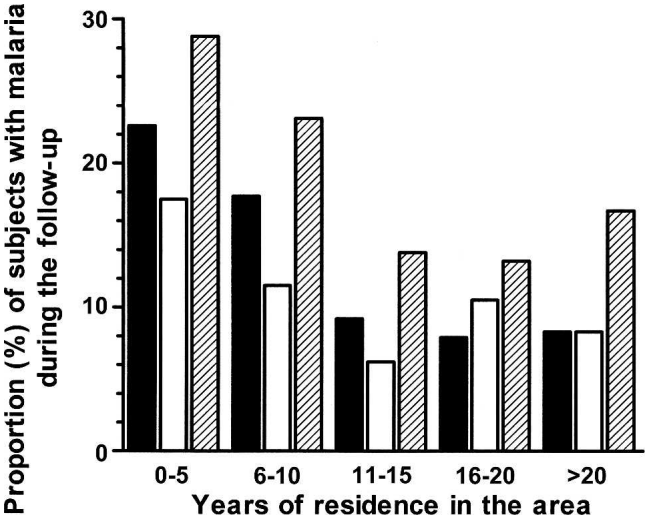

FIGURE 4. Proportions of subjects with one or more incident symptomatic infections with *Plasmodium falciparum* (black bars), *Plasmodium vivax* (white bars), and malaria episodes caused by any species (hatched bars), diagnosed between March 2004 and May 2005, according to the length of residence (in years) in the study area in rural Amazonia.

associated with reduced risk of morbidity with either species. Interestingly, the positive association between poverty and malaria morbidity suggested by the exploratory analysis either disappeared (*P. vivax*) or reversed (*P. falciparum*) after adjusting for more distal determinants of malaria risk in multivariate models.

The proportions of subjects with at least one episode of symptomatic malaria diagnosed during the follow-up decreased with increasing length of residence in the study area (Figure 4). Accordingly, multivariate models showed that the probability of having malaria caused by any species and the result of solely *P. vivax* during the follow-up increased during the first 5–6 years of residence in the study area and declined sharply thereafter (Figure 5). In contrast, the probability of having *P. falciparum* malaria varied slightly less markedly with duration of residence in the area, reaching a peak at 8–9 years and decreasing slowly thereafter (Figure 5).

Spatial distribution of malaria. Complete data for spatial analysis were available for 401 subjects, residing in 107 households, who were under continuous malaria surveillance between March–April 2004 and May 2005. Spatial scan statistics revealed a single significant cluster ($P = 0.0001$) comprising 75 subjects with at least one malaria infection of any type diagnosed during the study (*v*. 42.6 expected). Therefore, 78.9% of all subjects with incident malaria lived in just 47 households (43.9%), which were inhabited by a total of 180 people (44.9% of the sample). These households were within a 6.2 km radius of each other (Figure 5). A smaller cluster, involving 36 subjects (*v*. 12.1 expected) with at least one incident episode of *P. falciparum* malaria, was also highly significant ($P = 0.0001$). Twenty-one households (19.6%), inhabited by 87 people (21.7% of the sample), comprised 64.3% of all subjects with one or more incident *P. falciparum* malaria episodes. These households were situated within a 1.9 km radius of each other and had been included in the larger cluster of malaria caused by any species. *P. vivax* malaria was also spatially clustered; the spatial scan statistics detected a single significant cluster ($P = 0.0001$) comprising 58 subjects with at least one clinical episode of slide-confirmed *P. vivax* malaria during the study (*v*. 33.2 expected). Therefore, 82.9% of all subjects who had *P. vivax* malaria lived in the 50 households (46.7%) inhabited by 190 people (47.4% of the sample).

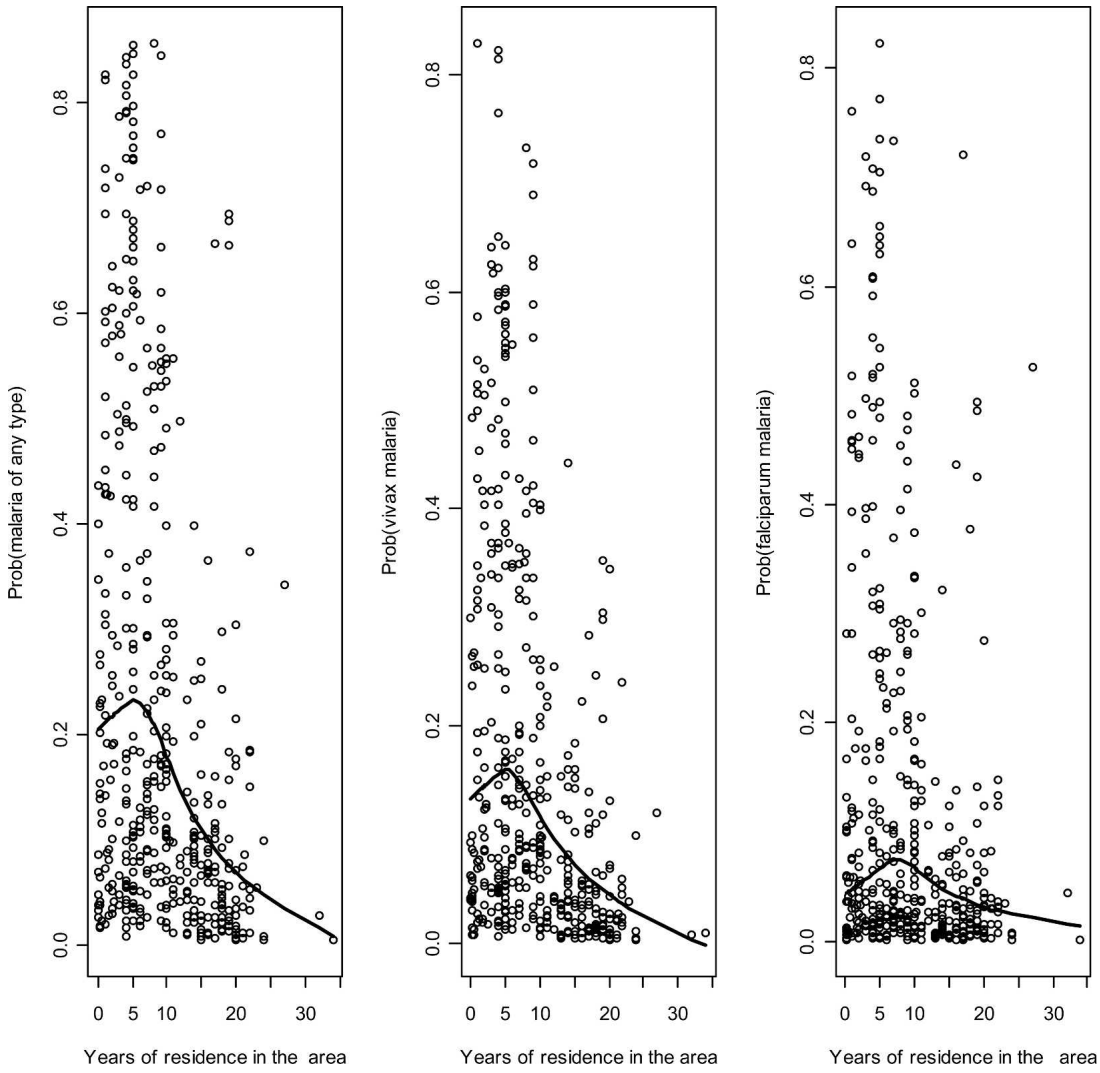

FIGURE 5. Lowess smoothed curve showing the association between length of residence in the study area (a proxy of cumulative exposure to malaria), and the probability of having of malaria of any type (left panel), *Plasmodium vivax* malaria (central panel), and *Plasmodium falciparum* malaria (right panel) during the follow-up. Circles represent individual probabilities derived from mixed-effects logistic regression models.

These 50 households were situated within a 6.6 km radius of each other; and only three of them were not included in the larger cluster of malaria (regardless of the species) (Figure 6).

We used logistic additive models to explore the effect of the place of residence ("house location") on the odds of having malaria during the follow-up. The shapes of the association between place of residence and risk of *P. vivax* and *P. falciparum* malaria clearly differed from each other (Figure 7): although the risk of having *P. vivax* malaria increased linearly with distance from the index house, that of *P. falciparum* morbidity increased sharply at a distance of 12–15 km from the index house, peaking at 14 km. This high-risk area roughly coincides with the spatial cluster of *P. falciparum* detected by spatial scan statistics (Figure 6). The covariate "house location" explained 16.0%, 12.2%, and 20.2% of the deviance observed in logistic additive models for malaria of any type, *P. vivax* malaria and *P. falciparum* malaria, respectively ($P < 0.0001$), indicating that the place of residence was a major determinant of malaria morbidity in the study area.

DISCUSSION

Few population-based prospective studies have focused on malaria morbidity and associated risk factors in communities originating from frontier agricultural settlements in the Amazon Basin of Brazil.[28,29] The classic description of frontier malaria in Amazonia, by Sawyer,[5] comprises three stages: epidemic, transition, and endemic. The first stage is characterized by high malaria rates and the inefficacy of traditional control measures, whereas the transition period is marked by declines in malaria rates that are partially explained by improved health infrastructure and reduced mobility of settlers. Malaria transmission becomes less intense and more stable in the final stages. Our study site provides an example of how the continuous influx of settlers to the forest fringes of the original settlements creates a heterogeneous pattern of malaria transmission with both epidemic and endemic features.

At least three findings of the present study have clear implications for designing appropriate interventions against malaria in our site and other settings with similar endemicity. First, malaria morbidity is clearly associated with forest-related activities such as land clearing and, to a lesser extent farming, which together represent the primary occupation of 35.9% study participants. In addition, sharing the house with a land clearer nearly doubled the odds of having malaria, after controlling for house location and several other covariates. Because land clearing also leads to changes in relative vector abundance that may favor malaria transmission in such a modified environment,[30] this activity plays a crucial role in maintaining malaria transmission in the entire commun-

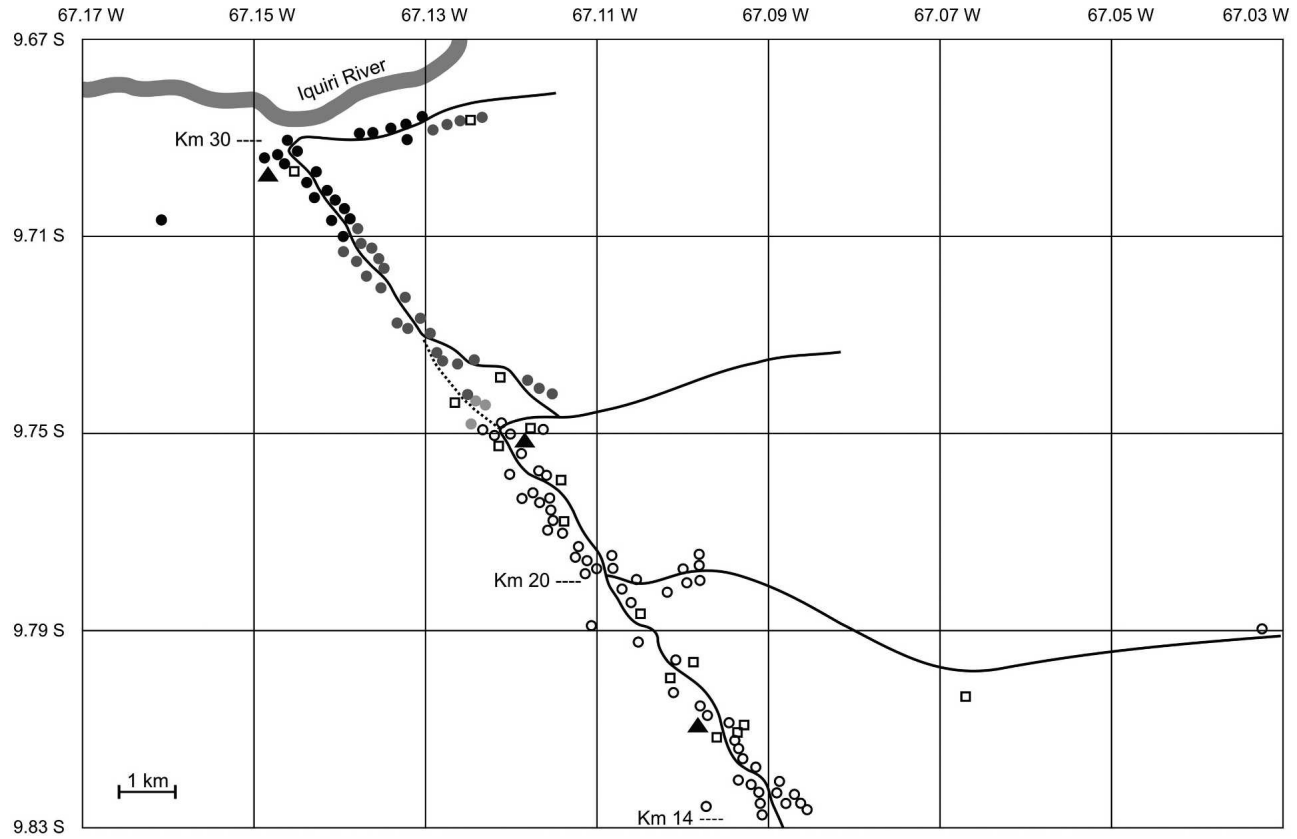

FIGURE 6. Spatial clustering of households with subjects having at least one malaria episode diagnosed during the follow-up, as detected with the spatial scan statistic.[22] Complete data for spatial analysis were available for 401 subjects distributed into 107 households, which are represented with either a black, dark gray, light gray, or white circles; households with incomplete follow-up data are represented with squares, and government-run malaria diagnosis outposts are represented with triangles. The 21 black circles show the Global Positioning System (GPS)-determined location of households included in the cluster of *Plasmodium falciparum* malaria; *Plasmodium vivax* malaria episodes were clustered into 50 households represented as either black, dark gray, or light gray circles, whereas malaria of any type was clustered into 47 households represented as either black or dark gray circles.

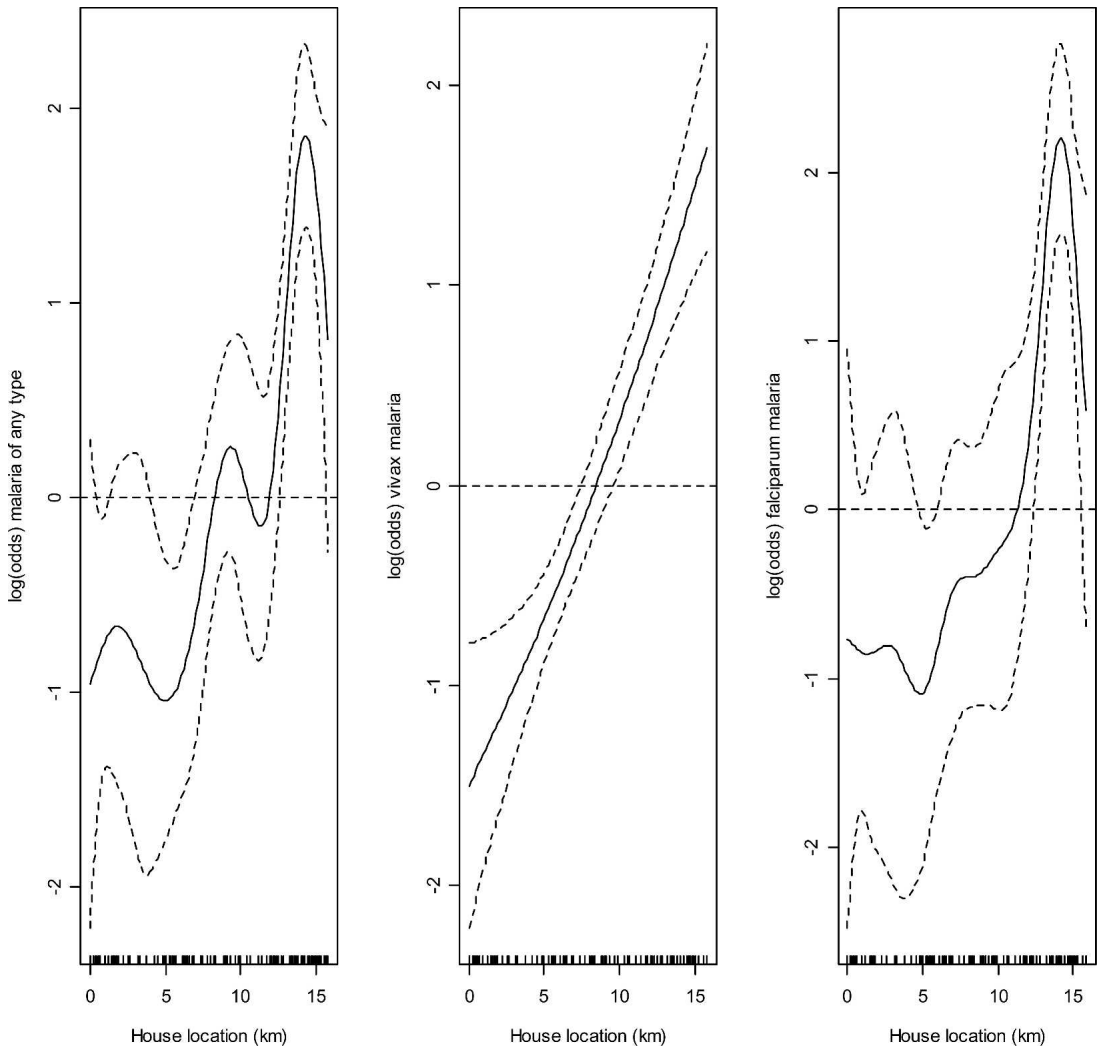

FIGURE 7. Risk of having malaria of any type (left panel), *Plasmodium vivax* malaria (central panel), and *Plasmodium falciparum* malaria (right panel) during the follow-up, expressed as log-transformed odds, according to the site of residence in the study area (house location). House location gives the linear distance in km between each dwelling and an index house situated in the sector of earliest human occupation in the study area. Data were fitted with logistic additive models with integrated smoothness estimation, using penalized regression splines; dotted lines represent the 95% confidence intervals (CI) around the spline. The horizontal dotted line corresponds to log (odds) of zero.

ity. These circumstances are reminiscent of the well-studied forest-malaria phenomenon in Southeast Asia[31,32] and call for specific individual- and community-level interventions to reduce the risk of forest-related malaria in frontier settlements of the Amazon Basin.

Second, the probability of having malaria decreases after five years of residence in the settlement, but is not affected by the subject's age. The decline in malaria morbidity with prolonged residence remains statistically significant after controlling for several factors that are potentially associated with increased malaria risk in newcomers, such as the place of residence (newcomers tend to settle in forest fringes, where transmission is more intense and environmental conditions differ from those in areas of earlier settling) and occupation (newcomers are often hired as land clearers before they can earn a living from their crops and cattle ranching). These findings suggest that gradual acquisition of clinical immunity to malaria exists under conditions of low endemicity that prevail in most communities originated from frontier settlements in rural Amazonia. Despite the relatively low levels of local malaria transmission, malaria-exposed subjects often harbor subclinical infections with very low parasite loads, most of them detected only by PCR.[33–36] Our data indicate that asymptomatic carriers may constitute a significant reservoir of malaria not only in isolated and sparsely populated riverine communities of native Amazonians,[33] but also in more densely populated agricultural settlements.

Third, we showed a significant spatial clustering of malaria risk in the study site. These results suggest that malaria control measures should be spatially targeted for maximum effect.[37] Significantly, two-thirds of subjects who had *P. falciparum* malaria episode(s) diagnosed during the follow-up lived in one-fifth of the dwellings, all situated in the more recently occupied areas of the study site. The spatial clustering of malaria revealed by this and other studies[6,7,37] indicates that selective house spraying and other household–specific interventions are possibly efficient malaria control strategies in this and other settings with similar endemicity.

Subjects with malaria episodes shortly before the baseline survey were at an increased risk for subsequent malaria infections

during the follow-up, most likely a result of the continuous exposure to some of the risk factors identified in our study. Interestingly, some predictors of malaria risk affect predominantly newcomers to the area in a synergistic way. First, the newcomers, before being able to earn the living from their own land, are often hired by local farmers to work in high-risk activities such as land clearing. Second, vacant land plots available for new settlers are often covered with native rain forest and are situated close to protected forest reserves. Extensive deforestation will thus be required before this land can be used for cultivation or cattle ranching. Finally, because most newcomers are migrants from malaria-free regions, they lack acquired immunity to malaria parasites. As a result, the continuous arrival of nonimmune settlers to occupy recently demarcated plots in the periphery of more structured colonization projects results in further environmental change that maintain malaria transmission in a number of communities in rural Amazonia.

Only two malaria parasite species, *P. vivax* and *P. falciparum*, are of public health importance in our study site. The absence of *P. malariae* in the baseline survey and the low incidence of *P. malariae* malaria during the follow-up (this parasite was found in only 1 of 195 infections) contrast with the high proportion of infections with this species (9.4–11.9%) found in other frontier areas of Brazil.[17,18] The reasons for these differences are unclear given that similar diagnostic methods were used in all studies. However, the proportions of mixed-species infections diagnosed in the baseline survey (15 of 60 PCR-diagnosed infections, 25%) and during the follow-up (33 of 195 infections, 16.9%) are within the previously reported ranges (determined by PCR) for native (5.4–36.1%)[33] and for migrant populations (25.7–30.2%)[17,18] across the Amazon Basin of Brazil.

Assessing malaria risk factors in frontier settlements is particularly challenging because of several factors: 1) there is no clear-cut correlate of cumulative exposure to malaria, as age is in rural Africa; 2) individuals cluster into households where several risk factors are shared; and 3) rural settlements usually consist of straight roads with linear or nonlinear gradients of time- and space-dependent covariates. These features were carefully considered in the present analysis, leading to the identification of several independent predictors of malaria risk in rural Amazonia that are potential targets for control measures. Further analyses of data generated by this ongoing cohort study are expected to provide additional insights into the phenomenon of frontier malaria in the Amazon Basin.

Received March 14, 2008. Accepted for publication July 7, 2008.

Acknowledgments: The authors thank the inhabitants of Ramal do Granada for their participation in the study; Sebastião Bocalom Rodrigues, Damaris de Oliveira, and Nésio M. Carvalho (Municipal Government of Acrelândia), Raimundo A. Costa and the malaria control teams in Granada and Acrelandia, for their logistic support, Adamílson Luís de Souza and Carlos E. Cavasini for help in fieldwork, Francisco das Chagas O. Luz (Ministry of Health, Brasília, Brazil) for reviewing all malaria slides, Marília Sá Carvalho and Oswaldo Gonçalves Cruz (Program of Scientific Computation, Oswaldo Cruz Foundation) for their helpful advice in the statistical analysis, Cassiano P. Nunes, for artwork, and Tatiana Havryliuk for a critical reading of the manuscript.

Financial support: CNPq (470067/2004-7) and FAPESP (03/09719-6 and 05/51988-0) to M.U.F.; scholarships from FAPESP (M.dS.N. and C.J.), and CNPq (N.S.dS. and M.U.F.) are also acknowledged.

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