Am. J. Trop. Med. Hyg., 66(6), 2002, pp. 641–648

HIGH PREVALENCE OF ASYMPTOMATIC *PLASMODIUM VIVAX* AND *PLASMODIUM FALCIPARUM* INFECTIONS IN NATIVE AMAZONIAN POPULATIONS

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Abstract. The epidemiology of malaria in 2 riverine localities in Rondônia, Brazilian western Amazônia, was assessed by a 1-year study at Portuchuelo, and a cross-sectional survey at riverine communities at Rio Machado (= Ji-Paraná). *Plasmodium* spp. infections were diagnosed by light microscopy and by polymerase chain reaction (PCR) amplification of ribosomal DNA. PCR was 6–7 times more efficient than microscopy for detecting plasmodial infections. Both *Plasmodium vivax* and *Plasmodium falciparum* infections occurred as asymptomatic and symptomatic forms of the disease. The relation between symptomatic and asymptomatic clinical forms was roughly similar for both species of *Plasmodium.* Symptomless patients were monitored for 2 months. The prevalence of symptomless infections was 4–5 times higher than the symptomatic ones—respectively, 20% and 4.6% for Portuchuelo and 49.5% and 10% for Ji-Paraná. Symptomatic malaria occurred mostly in patients in younger age groups. In contrast, there was a significant association of symptomless malaria with older age groups (medians of 26.5 and 21 years, respectively, for Portuchuelo and Ji-Paraná), whereas the age medians for symptomatic malaria were 14 and 8 years, respectively, in the 2 regions. Symptomatic malaria also was more prevalent in groups living for shorter times in Amazônia (13 and 4 years, respectively, for Portuchuelo and Ji-Paraná) as compared with symptomless malaria, which was more prevalent in groups living for longer periods in the region (medians of 25.5 and 18 years, respectively, for Portuchuelo and Ji-Paraná). The high prevalence of symptomless malaria may pose new problems for the currently adopted strategy for the control of malaria in the Amazonian region, which is essentially based on the treatment of symptomatic patients.

INTRODUCTION

In the last 150 years, 4 major malaria epidemics have been recorded in the region of the present Brazilian State of Rondônia, occidental Amazônia. Conspicuous episodic immigration was the common trait of these epidemics.

The first epidemic started at the time of the 1880s rubber boom. Most immigrants were peasants from Brazilian northeastern states who were trying to escape the drought that was ravaging their homeland.[1,2] The second epidemic took place at the turn of the century and plagued the workers of the Madeira-Mamoré railroad, which was being built to drain the Bolivian rubber production. In this case, many of the immigrants came from Caribbean countries, particularly Granada and Barbados.[1,3] Their health conditions were recorded by Oswaldo Cruz.[3] The third epidemic, which occurred at the time of the renewed rush for latex at the beginning of World War II, victimized voluntaries of the so-called rubber army, also mainly consisting of peasants from northeastern Brazilian states.[2,4] The present native Rondonian population is the offspring of these immigrants and of the aboriginal local populations.

The fourth and last epidemic exploded in the 1970s, when the federal government offered free land to a large contingent of settlers from the southern states of the country. This last episode is well documented.[2,5–8] In ~ 20 years, the population of Rondônia increased from 111.064 to 1.130.874 people (1970–1991, Brazilian Institute of Geography and Statistics, IBGE) whereas the number of malaria cases correspondingly increased from 5,772 to 278,408 cases per year (1970–1988, National Health Foundation, FUNASA, of the Brazilian Ministry of Health).

As in the preceding epidemics, immigrants got sick almost on arrival, and their often severe and lethal malaria was characterized by exuberant symptoms. In contrast, among native populations, malaria was generally mild and did not seem to spread as explosively as among migrants. Thus, the general idea that derived from this last, and still focally persisting, epidemic was that migrants were the agents responsible for malaria dissemination throughout the land.

However, while studying a riverine population of native Amazonians, we found that a large number of people, although carrying *Plasmodium* in their blood cells, remained symptomless through a follow-up of ≥ 30 days. These findings prompted the hypothesis that native populations could act as parasite reservoirs, and from there, malaria could spread to migrants.[9] Because the population studied was a small one, we decided to expand the sample and the period of study. In addition, we extended our studies to a quite distinct and remote region populated by native Amazonians living in small riverine settlements far apart from each other.

The results we describe herein confirm the widespread presence of asymptomatic malaria infections among native Rondonian populations. They also emphasize the need of an innovative strategy for the control of malaria that should take into consideration the diagnosis and treatment of healthy carriers of *Plasmodium* in the Amazonian region.

MATERIALS AND METHODS

Riverine population. Riverine communities are scattered along the margins of the Amazonian rivers and are constituted mostly by descendentis of aboriginal populations and migrants from northeastern Brazil. Presently, remotely located riverine communities support themselves by fishing and subsistence farming. Their houses have thatched roofs and are built in the riparian forest out of the abundant local wood. The modest surplus of agricultural goods, mainly cassava flour, is bartered for edible goods, remedies, and clothing,

which are carried by traders that periodically sail through the secondary Amazonian rivers.

Our studies were conducted at 2 different areas: the Portuchuelo community, at the Madeira River; and 6 communities along the Machado River (or Ji-Paraná, which means "ax river" in aboriginal language), a tributary of the Madeira River 180 km downstream from Porto Velho, the Rondonian capital (Figure 1). The climate of the 2 areas is similar, with 2 distinct seasons: a rainy season from October to April and a dry season from May to September. The temperature is in the range 15–38°C, and the humidity is always high, often > 90%. Heavy rains running over the impermeable clay soil of most riverine areas generate streams (*igarapés*) and deep ponds (*igapós*), which become excellent mosquito breeding places. Thus, the end of the rainy season is usually the period of high malaria transmission in riverine areas (Figure 2), but this may vary a little from year to year at each place, depending on microenvironmental peculiarities and pluvial and fluvial regimens.[10] The main malaria vector is *Anopheles darlingi,* which accounts for > 90% of the anopheline fauna of Rondônia.[4,11–14]

The Portuchuelo community (63°49′28″W, 8°37′44″S) is located on the right bank of the Madeira River, 34 km downstream from the capital, Porto Velho, and has been described in detail before.[15] It can be reached by boat throughout the year, and nowadays also by road during the dry season. The houses are distributed over 6 km along the riverside, and some are located up to 3 km inland. Part of the area has been cleared for agricultural production. The proximity to the capital facilitates communication between the 2 places. There are 38 families living in the community, making up a total population of ~ 180 inhabitants.

The Ji-Paraná (Rio Machado) communities are randomly distributed along the 2 riverbanks. After a preliminary visit to the area, we chose to study 6 communities (São João, Juruá, Trindade, Monte Horeb, São Pedro da Angustura, and Dois de Novembro) located 100–150 km upstream from the river mouth, at ~ 63–64°W, 8–9°S. The choice was based on the following characteristics: isolation, lack of population mobility, almost no access to health care facilities, and, apparently, a high incidence of malaria. The houses are sparsely distributed, and local transportation is by small boats similar to the canoes used by Amazonian natives. The area is reachable by large boats exclusively during the rainy season because in the dry season, the water level may drop 8–10 m. There are 32 families living in the area, with a total of ~ 180 inhabitants.

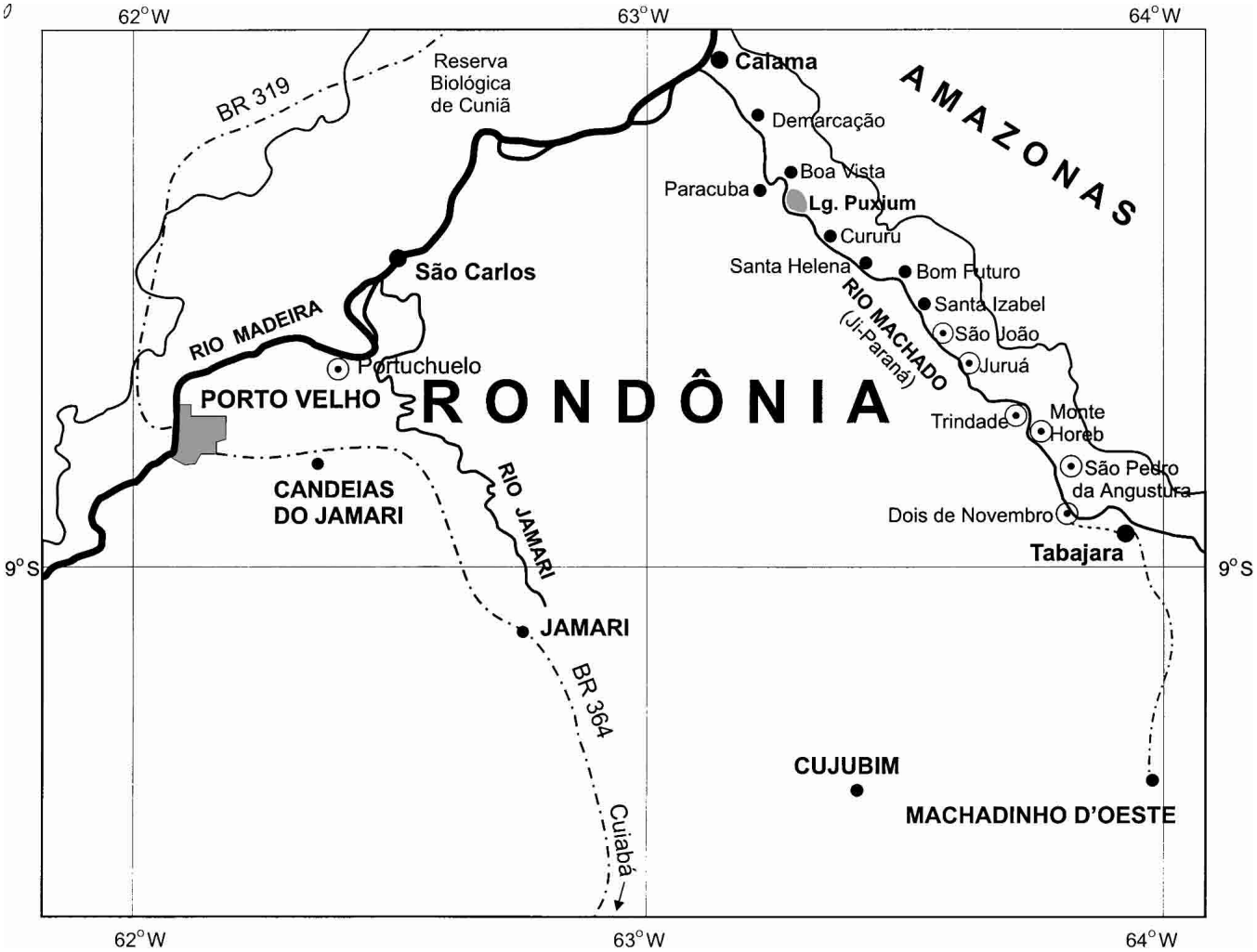

FIGURE 1. The study areas were Portuchuelo, near Porto Velho, and small localities **(open circle and dot)** at the banks of Rio Machado (Ji-Paraná).

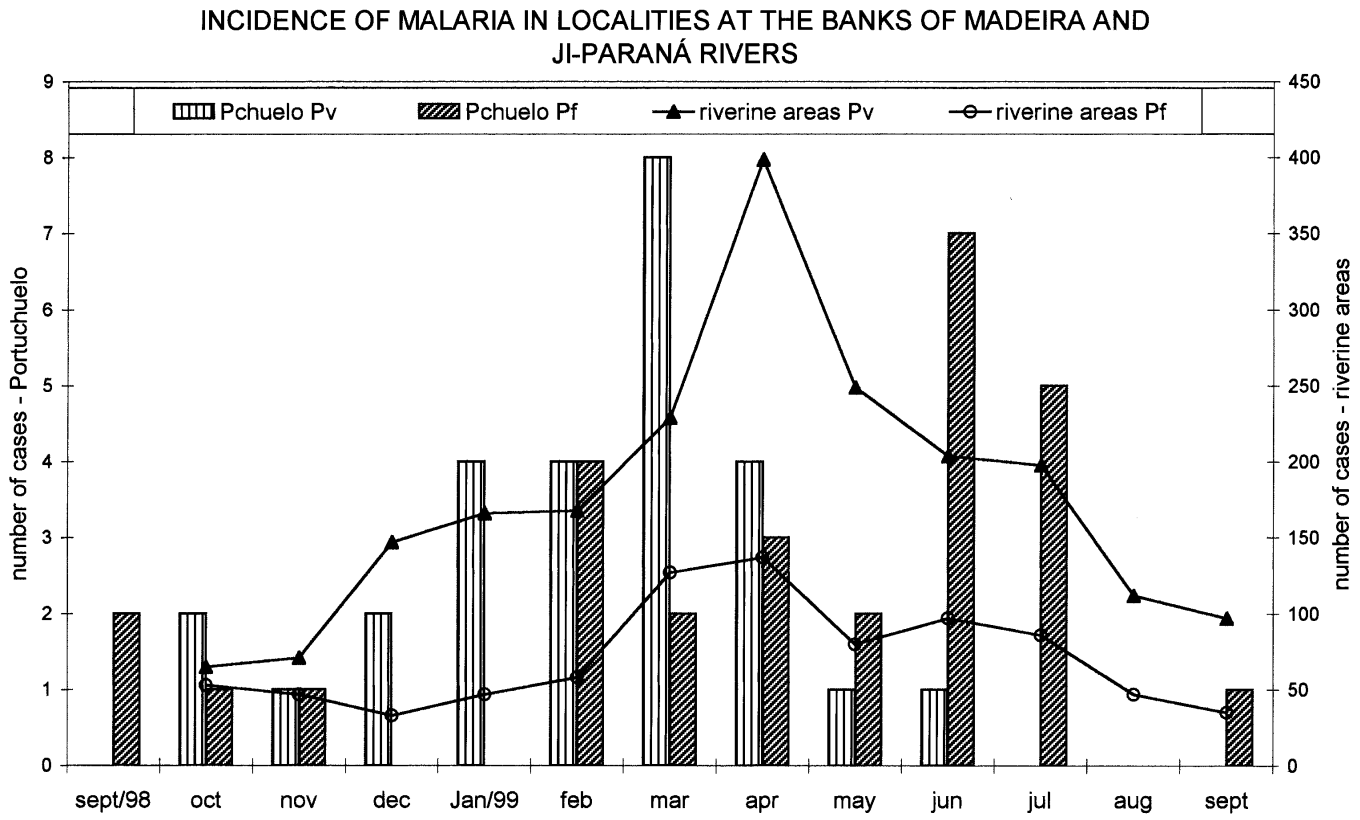

FIGURE 2. Incidence of Malaria in Portuchuelo and the riverine area of the Madeira River and tributaries. Diagnosis of infections by *Plasmodium falciparum* (Pf) and *Plasmodium vivax* (Pv) was performed by microscopic examination of blood smears. Data for the overall riverine population are from National Health Foundation, Brazilian Ministry of Health.

Schooling is rudimentary. There is no radio, electricity, sewage, or running water.

Cross-sectional studies. At Portuchuelo, cross-sectional surveys were carried out after repeated meetings with the community and discussions about the objectives of the project and its protocols. Informed consent was obtained from the study subjects, or in the case of children, their parents. The ethics committee of the Rondonian State Medical Council approved the protocols. Participants were interviewed and clinically examined. We collected ~ 5-mL blood samples in ethylenediaminetetraacetic acid (EDTA) from all. Malaria was diagnosed by light microscopy and by nested polymerase chain reaction (PCR) assay. Individuals with positive blood smears or positive PCR but without symptoms, were invited to participate in the follow-up protocol. Six and 12 months after the first survey, 2 other identical cross-sectional surveys were carried out.

At Ji-Paraná, protocols and consent giving were similar to those of Portuchuelo. All inhabitants were enrolled in the study. They were clinically examined, and ~ 5-mL blood samples in EDTA were collected for microscopy and PCR. Children younger than 4 were spared blood sampling. Instead, a blood smear was collected for malaria diagnosis by puncturing a fingertip.

Follow-up protocol. Microscopy- and/or PCR-positive but asymptomatic patients of Portuchuelo underwent 60-day follow up. The medical surveillance involved 2 weekly medical visits to the enrolled patients and immediate access to our central medical facilities at Porto Velho whenever necessary. In addition, the patients were under the constant attention of the health agents. During each visit, the patient was interviewed about malaria symptoms and clinically examined, and a blood smear was collected. A motorboat and a skilled boatman were on hand for emergencies, but they were never used. Patients who presented symptoms during the follow-up period were immediately treated and recorded as symptomatic (S^+).

At Ji-Paraná, in contrast to Portuchuelo, patients with positive smears and no symptoms were treated 10 days after diagnosis. This was because we could not provide adequate medical surveillance to the population for longer periods.

Definition of asymptomatic *Plasmodium vivax* and *Plasmodium falciparum* infections. Individuals who presented malaise, fever, or muscular pain and headache in addition to microscopy or PCR positivity were considered symptomatic.

At Portuchuelo, individuals with a positive microscopy and/or positive PCR who remained symptomless for 60 days, regardless of the onset of the infection, were considered asymptomatic (S^-).

At Ji-Paraná, our team could not carry out long-term observations. Symptomatic patients were immediately treated and recorded as S^+. Individuals without symptoms but with positive microscopy were observed for 10 days and then treated, even if they remained symptomless. Individuals with negative microscopy but with later evidence of positive PCR were visited after 2 months. Those who remained symptomless during this period were considered to be asymptomatic

patients. Those who presented symptoms were considered to be symptomatic.

Criteria for exclusion of the follow-up protocol. All symptomless patients were invited to participate in the follow-up protocols, except for 6 categories of individuals who were automatically excluded. These were as follows: 1) patients who expressed the slightest doubt about the safety of the procedures; 2) pregnant women; 3) patients who preferred to be treated in any circumstance; 4) patients who had received antimalarial therapy in the past 4 weeks and during follow-up; 5) children younger than 4 in Ji-Paraná; and 6) individuals of Ji-Paraná with positive microscopy who remained symptomless for 10 days but who were treated when our team left.

Microscopy. Well-trained microscopists were in charge of examining 200 fields of Giemsa-stained thick blood smears at ×1,000 magnification under immersion oil. Parasite density was determined by counting the number of asexual forms per 200 leukocytes, assuming a leukocyte number of 6,000/mm^3. Microscopy was performed on the spot at Ji-Paraná; the slides collected at Portuchuelo were brought to our central laboratory at Porto Velho for processing.

Diagnosis via PCR. The nested PCR was based on the Snounou protocols, with minimal modifications.[16,17] The target was the 18S rRNA gene, and genus- and species-specific primers were used in the assay.

Briefly, 300 μL of whole blood collected on EDTA was prepared for DNA extraction by the phenol-chloroform method followed by precipitation with sodium acetate and ethanol. The first PCR rDNA amplification was performed with *Plasmodium* genus-specific primers. Positive samples yielded a 1,200-bp fragment, which served as template for the nested reaction. The nested PCR amplification was performed with species-specific primers for 30 cycles at annealing temperatures of 58°C for *Plasmodium falciparum* and 65°C for *Plasmodium vivax.* The annealing temperature adopted for *P. vivax* was higher than that of the original protocol (58°C) for the sake of efficiency. The fragments obtained were of different sizes: 120 bp for *P. vivax* and 205 bp for *P. falciparum.* The products were visualized in 2% agarose gel stained with ethidium bromide.

All tests were performed at our headquarters in Porto Velho and confirmed at our main laboratory at the University of São Paulo. A few randomly chosen amplicons were sequenced as an additional control and were confirmed to correspond to the GenBank deposited sequences AF145334 and AF145335 of *P. falciparum* and *P. vivax* from Papua New Guinea.[18]

Surveillance in between cross-sectional studies. At Portuchuelo, 3 volunteers were recruited and trained as primary health workers. They were in charge of weekly house visits to detect acute febrile illnesses or other symptoms or signs of malaria. A blood sample was collected from every suspected case of malaria. Diagnosis was based on light microscopy. The medical team visited the community 2–3 times a week, examining all malaria patients. The confirmed malaria cases were treated according to the FUNASA (National Health Foundation) protocols.

At Ji-Paraná, independently of our presence, FUNASA technicians are routinely responsible for malaria diagnosis and treatment, and whenever possible, visit each community every 15 days. They are the only health care resource in the region. Malaria diagnosis was based on microscopy that used sunlight as the light source. Standard treatment was provided for positive cases. The technicians were also responsible for collecting and temporarily storing data on malaria incidence.

Treatment protocol. The malaria therapy protocol utilized was that of FUNASA. The treatment for *P. vivax* consisted of chloroquine phosphate (25 mg base/kg over a 3-day period) plus primaquine (0.25 mg base/kg/d, maximum 15 mg, for 14 days). Treatment of *P. falciparum* malaria consisted of quinine (30 mg/kg/d, maximum 2 g, for 3 days) plus doxycycline (3.3 mg/kg/d for people aged > 8 years, maximum 200 mg, for 7 days) and a single dose of primaquine to treat gametocytes (0.5–0.75 mg base/kg). For children < 8 years old, the standard treatment was quinine (25 mg/kg/d) for 10 days. Alternatively, mefloquine (20 mg/kg up to 1,000 mg in a single dose) plus primaquine was used for *P. falciparum* in special situations.

Analysis of data. Microsoft Access 2.0 (Microsoft, Redmond, WA) was used for database storage. Analysis was performed with the statistical programs SPSS 9.0 for Windows (SPSS Inc., Chicago, IL) and Epi Info version 6.04b (CDC, Atlanta, GA). Proportions and categorical data were compared by the chi-square test, with Yate's correction, in cases of 2 × 2 contingency tables, or by Fisher's exact test. Data that did not conform to normal distribution were analyzed by Mann-Whitney *U*-test.

RESULTS

Demographic analysis. *Portuchuelo.* Throughout the study period, up to 175 individuals distributed among 38 families participated in the 3 cross-sectional surveys. Ninety-two percent of the villagers were from the Amazonian region, and 75% were born in Rondônia. The age distribution was similar to that of the whole state of Rondônia (IBGE), with a predominance of youngsters (median age, 18 years). The distribution of the different age groups was as follows: < 5 years of age, 13.7%; 6–14 years, 26.3%; 15–20 years, 14.3%; 21–40 years, 21.1%; > 40 years, 24.6%. These age groups were chosen to make the data comparable to that of previous reports. The male/female sex ratio was 1.2.

Ji-Paraná. The 172 individuals studied belonged to 32 families. The male/female sex ratio was 1.2. Most inhabitants (95%) had been born in the Amazonian region, with 78% being from Rondônia, and 47% had been born in the Ji-Paraná region. The distribution of the different age groups was as follows: < 5 years, 27.3%; 6–14 years, 25%; 15–20 years, 11.6%; 21–40 years, 22.1%; > 40 years, 14%. The Ji-Paraná population had a statistically significant higher proportion of children < 14 years of age than the population of Portuchuelo (chi-square = 4.82, $P = 0.028$).

Prevalence of malaria in cross-sectional surveys. *Portuchuelo.* In the 3 cross-sectional surveys, the number of individuals enrolled was 175, 142, and 125 inhabitants, respectively. However, the relative composition of the population was the same in the 3 surveys. Physical examination in the 3 surveys respectively revealed the presence of palpable spleen in 0, 4, and 6% of the children < 10 years of age. References to previous malaria episodes at the first survey were: no malaria, 13.1%; 1–5 episodes, 56%; > 5 episodes, 30.9% (median, 3 episodes).

The first (September 1998) and third (September 1999) surveys were performed during the dry season and the second

one (March 1999) toward the end of the rainy season. The data on the prevalence of malaria are listed in Table 1. Malaria prevalence as diagnosed by microscopy was 4.6, 4.2, and 0%; and by PCR 23.8, 31.7, and 6.4%, respectively, in the 3 surveys. There was a slight predominance, although not statistically significant, of vivax over falciparum infections in the surveys of the dry season (chi-square = 3.32, $P = 0.068$ in the first survey, and chi-square = 3.75, $P = 0.053$ in the third survey), whereas in the rainy season, there was a practically equal frequency of the 2 types of infections (20 *P. vivax* and 21 *P. falciparum*). The observed frequency of mixed infections was similar to the expected frequency as if the 2 infections occurred independently, as estimated by the product of the prevalence of each parasite species (Fisher's exact $test_{2t} = 1$ for the 3 surveys). There was a remarkable reduction in the overall prevalence of malaria in the third survey compared with the first and second surveys (chi-square = 6.74, $P = 0.009$ for *P. falciparum* and chi-square = 4.87, $P = 0.03$ for *P. vivax,* comparing the first and third surveys).

Ji-Paraná. In the sole survey performed on the riverine population of the Machado River, 172 individuals were examined. The spleen was palpable in 5% of the children < 10 years of age. Two of the 3 children with a palpable spleen had *P. vivax* infection. References to previous malaria episodes were as follows: no malaria, 13.4%; 1–5 episodes, 62.2%; > 5 episodes, 24.4% (median, 3 episodes).

The Ji-Paraná survey was performed at the end of the rainy season (May 2000). The data on the prevalence of malaria are listed in Table 1. Malaria prevalence as diagnosed by microscopy was 16.9% and by PCR 64.8%. There was no significant difference between vivax and falciparum prevalence rates (chi-square = 0.32, $P = 0.57$). The high prevalence of mixed infections (23.4%) detected by PCR in this area was remarkable. Considering the infections to occur independently, we compared the observed versus the expected number of mixed infections, but did not observe a statistically significant difference (chi-square = 1.2, $P = 0.26$).

Microscopy versus PCR. In both study areas, detection of *Plasmodium* infection by PCR was severalfold higher than by microscopy (6.57-fold in Portuchuelo and 7.5-fold in Ji-Paraná). Individuals with symptomless malaria but with positive blood smears always had very low parasitemia and correspondingly low parasite counts (< 500 parasites/μL). If PCR is taken as the reference test, microscopy detected less than one-fourth of the infections. PCR is also ~ 30-fold more efficient in detecting mixed infections than microscopy alone, as shown by the Ji-Paraná data (Table 1). These data confirm previous observations about the superiority of PCR over microscopy for the detection of *Plasmodium* infections.[18–20] They also explain why routine microscopy failed to detect such a high incidence of symptomless infections among native Amazonians.

A PCR-negative sample never happened to be positive by light microscopy.

Asymptomatic *Plasmodium* infections and follow-up studies. At the cross-sectional surveys, all patients from Portuchuelo positive by microscopy or PCR, but without symptoms, were enrolled in the follow-up program, except those meeting the exclusion criteria. The prevalence of asymptomatic infections was 14.6, 21.7, and 6.4% in the 3 surveys, respectively (Table 2). Symptomless infections were more frequent than symptomatic ones. If we consider the null hypothesis that S^- and S^+ have the same chance of occurring, then the predominance of S^- over S^+ is highly significant for the first and second survey ($P < 0.001$, $P = 0.0029$). As to the third survey, the number of infected individuals was too small as to permit statistical analysis.

At Ji-Paraná, the prevalence of symptomless infections was 49.5% and of symptomatic malaria, 10%. However, 12 asymptomatic individuals with positive smears were observed for 10 days and then treated. They were not included in further analysis of comparison between S^- and S^+ groups. Children younger than 4 and patients meeting the exclusion criteria also are not included in Table 2. Among these were 12 children with positive smears and symptoms; these children were treated. Thus, the prevalence of symptomatic or symptomless infections listed in Table 2 may be underestimated.

Results of Table 2 show that asymptomatic malaria is highly prevalent in the 2 riverine communities studied. Actually, it is 4 times higher than symptomatic malaria in Portuchuelo and 5 times higher in Ji-Paraná.

Longitudinal survey and the incidence of malaria attacks. *Portuchuelo.* The cyclical nature of malaria incidence is a well-documented fact among riverine populations, as shown in Figure 2, which summarizes the incidence of malaria in all riverine localities of the Madeira River downstream from Porto Velho (data from FUNASA). Our data regarding Portuchuelo symptomatic malaria follow the same pattern, although the data are subject to erratic fluctuations that are the result of local environmental factors and the small size of the populations under study. Nevertheless, there is a remarkable seasonality in the incidence of malaria, which is higher at the end of the rainy season. This malaria peak is preceded by a peak in the density of the anopheline population (Gil LHS and others, unpublished data).

During 1-year of observations at Portuchuelo (September 1998–September 1999), 45 patients out of 175 individuals presented 57 episodes of symptomatic malaria, 27 *P. vivax,* 28 *P. falciparum,* and 2 mixed infections (Figure 2). This gives an Annual Parasite Index of 326 per 1,000 inhabitants. The cases of vivax malaria presented a peak in the end of the rainy season (74% of the vivax cases occurred between January and April 1999). Of the falciparum cases, 32% occurred during the rainy season, but 43% occurred in June–July 1999. However, 9 of these 12 falciparum cases occurred after the return

TABLE 1

Malaria cases diagnosed by microscopy and polymerase chain reaction and overall malaria prevalence*

Locality	Method	n	S^0	Pf	Pv	Mix	Prevalence (%)
Portuchuelo							
First survey	Microscopy	175	167	4	4	0	8 (4.6%)
	PCR	164†	125	15	23	1	39 (23.8%)
Second survey	Microscopy	142	136	2	4	0	6 (4.2%)
	PCR	142	97	21	20	4	45 (31.7%)
Third survey	Microscopy	125	125	0	0	0	0
	PCR	125	117	2	6	0	8 (6.4%)
Ji-Paraná	Microscopy	172	143	9	19	1	29 (16.9%)
	PCR	128†	45	28	25	30	83 (64.8%)

* Microscopy = light microscopy of blood smears; mix = number of mixed infections; PCR = polymerase chain reaction; Pf = number of infections by *Plasmodium falciparum;* Pv = number of infections by *Plasmodium vivax;* S^0 = number of negative individuals by microscopy and PCR.

† The differences in number between PCR and microscopy are due to the exclusion criteria (see Materials and Methods).

TABLE 2
Number of cases and overall prevalence of symptomless and symptomatic *Plasmodium* infections*

Locality	n	*P. falciparum*		*P. vivax*		Mixed		Overall prevalence (%)	
		S^-	S^+	S^-	S^+	S^-	S^+	S^-	S^+
Portuchuelo									
First survey	150	7	2	14	1	1	0	22 (14.6%)	3 (2%)
Second survey	138	14	5	14	4	2	2	30 (21.7%)	11 (8%)
Third survey	125	2	0	6	0	0	0	8 (6.4%)	0 (0)
Ji-Paraná	111	18	3	20	1	17	7	55 (49.5%)	11 (10%)

* n = number of individuals in the follow-up after applying the exclusion criteria (Materials and methods); S^- = number of symptomless infections; S^+ = number of symptomatic infections. Infections were diagnosed by light microscopy, polymerase chain reaction, or both.

home of a family that had spent a few months working elsewhere. This shows that for small populations, clustered malaria episodes may alter the standard seasonality of the infection.

Ji-Paraná. The data presented in Figure 2, from the riverine region of the Madeira River, include data from Ji-Paraná, where malaria also is seasonal.

Demographic and group characteristics of malaria patients. Table 3 summarizes the differences between the groups of individuals with symptomatic or asymptomatic infections in Portuchuelo and Ji-Paraná. It is interesting to note that in both populations, younger age groups were significantly more vulnerable to symptomatic malaria than older ones. There was also a strong relation between the length of time lived in the Amazônia, or in the current community, and the prevalence of symptomless malaria. All the differences between S^- and S^+ groups were statistically significant.

Table 4 presents the distribution of symptomatic or asymptomatic infections, according to age groups. As can be seen, the chances of getting a plasmodial infection are roughly similar for all age groups (chi-square = 0.32 with df = 4, P = 0.57). On the other hand, the chances of presenting symptomless infections are much higher in older age groups. In Portuchuelo, if we use the 0–4 years group as reference, the odds of getting S^- malaria are similar to that of the 5–14-year group, but increases thereafter to reach an odds ratio of 6.67 for people older than 40 years. Thus, the chances of presenting an asymptomatic infection increase significantly with age (chi-square for trend = 10.53, P = 0.001). The numbers of Ji-Paraná are too small for statistical analysis with all age groups. But if we divide the population at approximately its median—that is, in 2 groups (0–14 years and older than 15 years)—then we have an odds ratio of 5.82 of the older group over the younger to present an asymptomatic infection (chi-square = 9.52, P = 0.002). These facts unquestionably point toward the acquisition with age of a certain degree of immunity—that is, with time of exposition to malaria. This corroborates the data of Table 3 showing that longer the time lived in the Amazônia, the higher the chances of developing symptomless malaria.

DISCUSSION

From the 1970s on, malaria was omnipresent and responsible for much suffering and wretchedness in immigrant settlements in Rondônia. Newly arrived migrants experienced acute malaria symptoms.[7,21] In contrast, in riverine settlements of native Amazonians, malaria could pass unnoticed, were it not for a few patients with symptoms not nearly as exuberant as those of the immigrants.

There were more differences. Whereas the incidence of malaria, as determined by microscopy, was very high among immigrants (API_{1985} = 2,400 per 1,000 inhabitants in an forest settlement), it was much lower (242 per 1,000 inhabitants) among native riverine populations.[2,15,21–23] Among migrants, the incidence of malaria was higher in male subjects and in older age groups.[7,10,24,25] Children < 5 years old were generally spared. Among native riverine populations, the opposite was true.[15] Immigrants, but not native Amazonians, invariably presented malaria symptoms. The examination of thousands of malaria patients has consistently shown that migrants with *Plasmodium* in their blood always presented symptoms or would do so within hours, or 2–3 days at most. In cross-sectional surveys, there were a few parasite-positive patients without symptoms but who became symptomatic during follow-up. Thus, in our experience, as in the experience of others, malaria in Rondônia was always symptomatic among migrants.[24–26] Of course, there was anecdotal evidence about people, mainly gold diggers, who claimed to be resistant to malaria in spite of living permanently in endemic areas, but these cases have never been scientifically documented.

TABLE 3
Demographic parameters and malaria in native Amazonian populations*

Variable	Group	Portuchuelo			Ji-Paraná		
		n	Median	*U*-test	n	Median	*U*-test
Age (y)	S^-	44	26.5	547.5†	55	21	298†
	S^+	45	14		23	8	
Time living in Amazônia	S^-	42	25.5	468†	55	18	303.5†
	S^+	45	13		23	4	
Time living in the community	S^-	44	17	495.5†	55	12	272.5†
	S^+	45	7		23	3	

* S^- = symptomless infections; S^+ = symptomatic infections; *U*-test = Mann-Whitney *U*-test applied to compare the S^- and S^+ groups. Diagnosis of *Plasmodium* spp. infection was made by light microscopy, polymerase chain reaction, or both.
† $P < 0.001$.

TABLE 4
Distribution of symptomless *Plasmodium* spp. infections in native Amazonian populations according to age group*

Age (y)	Portuchuelo					Ji-Paraná			
	n	Infections, n (%)	S^-	S^+	OR	n	Infections, n (%)	S^-	S^+
0–4	24	11 (45.8)	3	8	1	47	12 (25)	1	11
5–14	46	23 (50)	6	17	0.9	43	23 (53)	17	6
15–19	25	15 (60)	9	6	4	20	10 (50)	9	1
20–39	37	19 (51)	11	8	3.67	38	20 (53)	17	3
≥ 40	43	21 (49)	15	6	6.67	24	13 (54)	11	2
Total	175	89 (51)	44	45		172	78 (45)	55	23

* OR = odds ratio; S^- = asymptomatic infections; S^+ = symptomatic malaria. For Portuchuelo, data are the average of 3 surveys. Chi-square for trend = 10.53; P = 0.001. For Ji-Paraná, the sample size was too small to perform chi-square trend analyses.

Thus, whereas in Africa asymptomatic *P. falciparum* infections are commonplace and widespread,[27–30] in Brazil, they were unheard of. Symptomless infections by *P. vivax* also have not been described in Brazil, except in southern coastal areas, where symptomless or oligosymptomatic infections by *P. vivax* have been reported.[31] However, in these areas, even symptomatic malaria is quite atypical, and the possibility has not been excluded that this regional malaria may be caused by a variant of *P. vivax* or *P. vivax*–like parasite.

Plasmodium infections in absence of symptoms have been often found in cross-sectional surveys.[32,33] However, absence of symptoms in a sectional study is not tantamount to asymptomatic malaria, which can only be ascertained through follow-up protocols. Studies carried out in Papua New Guinea and Thailand have clearly shown the existence of asymptomatic *Plasmodium* infections out of Africa.[34–37] Absence of symptoms upon infection by *Plasmodium* is also known from the attempts to treat neurosyphilis through malariotherapy. Interestingly, the acquired immunity in these cases was species-specific, and the protection afforded by a previous infection was higher when the next inoculation was performed with the same strain of the parasite.[38]

The results of the present study clearly show the existence of asymptomatic *P. vivax* and *P. falciparum* infections in Rondônia. This fact reverses the history of the Rondonian epidemics in the sense that native populations, not immigrants, are responsible for the constant presence of malaria.

First of all, it has been shown that the prevalence of *Plasmodium* infections is very high in native populations. This fact remained unacknowledged before the utilization of PCR because microscopy of blood samples detects only a fraction of the positive cases. In addition, symptomless people do not complain and do not seek malaria services, which thus register only clinical malaria cases, whereas in fact, at any given moment, most malaria infections of native populations are symptomless.

In native villages, people become infected with *Plasmodium* very early in life. Symptomatic malaria of native populations, in sharp contrast with the malaria of immigrants, preferentially affects young age groups and does not spare children < 5 years old. This is suggestive of indoor infection, a fact fully compatible with riverine houses made of wooden boards spaced far apart from each other to increase ventilation. Because mosquitoes are not known to have preference for children, one may infer that adults and children are equally exposed to mosquito bites. Thus, all age groups should be equally infected by *Plasmodium* spp., and indeed they are (Table 4). However, although symptomatic malaria is significantly more prevalent in younger age groups, the prevalence of asymptomatic infections is higher in older groups (Tables 3 and 4). These facts, taken together, indicate that individuals become immune to malaria as a function of age—that is, as a function of the number of exposures.

These observations draw a new picture of the Rondônia epidemics of malaria. Native populations, concentrated along the vast Amazonian riverbanks, are exposed since childhood to mosquito bites. Yet unpublished data (Gil LHS and others) have shown that the index of biting per person by *A. darlingi* (the principal Amazonian malaria vector) is indeed very high in riverine settlements and that a fraction of these mosquitoes harbor oocysts. We have also observed that not only symptomatic patients but also symptomless ones, with negative smear but positive PCR, can be a source of infection for mosquitoes (Alves FP and others, unpublished data). Thus, riverine populations are under permanent exposure to malaria and begin to show malaria episodes early in life. With time, they develop a certain degree of immunity but remain subject to infection until sterilizing immunity sets in—if it ever sets in. Until then, asymptomatic carriers of *Plasmodium* may act as reservoirs of the parasite and as a likely source of infection.

The present investigation was not conceived to disclose the immunological mechanisms that lie behind asymptomatic malaria. We have no hypothesis to forward, but we are sure that this will be a fruitful field of investigation. In spite of the nature of the underlying immune mechanisms, one question remains. Why do asymptomatic individuals eventually develop clinical malaria? We have observed this phenomenon in many instances. Particularly in Portuchuelo, it was commonly observed that individuals returning from visits to other settlements presented malaria. In some cases, as in the case of a microepidemic of clinical *P. falciparum* malaria reported above, there was a cluster of cases in a single family returning from elsewhere.

In Africa, it is known that it takes some years for an individual to be exposed to the *Plasmodium falciparum* strains of the area where he lives before becoming immune to clinical malaria. It is also known that whenever an "immune" individual becomes exposed to a new falciparum strain, he or she may develop clinical malaria.[28,29,39] If we consider isolated riverine communities as a microenvironment where only a few strains of the parasite circulate, we could hypothesize that the immunity could be achieved within a relatively short period of time and that immunity is area-specific and would not protect individuals against strains from other areas.

Answers to this and other questions can wait for future studies. What cannot wait are serious studies about alternative measures for the control of malaria in the Amazônia. Standard control measures, which have been successful elsewhere, have failed in the Amazônia.[2,5,7] Nowadays, treatment is the only way to keep malaria more or less under control in the Amazonian vastnesses. However, treatment is provided only to symptomatic patients, and these, according to our present findings, are a minority. Thus, it is reasonable to assume that symptomless but infected individuals could keep malaria spreading. On this basis, epidemiological studies of alternative control measures, which may even contemplate treatment of entire populations in remote, isolated areas, are urgently needed.

Acknowledgments: We thank Gerhard Wunderlich for helpful comments, the FUNASA team for excellent field assistance and for providing data from the municipality of Porto Velho, Mauro S. Tada for support at Porto Velho, and Roberto Penna A. Cunha for field assistance.

Financial support: This work was supported by Finep, Pronex/MCT, Fapesp, FUNASA, and CNPq.

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