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EPIDEMIOLOGY OF MALARIA IN A HYPOENDEMIC BRAZILIAN AMAZON MIGRANT POPULATION: A COHORT STUDY

ELISABETH CARMEN DUARTE, THERESA W. GYORKOS, LORRIN PANG, AND MICHAL ABRAHAMOWICZ

Special Program for Health Analysis, Pan American Health Organization, Washington, District of Columbia; Health Ministry, Fundação Nacional de Saúde/Centro de Epidemiologia, Cuiabá, Mato Grosso, Brazil; Universidade de Cuiabá, Cuiabá, Mato Grosso, Brazil; Department of Epidemiology and Biostatistics, McGill University, Montreal, Quebec, Canada; Division of Clinical Epidemiology, Montreal General Hospital, Montreal, Quebec, Canada; Walter Reed Army Institute, Washington, District of Columbia

Abstract. The present study describes aspects of the epidemiology of malaria in a migrant population living in a hypoendemic area in Brazil using an open cohort study design. Rural settlement residents in Leonislândia, Peixoto de Azevedo, Mato Grosso, Brazil were followed from September 1996 to April 1997. At baseline, an interview and malaria diagnoses were carried out and spleen size was measured. Incident cases were detected through follow-up visits and laboratory records. Cox regression was used to assess risk factors for time to malaria onset. Eighty percent (n = 414) of the study population (n = 521) contributed follow-up data. Overall, malaria prevalence during any study visit ranged from 0.3% to 5.4% and the malaria incidence rate (IR) was 4.49 (95% confidence interval = 3.66, 5.46) per 100 person-months. The IR of *Plasmodium vivax* malaria was approximately four times higher than the IR for *P. falciparum* malaria during follow-up. Among individuals who had had malaria during his or her lifetime, 14.03% reported hospitalization (median duration = 3 days) and 70.1% reported days of work lost (median duration = 4 days for *P. falciparum* malaria and 3 days for *P. vivax* malaria) related to the last malaria episode. No important risk factor was associated with the malaria IR. The fact that neither work-related factors nor age was associated with the risk of malaria indicates that indoor/peri-domiciliary transmission by the local vector is more important or as important as workplace-related transmission.

INTRODUCTION

Worldwide, more than 300 million cases of malaria occur every year. This disease remains a significant direct and indirect financial burden for both the affected family and for society due to costs of prevention, care, time costs, days of work lost, and premature deaths. Although approximately 90% of the world's malaria cases are reported from tropical Africa, malaria still claims more than 100,000 lives outside of Africa each year.[1] A total of 1.06 million new cases of malaria were reported in the Americas in 1997, and 393,000 (37%) of these cases were from Brazil.[2]

The political and administrative reorganization of the Brazilian Amazon Region during the 1970s has caused dramatic changes in malaria transmission, especially because of the existing optimal environmental conditions for malaria vectors and the massive introduction of new susceptible human populations. In 1999, approximately 631,000 malaria-positive blood smears were reported in Brazil, with approximately 99.7% of these originating from the Amazon region.[3]

Similar to other malaria-endemic countries, control measures in Brazil are based on early treatment of cases and different strategies for vector control that attempt as much as possible to be adaptive to the different presentations of the problem in different parts of the country.[4] These measures have also gradually been incorporated into general local health services within a national process of decentralization in the health sector. As in most malaria-endemic areas of Brazil, the endemic region of Mato Grosso State, one of the Brazilian Amazon States, is covered by a reasonable number of trained laboratory technicians distributed in several malaria laboratories. There are 140 malaria laboratories that provide a malaria diagnosis service for a population of 489,653 habitants. Diagnoses are free of charge and results are usually available a few hours after blood collection. For positive cases, full malaria treatment is also provided free of charge by the local malaria laboratory. Vector control measures include indoor insecticide spraying and outdoor spraying with non-residual insecticides for specific applications. Additional attention also focuses on health education, vector mapping, and studies of susceptibility to insecticides. Despite the morbidity and large numbers of malaria cases occurring each year in Mato Grosso State, the area is considered hypoendemic, as is most of the Brazilian Amazon endemic region (including the study site).[5,6]

A comprehensive description of malaria burden and risk factors in populations with a relatively reliable access to diagnosis and where malaria transmission is hypoendemic has not been adequately reviewed in Brazil. Therefore, the present study attempted to describe the occurrence and identify the risk factors related to malaria in a Brazilian migrant population to guide researchers and decision-makers in targeting intervention efforts. Moreover, the study may provide additional insight into the malaria transmission pattern in the study area.

MATERIALS AND METHODS

Study area. An open cohort design was carried out to address the study objective. This study was carried out in Leonislândia, a rural area of Peixoto de Azevedo City, in the Amazon region of Mato Grosso, Brazil. Peixoto de Azevedo is a city located at the northern frontier of Mato Grosso State, approximately 700 km north of Cuiabá, the state capital. It was founded in May 1986 after gold was discovered in the area, and during the 1980s this city was home to approximately 30,000 miners. Between the early 1980s and 1992, the price of gold dropped significantly and it became harder to extract from deeper veins, which increased the cost of mining.[7,8] As a consequence, miners had to be reallocated. Leonislândia was developed as a result of a settlement program initiated in 1993 to accommodate some of these ex-miners onto small farms, where they could live and work in agriculture. The Brazilian National Institute of Agrarian Reform officially recognized it as part of the countrywide agrarian reform in 1997. The designated settlement land was a large

uninhabited wilderness area located approximately 60 km from the center of Peixoto de Azevedo. In 1996, Leonislândia had approximately 4,000 inhabitants residing along nine parallel narrow roads, which are called travessôes. Homes were located very close to the forest itself, and, because proximity to a water source is considered essential, they would often be constructed close to rivers or streams. As soon as Leonislândia began to be occupied, malaria transmission was rapidly established as a consequence of the introduction of infectious human hosts. The main malaria vector in this area, as in most Amazon regions, is *Anopheles darlingi,* which is well known for being markedly anthropophilic.[9,10] There are two malaria laboratories in this area that are responsible for providing diagnostic services and complete treatment of malaria seven days a week, completely free of charge. Malaria diagnosis in this area follows the same standard procedures as defined by the Ministry of Health in Brazil (National Health Foundation). Malaria therapies were the following: chloroquine (25 mg/kg over a 2–3-day period; maximum adult dose = 1.5 g base over a three-day period) plus primaquine (0.25 mg/kg/day over a 14-day period; maximum adult dose = 2.1 g base over a 14-day period) for *P. vivax* malaria; mefloquine (15 mg/kg in a single dose; maximum adult dose = 1,000 mg in a single dose) for *P. falciparum* malaria; and mefloquine (15 mg/kg in a single dose; maximum adult dose = 1,000 mg in a single dose) plus primaquine (0.25 mg/kg/day over a 14-day period; maximum adult dose = 2.1 g base over a 14-day period) for mixed infections (*P. vivax* plus *P. falciparum*). Primaquine was not given to infants ≤ 6 months of age or pregnant women.[11]

Leonislândia was chosen as the study area because it had a relatively stable population of migrant individuals who previously had lived for different periods of time in different parts of the Amazon region.

Study population. Individuals of all ages living in two travessoes of Leonislandia were eligible to participate in the study. This comprised a population that fluctuated between 650 and 700 individuals during the study period. Parents or another adult from the family would answer interview questions on behalf of children if they were too young to do so.

Sample size and power calculation. Baseline assessments were obtained from 521 individuals distributed in 165 families. This number of subjects ensured a power of 90% to detect an absolute difference of approximately 15% in the prevalence of parasitemia (or other outcome of interest) among exposed and unexposed groups in any cross-sectional analysis. This sample size estimation was carried out assuming that the expected proportion of any event of interest among the unexposed group equaled 0.5 or 50%, and that the ratio of exposed to unexposed individuals in the study population equaled 1:3, i.e., approximately 25% are exposed. Some follow-up information was available for 414 individuals. It was assumed that between 20% and 25% of the study subjects would have at least one new malaria episode during the mean follow-up period of five months. Under these assumptions and using the method proposed by Lachin,[12] the study has excellent power (96% and 99%, depending on the frequency of outcomes) of detecting a strong effect (hazard ratio = 2.0) and an acceptable power (65–75%) of detecting a moderate effect (hazard ratio = 1.5) in survival analysis for a log rank test (equivalent to the score test in the Cox model) at the 0.05 significance level.

Data collection procedures. Three study visits were planned and carried out as follows: visit A in September, 1996, visit B in January 1997, and visit C, the last visit, in April 1997. During visits A and B, the research team was housed in schools in each travessâo. Visit C was carried out house-to-house.

Baseline data and procedures. Individuals could be enrolled into the study at any study visit (A, B, or C). For any new individuals entering the study at any visit, a detailed baseline interview was carried out. These individual interviews recorded information on demographic characteristics, socioeconomic characteristics, current use of individual protective measures against mosquito bites, current diseases, current medications taken, pregnancy, behaviors regarding hunting or fishing overnight, and travel and previous exposure to malaria. At the same time, spleen size was measured (as centimeters below the coastal margin in the mid-clavicular line in a supine position) by a trained physician, and blood samples were collected for malaria diagnosis.

Follow-up procedures. The study follow-up period spanned eight months from September 1996 (visit A) to April 1997 (visit C). To minimize loss to follow-up, houses with absent individuals were revisited as many times as possible during visit periods. Follow-up visits included malaria diagnosis and a short interview to collect information about malaria episodes and any malaria examination carried out since the last study visit. Moreover, all local government malaria laboratories (two in Leonislândia, two in Peixoto de Azevedo City and one in Matupá City) were monitored during the study period. The next nearest government malaria laboratory was located at least 120 km from the study area (Guarantã do Norte City). On study completion, the National Malaria database (SISMAL) system was searched to obtain supplementary laboratory data to complete the identification of malaria incident cases in the study population and to trace study individuals lost to follow-up. Most, if not all, malaria patients from the study area use government malaria laboratories for diagnostic investigation. This is because of their greater accessibility compared with any other alternative service, a preference confirmed in this study. Laboratory records and individual reports of malaria episodes were compared and the validity of self-reported malaria examinations are described elsewhere (Duarte EC, 1999. *Previous Exposure-Dependent Factors Related to Protection against Malaria in a Brazilian Amazon Migrant Population: An Open Cohort Study*. PhD thesis. Montreal, Quebec, Canada: McGill University).

Laboratory procedures for malaria diagnosis. Routine parasitologic examinations for malaria diagnosis were thick blood smears carried out on all study subjects at all study visits. In addition, for a person presenting with malaria symptoms, a ParaSight-F™ test (Becton Dickinson Advanced Diagnostics, Baltimore, MD) was also performed. Briefly, in the field, two thick blood smears were prepared on one slide and air-dried. In the laboratory, the smears were dehemoglobinized with methylene blue and stained with Giemsa. Well-trained laboratory technicians examined 100 high-power microscopic fields (approximately 0.20 mm^3 of blood) of the thick blood smear for identification and quantification of malaria parasites.[13] ParaSight F™ testing followed the manufacturer's instructions. A malaria positive examination result was defined based on the results of the thick blood smear test and taking into account the additional information provided by

the ParaSight-F™ tests when available for symptomatic individuals. ParaSight-F™ test for symptomatic individuals were carried out to rapidly deliver treatment of *P. falciparum* malaria cases.

Definitions of malaria outcomes. Two malaria outcomes were considered in this study: time to malaria onset and malaria frequency during follow-up.

Time to malaria onset. An incident malaria case was defined as an individual presenting at least one new episode identified by a positive malaria smear during the study follow-up. Three different sources of information were used to identify positive malaria smears: laboratory records (positive malaria smears at any government malaria laboratory during the study period, and recorded either on the laboratory form or identified in the SISMAL database); follow-up visit examinations (a positive malaria smear or a positive ParaSight-F™ test result at any follow-up visit); and individual reports (a positive malaria examination result self-reported by individuals at any follow-up interview during the study period). Therefore, time to malaria onset after baseline was defined as number of days without detectable malaria parasitemia after baseline (incident malaria case) up to the date of a new episode. Dates were recorded for all the following events occurring prior to or during follow-up: baseline interview, first *P. vivax* or first *P. falciparum* malaria episode after baseline, and last follow-up visit. If an individual did not have a malaria episode during the follow-up period or if an individual was lost to follow-up before experiencing any malaria episode, for the purpose of these analyses, this individual was censored at the time of his or her last follow-up visit. Therefore, the number of days between the baseline visit (time 0) and the time when he or she was censored (last follow-up visit) or the time when he or she had his or her first incident malaria episode was calculated for each individual and used as the dependent variable in the survival analysis. Moreover, if an individual presented with *P. falciparum* or *P. vivax* malaria at baseline, the first 30 and 15 days, respectively, were excluded from eligible time at risk for any malaria. This was necessary because based on selected pharmacokinetic parameters, it is expected that during this time period individuals were (completely or partially) not at risk for developing a new malaria episode due to antimalarial drug effects.

Malaria frequency during follow-up. A malaria episode in this study is defined as any positive malaria smear during study follow-up. The dates and examination results of all positive malaria smears carried out at any malaria laboratory or study visits during the study period were recorded and summed. A minimum period of seven days between two consecutive positive malaria smears for the same species of parasite was required to count the second positive smear as a new symptomatic episode. These episodes included new infections, relapses, and untreated malaria parasitemias, and are considered only as an indicator of the number of symptomatic episodes experienced during follow-up. To control for the duration of follow-up, malaria frequency was calculated as the number of malaria episodes experienced during follow-up divided by the number of days of follow-up.

Other variables related to the outcome of the last malaria episode were days of hospitalization, days of work lost (DWL) for adults (age $\geq$ 15 years old), and DWL over the last two years (based on DWL for the last malaria episode experienced multiplied by the number of malaria episodes reported for the last two years for each malaria species).

Potential risk factors. Variables selected as indicators of exposure to malaria in this study were season of follow-up, a time-dependent variable, categorized as 0 = the period from September 1 to October 31 (end of the dry season); 1 = the period from November 1 to December 31 (beginning of the rainy season); and 2 = the period from January 1 to April 30 (the rainy season). If an individual's follow-up period included more than one season, the value of this covariate changed accordingly and individual participation into the study was broken down by season; malaria (positive smear) at baseline; area of residence (travessâo 0 or II); skin color based on interviewer's assessment categorized nominally, and for analytic purposes categorized as 0 = white and 1 = non-white; formal education (continuous from 0 to $\geq$ 8th grade); monthly family income (applied to all family members); use of mosquito nets (or other protective measures); percent of time spent in more endemic areas (based on time spent out of the residential area and the specification of the area usually visited); hunting or fishing overnight; self-reported compliance with last malaria treatment; age in years; type of follow-up (categorized as 0 = follow-up through study visits; and 1 = follow-up through laboratory records).

Data analysis and statistical procedures. Data were analyzed using Epi-Info version 6.0[14] and STATA Statistical Software release 6.0.[15] Distributions of continuous variables were described using means and standard deviations when the distribution was close to normal or otherwise medians and first and third quartiles. Malaria prevalences and incidence rates (IRs) were estimated, and 95% confidence intervals (CIs) were calculated based on the exact binomial method (Fisher's 95% CI). Overall mean time to malaria onset after baseline was calculated, with the assumption of an exponential distribution of time-to-malaria (i.e., constant malaria hazard rate over time).[16]

Cox's proportional hazards regression was used to assess associations between risk factors and time to malaria onset after baseline.[16–18] Because inclusion of this time-dependent exposure, the season of follow-up, might create some degree of dependence between observations (clustering at the individual level), robust standard errors were used for all Cox regression models.

Multiple linear regression analyses were used to assess associations between risk factors and malaria episode frequency.[19] In this stage, analyses involved only incident cases (n = 101). This was done to prevent overrepresentation of the impact of the contrast between non-incident and incident cases on results, which would be a mere repetition of the analyses considering time to malaria onset after baseline as the outcome. Moreover, logarithmic transformations of the outcome (malaria frequency) using natural log (Y + 0.001) were performed to normalize the distribution of residuals.

The significance level for all hypothesis testing was set at $P \leq 0.05$. However, $0.05 < P \leq 0.10$ was considered marginally non-significant, especially when the sample size was less than 100.

Ethical considerations. This study received ethical approval from the Brazilian Ministry of Health, National Health Foundation and the Montreal General Hospital Ethics Review Committee. Study objectives, procedures, potential risks, and benefits were explained to all adult individuals and informed

consent from each individual was obtained. If the participant was a child, his or her parents or another adult from the family was asked to provide the consent. Treatment was provided for all malaria cases identified during the study.

RESULTS

Description of study population. The study population consisted of 521 individuals, of whom 33% and 67% were from travessâo 0 and II, respectively (Table 1). A total of 60.5% were male and 10.8% were five years old or younger, with a mean age of 26.3 years, ranging from < 1 to 70 years. The median monthly family income ($100.00 Reais or $98.45 U.S. dollars) was under the Brazilian minimum, which was $120.00 Reais ($118.14 U.S. dollars) in 1997. The majority of individuals more than seven years of age had attended school a mean of 2.6 years, and 21.2% never went to school. The two most reported past occupations were agriculture (83.8%) and mining (56.4%). Use of individual protective measures against mosquito bites was rarely reported, except for the use of regular work clothes. Hunting or fishing overnight was reported by 21.7% of adult males at baseline, with more than half of them (59.1%) undertaking these activities at least once every week. Only a very small proportion (7.0%) of individuals reported not having had experienced at least one episode of malaria during his or her lifetime. Proportions of individuals not reporting *P. vivax* (13.7%) or *P. falciparum* (14.8%) malaria during his or her lifetime were very similar. For the last two years, the median number of malaria episodes, when most of families were living in Leonislândia, was 3.0 episodes and 76.1% of individuals reported at least one malaria episode during the last two years.

Approximately 34.6% of the overall study population and 27.5% of the children between two and nine years old had palpable spleens (Table 1). A homogeneous pattern of behaviors regarding health care service use was observed in the study population. The great majority (98.7%) of individuals reported having had a malaria smear and had used government laboratories (Fundacao Nacional de Saude laboratories) for malaria treatment (85.4%) regarding the last malaria episode.

A common pattern of migration was noted among the study population (Table 2). Most (63.1%) individuals living in the study area were born in states of Brazil with very low or no malaria transmission (Maranhão = 30.2% and other Brazilian non-Amazon States = 32.9%). Only approximately one-quarter (27.8%) of the study population, mostly children (mean ± SD age = 8.3 ± 7.0 years) had been born in Mato Grosso State, after their parents' migration. The study population reported a mean time living in malaria endemic areas of approximately 11 years (135.1 months), and only a small proportion (15.2%) had lived in these areas for less than five years. They had lived approximately three and a half years (42.7 months) in mining environments, and a large variability around this value was noted (SD = 5.7 years or 69.0 months). A total of 69.3% had lived in mining areas for less than five years. Once they were settled in Leonislândia, most individuals rarely spent time out of their residential area (median percentage time spent out of residential area = 0.0%).

Overall, individuals living in travessâo 0 had indicators of a more recent malaria experience and a slightly worse health condition than those living in travessâo II. Travessâo 0 is closer to a river and had a more recent migration and, consequently, less deforestation than travessâo II.

Of 521 individuals enrolled in the study, 79.5% (n = 414) had both a baseline visit and some type of follow-up (n = 380, baseline and at least one follow-up visit with or without laboratory records; n = 34, baseline and laboratory records) and 20.5% had a baseline visit but no follow-up.

The prevalence and incidence of malaria during the study

TABLE 1

Description of characteristics reported by the study population at baseline (n = 521) in Peixoto de Azevedo, Brazil, September 1996 to April 1997

Variable*	Value†
Area of residency	
Travessao 0	33.0%
Travessao II	67.0%
Males	60.5%
Age, years	26.3 ± 18.0
Monthly family income (Reais)‡	100.0 (50–200)
< 120 Reais	62.6%
Years attending school§	2.6 ± 2.5
Occupation¶	
Agriculture	83.8%
Mining	56.4%
Use of protective measures against mosquito bites	
Long-sleeved shirts and long pants	41.0%
Mosquito nets	10.8%
Skin repellent oils	5.8%
Hunting or fishing overnight¶#	21.7%
Malaria episodes	
None	7.0%
None caused by *Plasmodium vivax*	13.7%
None caused by *P. falciparum*	14.8%
Spleen size > 0 cm	
Total population	34.0%
Children 2–9 years old	27.5%
Reported positive blood smear during last clinical episode of malaria**	98.7%
Service used for last malaria treatment**††	
FUNASA Laboratories††‡‡	85.4%

* The proportion of missing data for all variables ranged from 0.0% to 14.0%.
† Continuous variables are summarized as the mean ± SD or median (first–third quartiles).
‡ Monthly family income was applied to all members of the family. $1 = 1.057 Reais on September 2, 1996.
§ Excluded individuals less than seven years old (n = 76).
¶ Excluded individuals less than 15 years old (n = 199).
# Excluded women (n = 206).
** Excluded individuals reporting no malaria episodes (n = 33).
†† Excluded individuals reporting no malaria treatment for the last malaria episode (n = 3).
‡‡ FUNASA Laboratories = Health Ministry, National Health Foundation.

TABLE 2

Description of migrational patterns reported by the study population at baseline (n = 521) in Peixoto de Azevedo, Brazil, September 1996 to April 1997

Variable*	Value†
State of birth	
Mato Grosso	27.8%
Maranhâo	30.2%
Other Amazon States	9.1%
Other non-Amazon States	32.9%
Months lived in endemic environments	135.1 ± 87.5
Months lived in mining environments	42.7 ± 69.0
% of time spent outside residential area	0.0 (0–5)
% never out	71.5

* The proportion of missing data for all variables ranged from 0.0% to 10.0%.
† Continuous variables are summarized as the mean ± SD or median (first–third quartiles).

period is shown in Tables 3 and 4, respectively. Overall, among 1,067 blood smears examined during study visits, 32 cases of malaria parasitemia were identified: 16 of *P. vivax*, 14 of *P. falciparum*, and 2 mixed (*P. vivax* plus *P. falciparum*) malaria parasitemia. Sixteen malaria-prevalent cases were identified at visit A, 15 at visit B and 1 at visit C. Malaria prevalence during any study visit was relatively low (ranging from 0.3% at visit C to 5.4% at visit B). *Plasmodium vivax* malaria was twice as frequent as *P. falciparum* malaria during visit A, but its relative occurrence reversed to a ratio of 1:1.7 at visit B. During visit C, only one *P. vivax* malaria case was identified.

No deaths related to malaria were identified in the study population during the study period. A total of 101 malaria incident cases were identified during follow-up. Overall, the malaria IR was 4.49 (95% CI = 3.66, 5.46) per 100 person-months (Table 4). This rate became only slightly lower (4.07 per 100 person-months) when individuals not having at least one follow-up visit were excluded (i.e., individuals followed only with laboratory records). The observed malaria rate during follow-up (4.49 per 100 person-months = 0.54 per person-year or 1.08 per person every two years) agrees very closely with the questionnaire results regarding the number of malaria episodes reported in the previous two years (76.1%).

The *P. vivax* malaria IR was approximately four times higher than the *P. falciparum* IR during follow-up, and individuals living in travessâo 0 had higher malaria IR than those living in travessão II. Among the 101 incident cases, 151 malaria episodes (positive smear) during follow-up (2,247.93 person-months) were observed. The malaria-positive smear rate was 6.72 (95% CI = 5.69, 7.88) per 100 person-months. Assuming an exponential distribution of time-to-malaria, the overall mean time for malaria onset after baseline was calculated as 22.3 months.

Days of work lost and hospitalization related to malaria episodes prior to baseline are shown in Table 5. A proportion of 14.03% who had had malaria episodes during his or her lifetime reported hospitalization for the last malaria treatment during the last malaria episode with a median duration of three days. The majority (75.4%) of hospitalized patients needed no more than four days of hospital stay. However, 70.1% of adult individuals (≥ 15 years of age) who had had malaria during his or her lifetime reported one or more days of work lost (DWL, median = three days). As expected, the median DWL for *P. falciparum* malaria (4.0 days) was twice the median DWL for *P. vivax* malaria (2.0 days). In fact, using univariate linear regression, we noted that the last *P. falciparum* malaria episode was related to a more severe malaria episode than the last *P. vivax* malaria episode, as reflected by higher mean parasitemia levels ($\beta = 0.315$, $P < 0.005$), more days of work lost ($\beta = 2.69$, $P = 0.059$), and more days of hospitalization reported ($\beta = 0.725$, $P < 0.005$).

TABLE 4

Occurrence of malaria during follow-up in Peixoto de Azevedo, Brazil, September 1996 to April 1997 (n = 414)*

Travessâo	Category	n	Incidence rate per 100 person-months (95% Fisher's confidence interval)
Total	Person-months	2,247.93	– –
	Any malaria case	101	4.49 (3.66, 5.46)
	P. falciparum	22	0.98 (0.61, 1.48)
	P. vivax	88	3.91 (3.14, 4.82)
0	Person-months	783.63	– –
	Any malaria case	44	5.62 (4.08, 7.54)
	P. falciparum	17	2.17 (1.26, 3.47)
	P. vivax	33	4.21 (2.90, 5.91)
II	Person-months	1,464.30	– –
	Any malaria case	57	3.89 (2.95, 5.04)
	P. falciparum	5	0.34 (0.11, 0.80)
	P. vivax	55	3.76 (2.83, 4.89)

* Individuals who had a baseline visit and at least one follow-up visit (n = 380) or a baseline visit and at least one laboratory record during the study period (n = 34).

If children (n = 199) and individuals who did not know the number of malaria episodes per species experienced over the last two years (n = 39) were excluded, 208 (73.5%) of 283 individuals reported at least one malaria episode during the two years prior to baseline (Table 5). A total of 1,254 malaria episodes (median = 3.0, mean of 4.4 episodes per person) were reported and a ratio close to 1:1 for episodes of *P. vivax* (638 episodes) and *P. falciparum* (616 episodes) was identified. Assuming that the same median DWL applies to each malaria episode, 3,762 DWLs were attributed to malaria among the adult population during the two years prior to baseline. Overall mean DWLs due to malaria episodes (any species) over a period of two years per adult patient of at least 18 days were estimated. For an adult reporting *P. falciparum* or *P. vivax* malaria episodes over the last two-year period, the estimated mean DWL was at least 14.5 days and 7.6 days, respectively. Since immunity to malaria is known to develop over time under constant exposure and, as a consequence, symptoms are expected to decrease over time, the estimates based on the last malaria episode are likely quite conservative and should be considered the lower boundary of the mean DWL due to malaria episodes in the study population.

Risk factors for malaria occurrence. Using the Cox regression model and considering time to malaria onset as the outcome, we found that only the type of follow-up was significantly associated with the malaria incidence rate (hazard ratio = 4.048, 95% CI = 1.870, 8.766). Individuals identified only

TABLE 3

Description of malaria prevalence in the study population during study visits in Peixoto de Azevedo, Brazil, September 1996 to April 1997

Variable	Prevalence (95% CI)*		
	Visit A† Sep 1996 (n = 400)	Visit B† Jan 1997 (n = 279)	Visit C Apr 1997 (n = 388)
Any species	4.0%(2.3,6.4%)	5.4%(3.0,8.7%)	0.3% (0.0, 1.4%)
Plasmodium vivax	2.7%(1.4,4.9%)	2.2%(0.8,4.6%)	0.3% (0.0, 1.4%)
P. falciparum	1.5%(0.5,3.2%)	3.6%(1.7,6.5%)	0.0% (undetermined)
P. vivax:*P. falciparum*	1:0.5	1:1.7	1:0

* CI = confidence interval (exact binomial). Visit A = September 1996 (end of the dry season; visit B = January 1997 (rainy season); visit C = April 1997.
† Two cases of mixed (*P. vivax* plus *P. falciparum*) malaria infections were identified: one during visit A and one during visit B. They were included separately for each malaria parasite species.

TABLE 5

Days of work lost and hospitalization related to malaria in the study population prior to baseline in Peixoto de Azevedo, Brazil, September 1996 to April 1997

Variable	n*	Value†
Reported hospitalization due to last malaria episode‡	488	14.03%, 3 (3–4)§
Reported days of work lost due to last malaria episode‡¶	289	70.1%
Both species		3 (0–7)
Plasmodium vivax		2 (0–5)
P. falciparum		4 (1–8)
Reported a malaria episode in the last two years#	283	73.5% (n = 208)
Total episodes		1,254
P. vivax		638
P. falciparum		616
Estimated number of days of work lost due to malaria in the last two years#	208	
Total days lost		3,762
Days lost per person		18.1
Estimated number of days of work lost due to *P. vivax* in the last two years#	168	
Total days lost		1,276
Days lost per person		7.6
Estimated number of days of work lost due to *P. falciparum* in the last two years#	170	
Total days lost		2,464
Days lost per person		14.5

* The proportion of missing data ranged from 0% to 13.4%.

† Continuous variables are summarized as the median (first–third quartiles). Medians were used because variable distributions were markedly skewed (skewness = 3.0 and kurtosis = 10).

‡ Excluded individuals reporting no malaria episode (n = 33).

§ Excluded individuals who reported no hospitalization for the last malaria episode (n = 386).

¶ Excluded individuals less than 15 years old (n = 199).

The sample size (n = 283) was kept constant by excluding individuals less than 15 years old (n = 199) and those who did not know the number of malaria episodes per species in the last two years. Medians of days of work lost reported for the last malaria episode for each parasite species before baseline were multiplied by the number of reported malaria episodes in the last two years to estimate the total number of days of work lost in the last two years for each parasite species; 168 had *P. vivax* malaria and 170 had *P. falciparum* malaria in the last two years.

through laboratory records during follow-up had a malaria rate four times higher than individuals followed through study visits. All other potential risk factors were not statistically associated with malaria rates in this study population.

When malaria episode frequency (log-transformed) was considered as the outcome in the multi-linear regression model, only two variables were found to be statistically significant: type of follow-up and presence of malaria at baseline. Again, individuals followed only through laboratory records showed a frequency of malaria episodes per follow-up day ($e^{-5.025 + 2.227} = 0.0609$), which is 9.23 times higher than those followed through follow-up visits and laboratory records ($e^{-5.025} = 0.0066$) after adjusting for presence of malaria at baseline. Moreover, individuals presenting malaria at baseline (prevalent cases) had a lower malaria episode frequency during follow-up ($e^{-5.025-0.935} = 0.0026$) than those malaria-free at baseline ($e^{-5.025} = 0.0066$) after controlling for type of follow-up. These two variables together (type of follow-up and malaria at baseline) explained an important portion of the variability of the log-transformed malaria episode frequencies ($R^2 = 51.92\%$) among incident cases in this study.

DISCUSSION

Surprisingly, studies of risk and risk factors for malaria in Amazon Brazilian populations are relatively few (Duarte EC, 1999. *Previous Exposure-Dependent Factors Related to Protection against Malaria in a Brazilian Amazon Migrant Population: An Open Cohort Study*. PhD thesis. Montreal, Quebec, Canada: McGill University).[20–22] The present study provided an opportunity to examine the epidemiology of malaria among migrants living in a hypoendemic area of the Brazilian Amazon and to quantify the rate and risk factors of malaria in this situation.

Most (93%) of the study population had been previously exposed to malaria and most (76.1%) of them had also had a recent malaria episode (over the previous two years). The parasite (*P. falciparum* plus *P. vivax*) prevalence proportion was 3.00% (95% CI = 2.06, 4.21) and the malaria incidence rate and incidence density (malaria positive smear rate) were 4.49 (95% CI = 3.66, 5.46) and 6.72 (95 % CI = 5.69, 7.88) per 100 person-months of follow-up, respectively. Regardless of the high morbidity that can be attributed to malaria among residents in the study area, these indicators are low compared with those observed in most of the malaria-endemic areas of the world.[1,4,23] Even in Brazil in gold mining camps of Peixoto de Azevedo (Mato Grosso State), higher malaria parasite prevalences have been detected, for example, 20.5% by de Andrade and others.[24] In Colombia, in the village of Punta Soldado (Valle del Cauca), Terrientes and others identified a *P. falciparum* prevalence of 33.1%.[25] Similarly to our results, in Rondonia, another Brazilian hypoendemic Amazon State, prevalences between 0.5% and 4.2% have been documented.[20] Because, mathematically, prevalence is directly proportional to the incidence rate and/or the duration of the disease, the relatively low parasite prevalence observed in our study population may be a result of the low transmission profile in the area, or a short disease duration. Hypothetically, a short duration of an episode of malaria could be related to rapid diagnosis and treatment due to presence of symptoms and adequate coverage of malaria laboratories for rapid diagnosis and effective treatment. Moreover, the stability of agricultural workers compared with miners is expected to be an important advantage for malaria control interventions and case management. These are assumptions that should be addressed in future studies. In addition, the research team introduced mefloquine as the treatment of choice for *P. falciparum* malaria, replacing the government standard protocol of quinine plus tetracycline. Because *in vivo* resistance to mefloquine is considered absent in the study area and a single-dose treatment would ensure compliance, it was anticipated that this intervention would have an impact on *P. falciparum* transmission.[26] However, because this study was not designed to assess the impact of this or any intervention, a direct conclusion without assessing other determinants may be misleading.

Malaria infection in the study population is known as an acute event. Consequently, despite an active surveillance during the study period with visits every four months, incident malaria cases were mostly identified through routine laboratory examinations between study visits (passive surveillance). Again, this highlights the fact that the majority of malaria cases occurring in the study area were symptomatic. A wide range of prevalences of asymptomatic malaria in malaria-

endemic areas in Brazil has been previously documented. In a cross-sectional parasitologic, clinical, and sociodemographic survey, Camargo and others described the absence of asymptomatic malaria cases among individuals living in a hypoendemic village in Rondonia, Brazil (Candeias do Jamary).[21] However, a study of clinical immunity among gold miners living in an endemic rural area in Apiacás (Mato Grosso State) documented a prevalence of asymptomatic malaria of 7.2% (38 of 527) in the overall population and 43% among individuals with a positive smear (38 of 91) (Fontes CJF, 2001. *Epidemiologia da Malaria e Fatores Associados a Malaria Assintomatica por Plasmodio entre Garimpeiros da Amazonia Matogrossensse*. PhD thesis. Minas Gerais, Brazil: Minas Gerais Federal University).

In the study area, our findings support a low or null proportion of asymptomatic malaria infection and risk of malaria infection among all ages. This provides convincing evidence that the population's history of exposure, approximately one malaria episode every two years, was apparently not sufficient to fully develop naturally acquired immunity at any age.

As mentioned, based on the classification adopted by the World Health Organization and reported by Gilles, malaria transmission in most endemic areas in Brazil is usually considered hypoendemic.[5] In fact, evidence of hypoendemicity was found in this study: there was no mortality directly related to malaria during the follow-up period and the parasite prevalence proportion was less than 10% ($\leq$ 5%), the limit for hypoendemicity. However, the spleen rate among 2–9-year-old children exceeded the limit of 10% (27.5%). Camargo and others reported that in the hypoendemic State of Rondonia (Brazilian Amazon) the spleen rate in this age group was always less than 1%.[20] The high proportion of children presenting palpable spleens in our study area was probably not attributable to malaria alone. Other diseases frequently identified in this population may explain these findings, such as hepatitis B and iron deficiency anemia (due to intestinal parasitoses), among others.

In the World Health Organization classification of malaria transmission levels, it is mentioned that in hypoendemic areas malaria is an unimportant public health problem.[5] However, although malaria presents as a hypoendemic level of transmission in our study population, effects of malaria probably cannot be considered unimportant: during the last malaria episode prior to baseline, 14% of individuals had to be hospitalized, and the median of hospital stay was three days. Moreover, the cumulative DWL due to malaria episodes during the last two years was estimated to be 18.1 days per patient reporting at least one malaria episode during this time period. Besides the obvious implications on quality of life, hospital stay and days of work lost implies costs and may jeopardize the family income. These findings suggest that, regardless of its hypoendemicity, malaria remains an important public health problem and a relevant burden for the population living in this area.

Surprisingly, no important risk or protective factors were found associated with time to malaria onset after baseline or malaria episode frequency, except for type of follow-up and the presence of malaria at baseline. Individuals identified only through laboratory records during follow-up had a malaria rate higher than individuals followed through study visits. This was because some individuals who were lost to follow-up could have had a subsequent malaria laboratory examination sometime during study follow-up. Therefore, these individuals were tracked only though their laboratory records, which inflated positive cases among this presumably symptomatic population sub-group. Thus, a feature of the study design artificially generated the association between type of follow-up and malaria rate. Because this limitation was anticipated, the variable type of follow-up was present in all regression models to adjust for this confounder factor generated by a selection bias.

Another variable associated with risk of malaria in this study was prevalent cases. The fact that prevalent cases at baseline had a lower malaria episode frequency during follow-up than those malaria-free at baseline is probably due to protective effects of treatment given to those with malaria (on its re-occurrence after baseline).

The lack of other associations between malaria outcomes and expected risk factors, such as no use of mosquito nets, percent of time spent in more endemic areas, hunting or fishing overnight or some socioeconomic indicators, may be at least in part attributed to a low variability of these characteristics in the study population. A similar conclusion was reported by Snow and March in their review of the epidemiology of malaria relevant for disease control.[27] In addition, in a cross-sectional survey carried out in 1992 in a community of low endemicity in The Philippines, Lansang and others found that among 11 demographic and socioeconomic factors examined, only two (immunofluorescent antibody–based scores of the residence area and frequent nocturnal visits to the forest) were identified as associated with parasite prevalences.[28]

Our results cannot advance the observation of protective factors for malaria due to the homogeneity of our study population, especially with respect to preventive behaviors. For example, only a very small proportion of the study population reported the use of bed nets (10.8%) or use of some type of skin repellent against mosquito bites (5.8%). Bloland and others studying pregnant women living in western Kenya, where malaria is holoendemic, also reported that use of anti-mosquito measures was rare: 8.5% reported using a bed net regularly and 2.7% reported using an insecticide spray.[23]

Camargo and others reported that in an unstable hypoendemic malaria area of Rondonia State in Brazil, malaria affects especially the adult male population, pointing out the place of work as the area of higher transmission.[20] In our study population, the fact that age was not statistically associated with malaria outcomes indicates that indoor or peridomiciliary transmission of malaria is more important or as important as workplace-related transmission. These observations indicate the need for specific local interventions, and may guide the selection of effective malaria control measures for this population.

During the study follow-up, healthier individuals probably were the most likely to be out of the study area for work-related reasons, and malaria occurrence may be slightly overestimated. However, the number of individuals lost to follow-up was not exceptionally large (20.5%). Although malaria transmission in Brazil is present during the entire year, it may moderately increase during the beginning and end of the rainy season. The study follow-up spans eight months that include the end of the dry season and most of the months of the rainy season. The period from May to August was not included in this study, which may have excluded some seasonal variation of low malaria transmission, and may have

slightly inflated estimations of malaria rates and prevalences. Therefore, study results should not be generalized to the entire year. Because selection bias was identified in this study due to follow-up type, all multivariable models assessing associations with malaria outcomes during the study period were adjusted accordingly. Because some study variables, such as those regarding days of work lost and malaria episodes during lifetime, rely on individual recall, a certain degree of recall error may be present in this study. However, previous studies have demonstrated a high validity of self-reported malaria examinations by the study population.[13,29]

This study provided relevant information on the epidemiology of malaria in a hypoendemic Brazilian Amazon setting. It describes the sociodemographic characteristics of the individuals affected by malaria, their history of previous exposure and migration, present behaviors, and patterns of use of the malaria services, as well as hypotheses of the type of transmission expected to be more relevant in the area. Moreover, the study findings support the hypothesis that malaria transmission in this area was not sufficient to fully develop naturally acquired immunity against clinical manifestations. This information may help guide decision makers to specify effective control measures targeted to this population and transmission situation. Moreover, it provides measurable evidence of the burden of malaria in this area, especially regarding the days of work lost and days of hospital stay, which may influence political commitment with regard to this relevant public health problem. In addition, for researchers in particular, these results may be useful in guiding them in the choice of study variables and in providing background data and estimates for future studies in similar settings.

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Authors' addresses: Elisabeth Carmen Duarte, Pan American Health Organization, 525 23rd Street NW, Washington, DC 20037, Telephone: 202-974-3481, Fax: 202-974-3674, E-mail: eduarte@terra.com.br. Theresa W. Gyorkos, Division of Clinical Epidemiology, Montreal General Hospital, 1650 Cedar Avenue, Montreal, Quebec, Canada H3G 1A4, Telephone: 514-934-1934 extension 44721, Fax: 514-934-8293, E-mail: theresa.gyorkos@mcgill.ca. Lorrin Pang, Maui County Hawaii Department of Health, 54 High Street, Room 301, Wailuku, HI 96793, Telephone: 808-984-8200, Fax: 808-984-8222, E-mail: panghil@mauigateway.com. Michal Abrahamowicz: Division of Clinical Epidemiology, Montreal General Hospital, 1650 Cedar Avenue, Montreal, Quebec, Canada H3G 1A4, Telephone: 514-934-1934 extension 44712, Fax: 514-934-8293, E-mail: michal.abrahamowicz@mcgill.ca.

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