Factors associated with recurrent *Plasmodium vivax* malaria in Porto Velho, Rondônia State, Brazil, 2009

Fatores associados às recidivas de malária causada por *Plasmodium vivax* no Município de Porto Velho, Rondônia, Brasil, 2009

Factores asociados a las recidivas de malaria, causada por *Plasmodium vivax*, en el municipio de Porto Velho, Rondônia, Brasil, 2009

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Abstract

This open retrospective cohort study aimed to describe the incidence of recurrent Plasmodium vivax *malaria and associated factors in Porto Velho, Rondônia State, Brazil, in 2009. Data were collected from the National Information System for Malaria Epidemiological Surveillance. There were 23,365 reported* P. vivax *malaria cases in 2009, 23% of which were classified as relapses. Incidence density of* P. vivax *recurrence was 45.1/100 patient-years, mostly occurring between the 4th and 13th week after initiating treatment. Male gender, shorter time since onset of symptoms, and higher parasitemia in the initial infection increased the risk of relapse during the year, with a 10% reduction in relative risk for longer symptoms and 11% and 15% increases in relative risk for males and higher initial parasitemia, respectively. However, the results show low clinical relevance for these associations, thereby limiting their applicability to decision-making at the public health level.*

Recurrence; Plasmodium vivax; *Malaria*

Resumo

Estudo de coorte histórica aberta, construída com informações registradas no Sistema de Informação de Vigilância Epidemiológica da Malária, com objetivo de descrever a incidência e os fatores associados à recidiva de malária causada pelo Plasmodium vivax *em Porto Velho, Rondônia, Brasil. Foram notificados 23.365 casos de malária por* P. vivax *no município, sendo que 23% deles apresentaram recidiva no decorrer de 2009. A densidade de incidência de recidivas foi de 45,1/100 pacientes-ano, ocorrendo, principalmente, entre a 4ª e a 13ª semana após o início do tratamento. Ser homem, ter menos tempo de sintomas e ter maior nível de parasitemia na infecção inicial aumentaram o risco de recidiva no decorrer do ano, com 10% de redução relativa do risco para maior tempo de sintomas e de 11% e 15% de incremento relativo do risco para o sexo masculino e maiores níveis de parasitemia, respectivamente. Contudo, os resultados demonstram baixa relevância clínica das associações encontradas, comprometendo a sua aplicabilidade na tomada de decisão em nível de saúde pública.*

Recidiva; Plasmodium vivax; *Malária*

Introduction

The Brazilian Ministry of Health recorded 293,794 cases of malaria in the country in 2011, 99.9% of which in the Legal Amazonia. Of this total, 257,172 (87.5%) were caused by *Plasmodium vivax*, and the highest incidence rates were in the States of Pará (44.5%), Amazonas (21.8%), and Rondônia (12.8%). Rondônia had 42,761 malaria cases in the year 2010, representing 13.1% of all cases reported in Brazil that year. *P. vivax* was the most prevalent species in the region, with 89% of cases, followed by *Plasmodium falciparum* with 10.1% and less than 1% of mixed infections. Annual malaria incidence in the Municipality (County) of Porto Velho has reached high levels, ranging from 20 to 400 cases per 1,000 inhabitants [1].

Recurrent malaria is defined as recurrent asexual parasitemia following treatment of the disease, after having observed negative parasitemia during a varied period of time [2]. Recurrence is due to one of the following: (a) therapeutic failure resulting from non-adherence to treatment [3], resistance of the parasite to the drugs used [4], poor quality of the medication [5], or sub-therapeutic doses of the drugs [4]; (b) reactivation of hypnozoites [2]; and (c) exposure to new infection by the mosquito vector. Conceptually, recurrent malaria can result from recrudescence, relapse, or reinfection.

Recrudescence of malaria occurs when the bloodstream forms of the parasite are not completely eradicated by treatment and re-expand in number after a decline in serum concentration of the drugs. This occurs more frequently in malaria from *P. falciparum* and *P. vivax* and rarely with *P. malariae* [6].

Relapse is defined as resurgence of parasitemia and clinical manifestations due to reinvasion of the erythrocytes by merozoites from dormant hypnozoites in the liver. Relapses are believed to occur 21 to 140 days after treatment of the tropical strain and 180 to 420 days after treatment of the temperate strain [2]. The main cause is treatment failure. Based on clinical observation alone, it is very difficult to distinguish recrudescence from relapse or relapse from reinfection. In some situations the distinction can be made by identifying a parasite genotype in the relapse which is identical to that of the primary infection. Still, genotyping parasites from the initial infection and relapse has not proven efficient in distinguishing between relapse and reinfection [6,7,8,9,10,11].

Reinfection with *P. vivax* is a new infection acquired by patients treated for malaria after a time interval inconsistent with recrudescence or relapse. In genotyping, reinfection can be defined

by finding a parasite that is genetically different from the one that caused the primary infection [12]. Since the intervals between episodes overlap, even with genotyping it is also difficult to distinguish precisely between relapse and reinfection.

Studies have agreed that up to 28 days, when there is still drug circulating after treatment, recurrences can be considered recrudescence. From 29 days (or even earlier in the case of resistant parasites) up to six months, recurrences can be considered relapses in the case of the tropical strain. Meanwhile, reinfections can occur at any moment after elimination of the drug from the system [2,13].

The Brazilian National Malaria Control Program (PNCM) conducts systematic investigation of recurrences by monitoring parasitemia at various intervals after the conclusion of chemotherapy. The PNCM defines recrudescence, probable relapse, and reinfection with *P. vivax* as the resurgence of parasitemia at intervals of 7 to 28 days, 29-60 days, and greater than 60 days, respectively, after initiating treatment of the primary infection. These cutoff points are arbitrary and can result in erroneous patient classification [14].

Due to the operational difficulty in distinguishing relapses from reinfections, the current study aimed to describe and analyze all recurrences of *P. vivax* malaria in an endemic municipality (county) in the Brazilian Amazon, in order to better understand the phenomenon of repeated illness in the population due to this parasitic infection and to promote discussion of its impact on the prevailing control strategy.

Methodology

This was an open historical cohort study constructed from secondary data on malaria and its recurrences from January to December 2009 in the Municipality of Porto Velho, Rondônia, Brazil. The data were obtained from the National Information System on Malaria Epidemiological Surveillance (SIVEP-Malaria). The databank was first organized in Excel (Microsoft Corp., USA) and classified in alphabetical order by patient's name, mother's name, date of birth, and date of notification to ensure identification of recurrences. Duplicate notification was defined as the entry of data for the same patient on the same date of diagnosis or treatment for malaria, and was then excluded from the databank. Recurrent cases were defined as patients present in the databank with different diagnostic and treatment dates and which showed agreement between the patient's name, mother's name, and date of birth. Only those which showed agreement between

at least three of these four variables were classified as recurrent cases. This stage of the study was completely manual and conducted entirely by the study's principal investigator.

For the analysis proposed in the study, the data were subsequently exported to Epidata Analysis, version 2.2.1.171 (Epidata Assoc., Odense, Denmark) and Stata, version 6.0 (Stata Corp., College Station, USA). Stratification of recurrent cases into different types used the cutoff points for time elapsed since last malaria treatment, according to PNCM guidelines [9]. Incidence of recurrences and respective 95% confidence intervals (95%CI) during the target year were analyzed in relation to demographic (gender, age, and schooling) and clinical variables (time since onset of symptoms and parasitemia level in the primary infection), drawing this available data from SIVEP-Malaria. Determination of individual exposure time to the target event was based on the number of days elapsed since the first episode of the disease in 2009 and the first recurrence. The sum of these times calculated for each individual represented the person-time sampling universe, used to calculate the incidence density of recurrences. Thus, the time counted for patients that presented recurrent malaria during the year referred only to the date on which the patient entered the system for the first time, i.e., at the first malaria episode during the study year and the number of times they returned to the system.

Statistical analysis began with estimation of simple frequencies for the target variables. Demographic, clinical, and laboratory factors associated with incidence density of recurrences were identified by univariate analysis, determining relative risk (RR) and respective 95%CI. Proportions between groups were compared using Pearson chi-square (χ^2) or Z-score. Means were compared using the Student *t*-test with the respective descriptive levels (p-value). The effect of the accumulated number of repeated recurrences in the same individual during the time elapsed between each episode of recurrence was analyzed by simple linear regression. Significance was set at 5% for all the conclusions on the analysis of factors associated with recurrence.

A comparative evaluation of the incidence densities of recurrences between groups used survival analysis, constructing overall and group survival function curves with the Kaplan-Meier product estimator. In this analysis the accumulated risk of an individual presenting a recurrence over time after treatment of the initial symptomatic infection assumed independence of the event's occurrence between the different moments in time. Comparison of the survival curves without recurrence, constructed for the

subgroups, used the Mantel-Haenszel log-rank test. Finally, the Cox proportional risks model was used to estimate the effect of the study's covariates on time until recurrence.

Access to the SIVEP was authorized by the PNCM, respecting non-disclosure of the patients' identity. The study was approved by the Ethics Research Committee at the Júlio Muller University Hospital, Federal University of Mato Grosso (case review 600/CEP/HUJM/09, March 18, 2009).

Results

During the year 2009, 26,296 patients with malaria were notified in the Municipality of Porto Velho. Analysis of the information recorded in SIVEP-Malaria showed duplicate notification of 290 patients, which were excluded from the database constructed in this study. Thus, 26,006 patients were analyzed, of whom 23,365 (89.8%) were treated for malaria caused by *P. vivax*, 2,580 (9.9%) for *P. falciparum*, and 61 (0.2%) for mixed infection, that is, simultaneous infection with *P. vivax* and *P. falciparum*. The majority were males (64.0%), young adults from 20 to 49 years of ages (57.4%), with low schooling (79.2%), and living in the Municipality of Porto Velho (94.1%). Malaria transmission was relatively stable in 2009, with a slight increase in *P. vivax* cases in July and a drop from August to December (Figure 1). The chemotherapy regimen based on chloroquine + primaquine, recommended by the Brazilian Ministry of Health, was used to treat all patients with *P. vivax* malaria.

Of the 26,006 patients included in study, 5,958 (22.9%) presented recurrences, of which 5,363 (90%) were caused by *P. vivax*; 572 (9.6%) by *P. falciparum*; and 23 (0.4%) by mixed infection. During the year 2009, multiple recurrences (as many as eleven) were observed in various patients and for both *Plasmodium* species. For *P. vivax*, recurrences occurred as follows: one only in 67% of the patients, two in 21.5%, three in 7.4%, and four or more in 4.1%. The most frequent type of recurrence in 2009 was probable reinfection with *P. vivax*, representing 65.3% of the recurrent cases. Probable relapses occurred in 1,760 patients (29.5%) and recrudescence in 279 (5.2%) (Table 1).

P. vivax recurrences were proportionally more frequent in males (64.7%), in individuals from 12 to 49 years of age (73.1%), and in those with low schooling (88.9%). Mean age of patients with recurrence was 26.8 years (SD: 15.7). The most frequent level of parasitemia at diagnosis was 100 to 500 parasites/mm³. Among the 1,018 childbear-

Figure 1

Monthly distribution of number of patients with diagnosis of *Plasmodium vivax* and *Plasmodium falciparum* malaria. Municipality of Porto Velho, Rondônia State, Brazil, 2009.
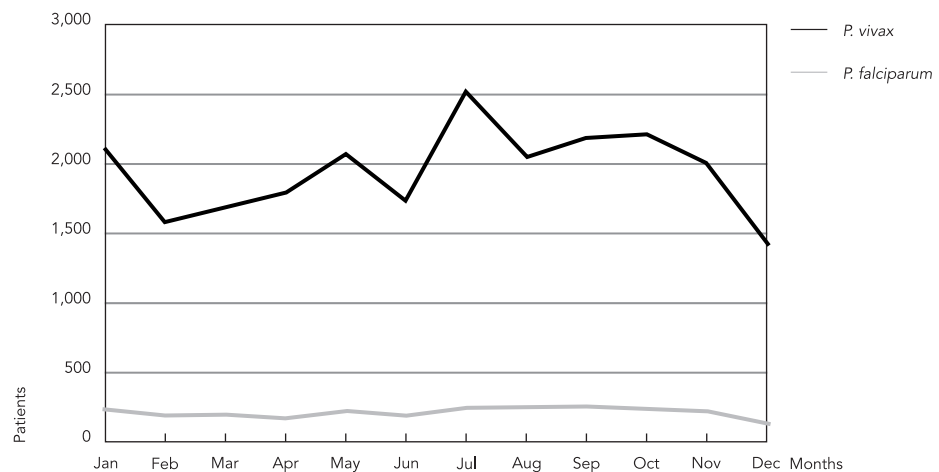
ing-age women analyzed, 215 were pregnant, of whom 26 (12.1%) presented recurrent *P. vivax* infection. *P. falciparum* was the causative species of previous infection in only 6.6% of the patients that presented recurrence (Table 2).

Analysis of the time elapsed until the first recurrence with *P. vivax* in Porto Velho (2009) showed an asymmetrical shift to the right in the distribution, with a mean of 87.5 days (SD: 59.0), median of 69.5 (Q1-Q3: 48-106), and mode of 34 days. This distribution differed from that observed in recurrent *P. falciparum* malaria, which also showed a shift to the right, but with a median of 42 days and mode of 14 days (Figure 2). The difference in the intervals between each recurrence was progressively smaller [β = -7.57; 95%CI: -9.19; -5.95; p < 0.0001] as the number of repetitions in recurrences per patient increased over the course of the year (Figure 3).

The 23,365 patients in the study had their first symptomatic episode of *P. vivax* infection in different months of 2009, resulting in variation in individual follow-up time in this historical cohort until occurrence of the target outcome, namely recurrence. Total follow-up time was thus 2,908,181 person-days, or 7,967.2 person-years. A total of 3,595 patients experienced at least one *P. vivax* recurrence in 2009. This resulted in an incidence density of 45.1/100 person-years (95%CI: 44.4; 45.3)/100.

Incidence of first recurrence was higher in men (RR = 1.10; 95%CI: 1.02; 1.17; p = 0.009) and was only associated with age greater than 50 years. Recurrence rate was also associated with level of education in patients with four to seven years of schooling (p = 0.002). However, no association was observed with level of schooling less than three years (p = 0.214) or eight years or more (p = 0.897). Incidence of first recurrence in pregnant and non-pregnant women was similar (RR = 0.91; 95%CI: 0.62; 1.34; p = 0.641). Patients' clinical characteristics showed that first recurrence was less frequent in patients with more than 72 hours since onset of symptoms and more frequent in those with parasitemia greater than 10,000/mm³ at the first symptomatic infection in 2009 (Table 3).

After adjusting the probability of recurrence over time, the following remained significantly associated with the outcome: male gender (HR = 1.10; 95%CI: 1.03; 1.18; p = 0.006), time since onset of symptoms 48-72 hours (HR = 1.21; 95%CI: 1.08; 1.35; p = 0.001) or less than 48 hours (HR = 1.36; 95%CI: 1.25; 1.49; p < 0.0001), and having peak parasitemia at diagnosis of 501-10,000/mm³ (HR = 1.32; 95%CI: 1.23; 1.43; p < 0.0001) or greater than 10,000/mm³ (HR = 1.44; 95%CI: 1.19; 1.75; p < 0.0001) (Table 4; Figure 4).

Table 1

General characteristics of recurrent *Plasmodium vivax* malaria in the Municipality of Porto Velho, Rondônia State, Brazil, 2009.

Characteristics	n	%
Recurrences (n = 23,365)		
Yes	5,363 *	23.0
No	18,002	77.0
Number of recurrences per patient in 2009		
One	3,595	67.0
Two	1,154	21.5
Three	396	7.4
Four	148	2.8
Five or more	71	1.8
Type of recurrence **		
Recrudescence (< 28 days)	186	5.2
Probable relapse (28-60 days)	1,061	29.5
Probable reinfection (> 60 days)	2,348	65.3

* Some patients had more than one recurrence during the study period;

** Classification based on the guidelines of the Brazilian National Malaria Control Program, Ministry of Health.

Discussion

The recurrence rate for *P. vivax* infection in Porto Velho was high in 2009. The initial hypothesis for these recurrences was reactivation of hypnozoites, which are not always eliminated by primaquine, due either to resistance to this drug or another factor that interferes with its action [15]. From the epidemiological point of view, little has been published on the magnitude of recurrences after treatment of *P. vivax* malaria in a given endemic region, based on the interpretation of this phenomenon as the combination of recrudescence, relapse, and reinfection, as defined above. However, the proportion of *P. vivax* infections that evolve to relapses has been well-studied, and it is known to be an intrinsic property of the malaria parasite. The proportion varies considerably between geographic regions and is higher with the species' tropical strain when compared to the temperate strain. It is also a function of the size of the sporozoite inoculum and the patient's immunity, when the latter exists [2,15,16]. The probability of relapse with a single genotype strain is also known to depend on the time since the patient has been ill, which also suggests the effect of immunity on the occurrence of this phenomenon [2].

The high proportion of malaria cases caused by *P. vivax* as compared to *P. falciparum* in Porto Velho was no different from that recorded in the Amazon Region as a whole that same year. The same was true for the predominance in males, young adults, and individuals with low school-ing [2]. The 23.0% overall recurrence for *P. vivax* malaria in this municipality in the year 2009 can be considered high, although not very different from the levels for the Legal Amazonia during the same period, namely 20.8%, identified as LVC (lâmina de verificação de cura, or cure verification smear) in the SIVEP-Malaria database [1]. It was also similar to the rates observed by Prasad et al. [15] in a study of reinfection/relapse in India (23.2%), Adak et al. [17] in a study of *P. vivax* recurrence patterns, also in India (23.3%), and Haghdoost et al. [18] in a study in Iran (16.8%), also in one-year population follow-up.

In Brazil, a retrospective study of cases of symptomatic *P. vivax* infection reported in São Paulo in the 1980s (when malaria was still autochthonous in that state) showed a similar recurrence rate after treatment of symptomatic *P. vivax* infection (24.5%) [19]. Meanwhile, in a study in a non-endemic area without risk of transmission, *P. vivax* recurrence (observed as relapse after treatment with primaquine) occurred less frequently (14%) than in the current study, probably due to treatment supervision [3].

Overall incidence of *P. vivax* recurrence in Porto Velho (2009) was 45.1/100 person-years. The high rate expresses the risk of an individual with symptomatic *P. vivax* infection in that municipality presenting a new episode of malaria from the same species over the course of a year. In countries like Thailand and Papua New Guinea, the *P. vivax* recurrence rate after treatment with chloroquine and primaquine can reach 65% in 30 to 180 days

Table 2

Demographic and clinical characteristics of patients with at least one recurrence (R1) of *Plasmodium vivax* malaria. Municipality of Porto Velho, Rondônia State, Brazil, 2009.

Characteristics	n	%
Sex (n = 3,595)		
Male	2,327	64.7
Female	1,268	35.3
All females (n = 1,018)		
Pregnant	26	2.6
Not pregnant	992	97.4
Age (years)		
Mean (SD)	26.8 (15.7)	
Median (Q1-Q3)	25.0 (14.0-38.0)	
Age bracket (years) (n = 3,595)		
< 12	652	18.1
12-19	678	18.9
20-49	1,950	54.2
> 50	315	8.8
Years of schooling (n = 3,376)		
None	301	8.9
1-3	2,701	80.0
4-7	239	7.1
8-11	135	4.0
Species of infection prior to the recurrence (n = 3,595)		
Plasmodium falciparum	238	6.6
Plasmodium vivax	3,357	93.4
Parasitemia at first episode/mm3 (n = 3,595)		
100-500	2,059	57.3
501-10,000	1,393	38.7
> 10,000	143	4.0
Time since onset of symptoms (hours) (n = 3,079)		
< 48	1,741	56.5
48-72	581	18.9
> 72	757	24.6

Note: variation due to missing information for the variable.

of follow-up [20,21]. However, the rate had not been studied in the endemic region of the Brazilian Amazon. The few available studies were conducted in an area without transmission [3] and in the endemic area of the Atlantic Forest, where the risk of new infections is very low [19]. A recent study by Orjuela-Sanchez et al. [9] evaluated *P. vivax* incidence and time to recurrence in two cohorts of patients living on the Brazil-Peru-Bolivia border, treated with chloroquine + primaquine. The authors found 180-day accumulated incidence rates of 26% and 40% for *P. vivax* recurrence in these two patient cohorts.

The following factors may explain the high recurrence rate in the current study. The first was the high level of *P. vivax* transmission in Porto Velho in 2009, favoring new infections. During that year, malaria incidence in Porto Velho (as recorded in the SIVEP-Malaria database) exceeded 50 cases/1,000 inhabitants, characterizing high transmission risk in the municipality [1]. The second was the high recrudescence/relapse rate, due to possible treatment failures with the chloroquine + primaquine regimen recommended by the PNCM for treatment of this species throughout the Amazon [3]. Since malaria treatment is not supervised in the Amazon Region and the antimalarial dispensing system in health services is often precarious [2], this hypothesis cannot be overlooked. Neither is it possible to rule out factors associated with the total

Figure 2

Distribution of time elapsed between initial episode and first recurrence of *Plasmodium vivax* (2a) and *Plasmodium falciparum* malaria (2b). Municipality of Porto Velho, Rondônia State, Brazil, 2009.
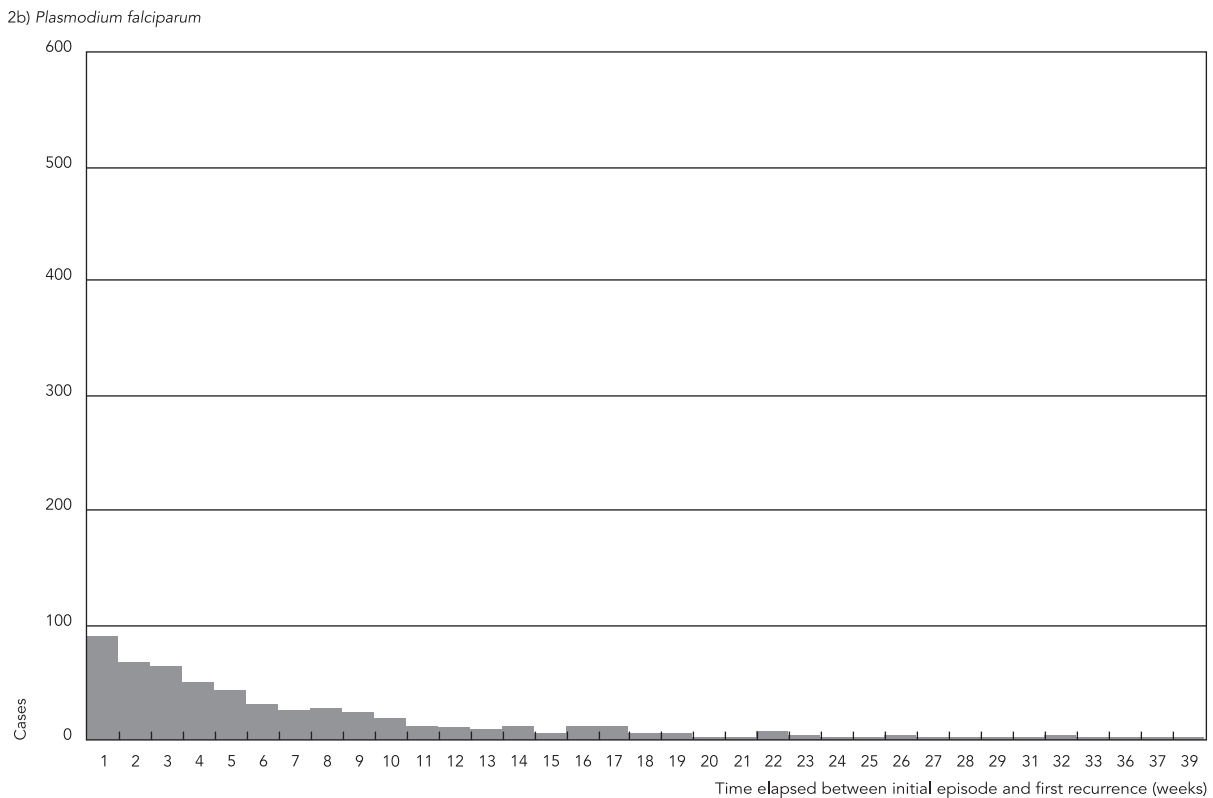

2a) *Plasmodium vivax*
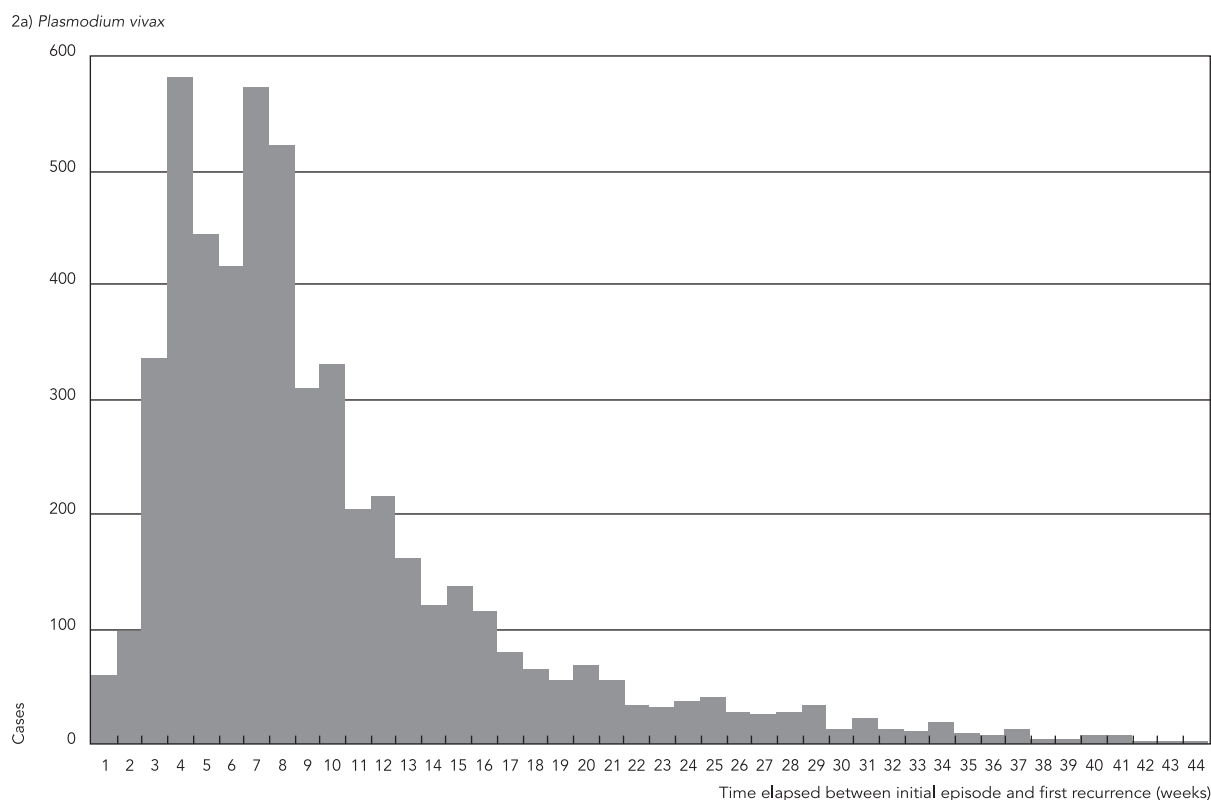
(continues)

administered dose of primaquine and the development of hypnozoite resistance to this drug in the region, as other researchers have suggested [3,22]. Another issue that cannot be overlooked is Brazil's recommendation of the short (7-day) primaquine regimen, unlike the 14-day regimen recommended in most endemic regions of the world [3,4].

The number of recurrences in patients with recurrent *P. vivax* malaria in 2009 in Porto Velho varied from 1 to 11. This high rate of recurrent malaria in such a short time interval had already been reported previously, as the exclusive result of hypnozoite reactivation [2]. During World War II, 70% to 80% of American soldiers stationed in Southeast Asia experienced relapses of *P. vivax* malaria (tropical strain) after returning to the United States, many of whom (9%) more than ten times in a year [23]. Other authors also observed the predominance of recurrences (and in varying numbers) within the first year after treatment of the primary infection, despite correct primaquine dosage [3]. In

areas where the long and short latency strains are known to occur simultaneously, as in India, radical treatment of *P. vivax* is more effective for the temperate strain than the tropical strain [2]. These findings shed doubt on the efficacy of primaquine and raise hypotheses on possible phenotypic characteristics of *P. vivax*, making hypnozoites more resistant to eradication [24].

Prospective studies in India in the last 25 years showed relapse rates varying from 8.6% to 40.1% following treatment of *P. vivax* exclusively with chloroquine (without combined primaquine) [17,25]. It is suggested that without radical treatment with primaquine, a constant proportion of patients experience one, two, three, or more relapses, with an exponential ratio [2]. It is thus estimated that in areas with a relapse rate of 50% or more, approximately 6.25% of patients will have four or more relapses after a single symptomatic primary infection. This information is extremely relevant for *P. vivax* malaria control, since it provides an indication of the

Figure 2 (continued)

2b) *Plasmodium falciparum*

Time elapsed between initial episode and first recurrence (weeks)

circulating hypnozoite burden in a given area. That is, if the relapse rate exceeds 50%, the principal determining factor in the persistence of malaria in an area is hypnozoite reactivation in patients previously infected with *P. vivax*. This should therefore be the priority target for control measures [2].

The monthly recurrence rate increased rapidly in the first three months of 2009, stabilizing around 22% in the subsequent months, without displaying a seasonal pattern. Studies in American soldiers showed that the first relapse occurred 60 days after the primary attack by *P. vivax*, recurring some 300 days later [2]. The low recurrence rate in the initial months of this cohort was expected, due to the definition of recurrence used in the study. Many of the malaria episodes during this period may actually have been recurrences of initial infections acquired in the previous year. In fact, analyzing only the period from April to December 2009, the monthly recurrence rate accompanied the malaria endemic curve in Porto Velho during the same year, showing that recurrences in the current study also resulted from reinfections that occurred simultaneously with relapses of the symptomatic *P. vivax* infection.

The mean time elapsed between the first symptomatic infection in 2009 and first recurrence, as well as between the first subsequent recurrence and the previous treatment episode, varied and decreased with the increase in the frequency of recurrent episodes. Lysenko et al. [26], reviewed by White [2], also found a progressive reduction in the *P. vivax* relapse intervals, in both the temperate and tropical strains of the parasite. A plausible theory to explain variability in the intervals between relapses is that the sporozoites inoculated by the vector are actually a mixture of genetically distinct parasites that induced two populations of hypnozoites, i.e., with short and long latency [26].

The highest risk of recurrent *P. vivax* in a patient in 2009 occurred between the fourth and ninth

Figure 3

Distribution of recurrences of *Plasmodium vivax* malaria according to mean time between episodes. Municipality of Porto Velho, Rondônia State, Brazil, 2009.
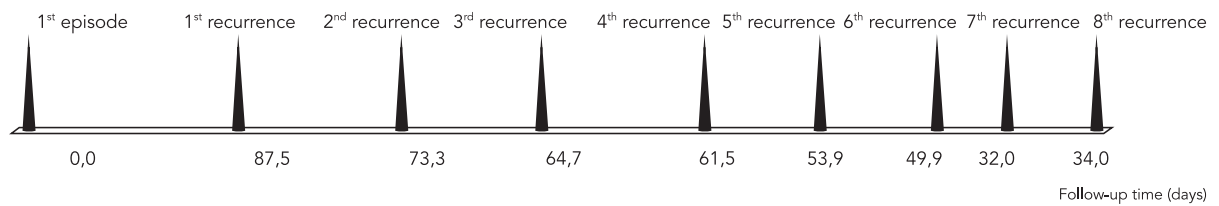
$\beta = -7.57$; 95%CI: -9.19; -5.95 ($p < 0.0001$).

Table 3

Analysis of demographic and clinical factors associated with first recurrence of *Plasmodium vivax* malaria. Municipality of Porto Velho, Rondônia State, Brazil, 2009.

Characteristics	Recurrence		RR	95%CI	p-value *
	n	Incidence density (per 100 person-years)			
Sex					
Female	1,268	42.6	1.10	1.02; 1.17	0.009
Male	2,327	46.6			
Pregnant (n = 1,018)					
No	992	41.9	0.91	0.62; 1.34	0.641
Yes	26	38.4			
Age bracket (years)					
< 12	652	45.8	1.00	-	-
12-19	678	47.1	1.03	0.93; 1.14	0.620
20-49	1,950	46.1	1.01	0.92; 1.10	0.888
> 50	315	35.8	0.78	0.68; 0.89	0.0003
Schooling (years)					
None	301	40.2	1.00	-	-
1-3	2,701	43.4	1.08	0.96; 1.21	0.214
4-7	239	52.6	1.30	1.10; 1.55	0.002
8-11	135	40.8	1.02	0.83; 1.24	0.897
Onset of symptoms (hours)					
> 72	757	36.8	1.00	-	-
48-72	581	43.9	1.19	1.07; 1.33	0.001
< 48	1,741	49.4	1.35	1.24; 1.47	< 0.0001
Parasitemia at first episode (mm^3)					
100-500	2,059	40.6	1.00	-	-
501-10,000	1,393	52.7	1.30	1.21; 1.39	< 0.0001
> 10,000	143	58.6	1.44	1.22; 1.71	< 0.0001

95%CI: 95% confidence interval; RR: relative risk.

* Descriptive test level (p-value) calculated by Z-score.

Table 4

Multivariate analysis of demographic and clinical factors associated with probability of first recurrence of *Plasmodium vivax* malaria. Municipality of Porto Velho, Rondônia State, Brazil, 2009.

Characteristics	First recurrence		HR	95%CI	p-value *
	n	%			
Sex					
Female	1,268	19.4	1.10	1.03; 1.18	0.006
Male	2,327	20.8			
Age bracket (years)					
0-12	652	20.6	1.00	-	-
12-19	678	21.5	1.18	1.01; 1.37	0.036
20-49	1,951	20.5	1.31	1.13; 1.53	< 0.0001
> 50	315	16.6	1.27	1.12; 1.46	< 0.0001
Schooling (years)					
None	301	19.0	1.00	-	-
1-3	2,701	20.2	1.05	0.91; 1.20	0.501
4-7	239	22.3	1.13	0.93; 1.37	0.211
8-11	135	19.8	0.95	0.76; 1.19	0.640
Onset of symptoms (hours)					
> 72	757	24.6	1.00	-	-
48-72	581	18.9	1.21	1.08; 1.35	0.001
< 48	1,741	56.5	1.36	1.25; 1.49	< 0.0001
Parasitemia (mm^3)					
100-500	2,059	57.3	1.00	-	-
501-10,000	1,393	38.8	1.32	1.23; 1.43	< 0.0001
> 10,000	143	3.98	1.44	1.19; 1.74	< 0.0001

HR: hazard ratio; 95%CI: 95% confidence interval.

* Cox regression

week after treatment of the first malaria. Since low adherence to malaria treatment it not that common in the Brazilian Amazon [2], these early recurrences may actually result from relapses due to hypnozoite activation. The so-called high relapse frequency strains of *P. vivax* (including the tropical strain) have already been recorded in South America, India, Southeast Asia, and Oceania. However, the strains with long latency hypnozoites also occurred in these same areas [2]. This overlapping could thus explain the wide range in time to recurrence observed in the current study, i.e., the early recurrences caused by relapses of tropical strains and the later ones by relapses of long latency strains. In Brazil, the predominant situation is presumed to be that the tropical strain of *P. vivax*, characterized by early primary attack, is followed by a short latency period until the relapses that appear during the subsequent year. However, no scientific evidence is available on this issue in Brazil or in the rest of South America. Researchers in Rio de Janeiro and Brasília have recently reported travelers with long incubation periods for malaria, varying from 3 to 12 months after returning from trips to the Amazon. They suggested that temperate strains coexist with the tropical strain in the country [22,27]. This hypothesis is corroborated by the observation of relapses at days 33, 73, 82, 88, 113, 121, and 137 after initiating therapy in a *P. vivax* malaria treatment cohort in 1990 in Cuiabá (Mato Grosso State), where malaria transmission does not occur [3].

Based on the operational classification of recurrences proposed by the PNCM, 29.5% of *P. vivax* recurrences in Porto Velho in 2009 were probable relapses. It is quite probable that this estimated proportion is close to the true proportion of relapses in the study population, because the reappearance of *P. vivax* in relapses after a primary infection occurred between 28 days (3%) and 63 days (63%) in a study in Thailand [28]. Furthermore, *P. vivax* recurrence was observed between 28 and 90 days in 70% of patients studied in French Guiana, although recrudescence and

Figure 4

Kaplan-Meier curves for probability of remaining without recurrence of malaria, according to age bracket (4a), time since onset of symptoms (4b), and level of parasitemia (4c). Municipality of Porto Velho, Rondônia State, Brazil, 2009.
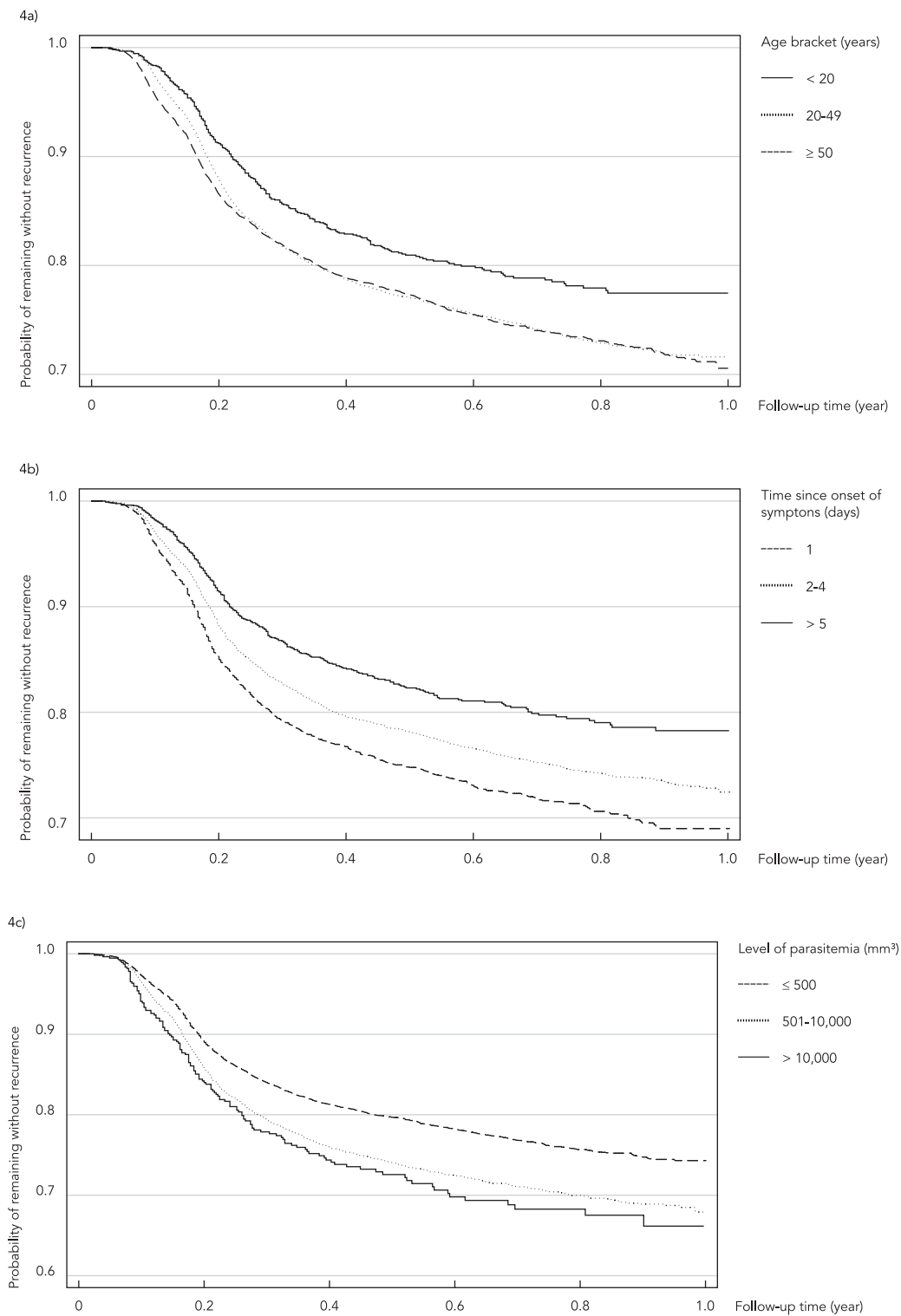
reinfections could not be ruled out, as occurred in the present study [29].

A higher recurrence rate was expected among pregnant women. The risk of relapses from *P. vivax* malaria in individuals that do not receive primaquine is known to range from 5% to 80%, depending on the geographic region [30]. A likely explanation for the low recurrence rate observed in this group is the use of hypnozoite suppressive therapy with weekly chloroquine (5mg/kg), recommended since 2008 by the PNCM for patients in whom primaquine is contraindicated [14].

Although some studies have not found a higher recurrence rate in men [18], this finding was observed by Prasad et al. [15], who suggested low treatment adherence as the probable explanation for this difference, highlighting greater risk of treatment dropout by men due to low schooling and longer workweeks (as contributing factors). Thus, recurrences in women would tend to be due more to relapses or reinfections. In addition to relapses and reinfections, men would also show a higher recrudescence rate due to low treatment adherence.

No age bracket in the patients studied here was associated with greater risk of recurrences in Porto Velho (2009). In endemic areas in Thailand, the age profile of patients with *P. vivax* malaria suggests more rapid and intense acquisition of immunity with this species than with *P. falciparum* [30]. Various studies on this issue have suggested that both the proportion of *P. vivax* relapses and the rate of entomological inoculation decline in elderly individuals. Immunity and thus age are probable confounders in epidemiological evaluations of recurrence and relapse from *P. vivax* malaria [2,31].

The recurrence rate for *P. vivax* malaria was low following symptomatic infection with *P. falciparum*. In Southeast Asia, the incidence of this phenomenon is substantially greater than expected according to the entomological inoculation rate, i.e., with no relationship to previous infection with a different species [32]. In Ethiopia, where the early relapses rates for *P. vivax* malaria are lower, as in the current study, the phenomenon occurred in only 7% of patients [33]. Since the two species coexist in Porto Velho, recurrences from *P. vivax* following infections with *P. falciparum* probably result merely from the greater risk of transmission of the first species, as recorded in recent years.

Higher parasitemia in the first infection with *P. vivax* diagnosed in 2009 was associated with greater risk of recurrence. Recurrence also occurred earlier in patients with hyperparasitemia. In hyperendemic areas, higher parasite density for any *Plasmodium* species is seen in children, pregnant women, and adults with primary infection, due to the lower level of naturally acquired immunity in these groups [34]. Therefore, the observed association is actually presumed to be the effect of lower immunity in patients with hyperparasitemia and consequently the effect on recurrence rate, as described for *P. vivax* relapses [2].

Meanwhile, the longer the time since onset of symptoms (as reported by patients at time of diagnosis), the lower the rate of *P. vivax* recurrences, which also occurred later. One cause of longer time since onset of symptoms that cannot be ruled out is delay in diagnosis resulting from difficult access to health services, especially distance from the patient's home to the laboratory. However, the patients with the longest delays in seeking malaria diagnosis are probably the least symptomatic, having already developed some degree of clinical immunity, already described for malaria in individuals from endemic areas, causing a negative impact on the incidence of recurrence [2]. The current study also corroborates this with the lower recurrence rate among patients in higher age brackets, who are more likely to have naturally acquired immunity to malaria [35].

In short, the study showed that male gender, shorter symptom time, and higher parasitemia at diagnosis of *P. vivax* malaria increased the risk of recurrence over the course of a year. The strength of association for each of these risk factors after the adjusted analysis included 11% and 15% increases in relative risk for males and patients with higher parasitemia, respectively, and a 10% reduction in risk of recurrence in patients with longer symptom time. Although these values for increased and decreased risk are not that large, they could help orient malaria control programs in choosing groups of patients to focus greater attention, in order to minimize their suffering from recurrences.

It is important to highlight some inherent limitations to this type of epidemiological study. Since the data were obtained from a secondary source, the results are not exempt from a possible selection bias due to underreporting of cases in the SIVEP-Malaria base. But this bias is likely to have been minimal, since the SIVEP-Malaria base is known as a sensitive and timely information system with good completeness. Another important issue that can underestimate the recurrence rate in the study is the patient's mobility in the endemic area, and the same patient may have had one or more recurrences diagnosed in a neighboring municipality or state. Although this possible loss was not measured, if it does exist, its influence on the study's result must not have interfered in the objective, since the observed recurrence rate was already high.

The low values for the risk measures in the multivariate analyses can be interpreted as hav-

ing low clinical relevance for the observed associations, limiting their causal potential for predicting recurrence rates or justifying decisions at the public health level. Although further cohort studies including information on patients' clinical and behavioral patterns are necessary to better evaluate the real factors related to recurrent malaria, the current study's results are relevant for calling the attention of the PNCM to the high rate of recurrences from *P. vivax* malaria in Brazil and for reflecting on more effective control measures.

Resumen

Estudio de cohorte histórica abierta, construida con información registrada en el Sistema de Información de Vigilancia Epidemiológica de la Malaria, con el objetivo de describir la incidencia y los factores asociados a la reaparición de malaria, causada por el Plasmodium vivax, *en Porto Velho, Rondônia, Brasil. Se notificaron 23.365 casos de malaria por* P. vivax *en el municipio, donde un 23% de ellos presentaron recidiva a lo largo del 2009. La densidad de incidencia de recidivas fue de 45,1/100 pacientes-año, produciéndose, principalmente, entre la 4ª y la 13ª semana tras el inicio del tratamiento. Ser hombre, tener menos tiempo síntomas y tener un mayor nivel de parasitemia en la infección inicial aumentaron el riesgo de recidiva a lo largo del año, con un 10% de reducción relativa del riesgo, para un mayor tiempo de síntomas, y de un 11% y 15% de incremento relativo del riesgo para el sexo masculino y mayores niveles de parasitemia, respectivamente. No obstante, los resultados demostraron baja relevancia clínica de las asociaciones encontradas, comprometiendo su aplicabilidad en la toma de decisiones dentro de la salud pública.*

Recurrencia; Plasmodium vivax*; Malaria*

Contributors

C. J. F. Fontes participated in the study design, elaboration of the research project, data analysis, and writing of the manuscript. L. R. Simões participated in the data organization and analysis and writing of the manuscript. E. R. Alves-Jr participated in the organization of the databank and critical revision of the manuscript. D. Ribatski-Silva participated in the data analysis and writing of the manuscript. L. T. Gomes participated in the data analysis and critical revision of the manuscript. A. F. Nery verified and conducted the final revision of the databank and critical revision of the manuscript.

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