Prevalence and Force of Plasmodium Vivax and Plasmodium Falciparum Blood Stage Infection and Associated Clinical Malaria Burden in the Brazilian Amazon

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Abstract

Background: *Plasmodium vivax* and *Plamodium falciparum* are co-endemic in much of the Brazilian Amazon, with *P. vivax* comprising greater than 80% of clinical cases, especially in low transmission settings. The molecular force of blood-stage infection of *P. vivax* (molFOB) can provide a detailed picture of *P. vivax* transmission in low transmission settings and help improve malaria measures control and elimination efforts.

Methodology: Monthly samples were collected in a cohort of 1,274 individuals of all ages between April 2013 and March 2014 in three peri-urban communities in the Brazilian Amazon. Regression analyses were used to test how factors including age and community were associated with *P. vivax* molFOB, parasite positivity and clinical episodes.

Principal Findings: Respectively, 77.8% and 97.2% of the population remained free of *P. vivax* and *P. falciparum* infection. Expected heterozygosity for *P. vivax* was 0.69 for MSP1_F3 and 0.86 for MS2. Multiplicity of infection in *P. vivax* was close to the value of 1 as determined with both markers (1.06 for MSP1_F3 and 1.04 for MS2). Season was associated with *P. vivax* positivity [adjusted hazard ration (aHR) 2.6 (1.9-5.7)] and clinical disease [aHR 10.6 (2.4-47.2)]. *P. falciparum* infection was associated with previous malarial episodes [HR 9.7 (4.5-20.9)]. Subjects who reported bednet possession [incidence rate ratio (IRR) 1.6 (1.2-2.2)] or previous malaria episodes [IRR 3.0 (2.0-4.5)] were found to have significantly higher *P. vivax* molFOB.

Conclusions: Overall, *P. vivax* infection prevailed in the area and infections were mostly observed as monoclonal. High proportions of symptomatic and submicroscopic infections were found. Previous malaria episodes were associated with significantly higher *P. vivax* molFOB, likely indicating that effective radical cure is an important strategy to be addressed in these endemic communities.

Introduction

The global burden imposed by malaria remains high, with an estimated 219 million cases and 435,000 related deaths in 2017 [1]. Although malaria remains endemic in 91 countries, the majority of the burden (90%) is confined to the African Region where *Plasmodium falciparum* predominates [1]. In the Americas, *Plasmodium vivax* is the predominant species causing over two thirds (69%) of all cases in 2017. Despite most endemic countries in Latin America having achieved a reduction of incidence since 2010, there were still more than half a million (562,300) cases in 2017, with Venezuela and Brazil accounting for more than half of these cases (65%) [1]. Between 2005 and 2017 Brazil has achieved a 70% reduction of malaria cases, with 99% of all cases occurring in the Amazon region in 2017 [1,3]. Of special vulnerability are malaria-naïve individuals recently arrived from malaria-free areas and engaged in agricultural and forest-related activities such as logging, fishing and mining [4–7]. In the 1970s, increased industrial development demanded a large labor force, which evoked a large migratory influx to the peripheries of

bigger cities such as Manaus. Such largely uncontrolled settlements have led to a gradual increase of malaria transmission in peri-urban areas [3].

Anopheles darlingi is the major malaria vector in the Amazon region, and has highly anthropophilic and endophagic behavior [8–11]. Current vector control measures primarily include the use of long-lasting insecticide-treated nets (LLINs) and indoor residual spraying (IRS), but there are little data from Latin America on the impact of vector control. One study showed that the use of LLINs was associated with malaria protection in the Amazon, however, it seemed to have a more protective effect against *P. falciparum* malaria in comparison with *P. vivax* malaria [7]. As in other regions, recent progress in malaria control in Brazil has been accompanied by an increasing proportion of cases caused by *P. vivax*, underscoring the challenges, namely relapses, yet to be addressed in control and management this species [12]. Understanding the epidemiology of relapses after primary infection is challenging, with an estimated 14-40% of individuals having detectable recurrences, even after treatment with primaquine [7,13–16]. A second challenge is the early production of *P. vivax* gametocytes, along with asymptomatic cases, which enables the transmission of parasites even before the infected individual develops clinical symptoms. Thirdly, *P. vivax* parasite densities are generally lower than those seen in *P. falciparum* infections, especially in hypoendemic areas such as in Brazil, therefore requiring more sensitive diagnostic tools [17]. Research has shown that a high proportion of submicroscopic and asymptomatic *P. vivax* infections usually exist in such settings, and that these infections can easily be missed by routine active and passive case detection [17]. It has been shown that asymptomatically infected individuals are able to infect mosquitoes of the Amazon region, therefore these infections might fuel residual malaria transmission, thereby complicating malaria elimination [18–21].

In view of these challenges to malaria elimination, it is important to gather data of spatial and temporal patterns of asymptomatic Plasmodium infections as well on gametocyte carriage from low-transmission settings. Therefore, in this study, we investigated risk factors and spatial-temporal patterns of incidence of Plasmodium infection and clinical malaria episodes in a peri-urban area of Manaus, Western Brazilian Amazon. We paid special attention to submicroscopic and asymptomatic infections by measuring the prevalence of *P. falciparum* and *P. vivax* blood-stage infections by PCR, the incidence of clinical cases, and estimating the molecular force of *P. vivax* blood-stage infection (molFOB) derived from molecular detection and genotyping of infections. Understanding the malaria epidemiology through *P. vivax* molFOB may provide a detailed assessment of malaria transmission by measuring individual exposure and its burden. Such information is of paramount importance when measuring intervention efficacy, host susceptibility and transmission patterns in low transmission and pre-elimination settings such as Brazil.

Methods

Ethics statement

This study was approved by the Brazilian National Committee of Ethics (CONEP) (349.211/2013) and the Committee of Ethics for Clinical Investigation of the Barcelona Hospital Clinic (7306/2012). All

participants were informed about the objectives of the study as well as the potential risks and benefits of their participation in the study. An informed consent form was signed by all study participants or by a parent or legal guardian in case of participants that were under 18 years of age. Children between 12 and 17 years of age signed an additional assent form. As routinely done, malaria patients received treatment with 25 mg/kg of chloroquine over a 3-day period (10 mg/kg on day 0 and 7.5 mg/kg on days 1 and 2. Primaquine was prescribed at the dosage of 0.5 mg/kg per day, during 7 days.

Study design and subjects

This cohort study was conducted in the Brasileirinho, Ipiranga and Puraquequara communities, located in Manaus peri-urban area, between April 2013 and March 2014 (Figure 1A). A detailed description of the study area has been presented elsewhere [17]. According to a census performed by a Fundação de Medicina Tropical Doutor Heitor Vieira Dourado (FMT-HVD) field team, the population of the study area was estimated to be approximately 2,400 inhabitants before the start of the study, in 2012. Each community has access to a malaria clinic for microscopy-based malaria diagnosis and treatment. A total of 1,200 participants of any age were enrolled into the study in April 2013.

Data and sample collection

For each study participant, a questionnaire was completed containing personal information such as age, gender, occupation, pregnancy, history of travel, as well as information on malaria preventive measures, previous malaria episodes and current health status. Upon enrolment and monthly during follow-up, finger-prick blood 151 samples (~300 μL) were collected using Microtainer® tubes containing EDTA and sodium fluoride (Becton Dickinson, USA). In infants, blood was obtained by either heel or toe puncture. Within one hour of collection, 50 μL of blood were transferred into a reaction tube containing 250 μL of RNAprotect (QIAGEN, Germany) in order to preserve RNA for downstream analyses [22] and 200 μL of whole blood was transferred to another reaction tube. Samples were stored in cooling boxes until arrival in the laboratory, where the 200 μL sample was separated into a red blood cell (RBC) pellet and plasma. All samples were frozen at -80ºC until further processing. If the collected blood volume was <250 μL, the actual volume was recorded.

Clinical symptoms

In the case of symptoms related to malaria or in 161 the case of increased body temperature (>37.5ºC), a thick blood smear (TBS) was prepared according to World Health Organization guidelines [23]. When positive for malaria, appropriate treatment was provided in accordance with the national guidelines of the Brazilian Ministry of Health [24]. An asymptomatic infection was defined as presence of a malarial infection by TBS, but absence of fever and any other malaria related symptoms (chills, sweating, headache, vomit, abdominal pain) at the moment of sample collection, or anytime in the preceding 48 hours.

Plasmodium spp. infection, clones and gametocyte carriage

Pelleted RBCs, obtained from 200 µL of whole blood, were re-suspended in PBS and genomic DNA was extracted using FavorPrep 96-well Genomic DNA Kit (Favorgen, Taiwan) according to the manufacturer's instructions. DNA was eluted with 2x 100 µL of elution buffer and stored at -20ºC until assayed by PCR. If the amount of whole blood available for DNA extraction was ≤100 µL, the DNA volume was vacuum concentrated until reaching the original blood volume recorded. RNA from 50 µL whole blood, stored in RNAprotect, was extracted using RNeasy Plus 96-well kit (QIAGEN, Germany) and eluted in 50 µL RNase-free dH2O as described previously [22]. All DNA samples were subject to a generic Plasmodium species (QMAL) qPCR targeting a conserved region of the 18S rRNA gene [22]. QMAL-positive samples were further analysed by species-specific qPCR assays targeting the18S rRNA genes of *P. falciparum* and *P. vivax*, as previously described [22,25]. For detection of *P. falciparum*, a modified reverse primer was used [26]. For quantification of 18S rRNA gene copy numbers, in each experiment three dilutions of control plasmids containing the respective amplicons were included in triplicates (102, 104 and 106 copies/µL). For genotyping individual *P. vivax* clones, the molecular markers msp1F3 and MS16 were typed using capillary electrophoresis for highly precise fragment sizing allowing for longitudinal follow up of individual parasite clones. Details of the genotyping methods have been described previously [27]. RT-qPCR assays were performed on RNAs from all *P. vivax* and/or *P. falciparum* positive samples to detect gametocyte-specific transcripts of the pvs25 (*P. vivax*) and pfs25 (*P. falciparum*) genes. For quantification of pvs25 and pfs25 transcript numbers, control plasmids containing the amplified region were included as standards in each run. All qPCR and RT-qPCR assays were performed on a 7500 Fast Real-Time PCR System (Applied Biosystems).

Statistical analysis

Data from questionnaires were imported into databases using Cardiff TeleForm version 10.4.1 (Cardiff Software). Individual databases were combined in Microsoft Access 2010. For incidence calculations (molFOB and clinical malaria incidence), subject data were censored on the last visit before two consecutively missed scheduled follow-up visits in order to reduce bias [28]. Differences in proportions were tested for statistical significance using the McNemar X2 test with continuity correction. To achieve normal distribution, qPCR densities were expressed as log10-transformed 18S rRNA genomic copies/µL blood for asexual parasites, and log10-transformed pfs25 or pvs25 transcripts/µL blood for gametocytes. Geometric means of densities were calculated. Differences in densities of asexual or sexual-stage parasites were tested for statistical significance using Welch's two-sample t-test.

The molecular force of new *P. vivax* blood-stage infections (molFOB) was calculated by counting the number of genotypes observed at each visit, that had not been present in the preceding two visits (0-0-1 patterns) and adjusting these counts by the respective times-at-risk. molFOB for *P. vivax* was determined for both genetic markers combined. Negative binomial regression models were used to assess the influence of different risk-factors on the incidence of *P. vivax* and *P. falciparum* gametocyte positivity as previously described, using positivity counts and times-at-risk over the entire period of observation [28]. Since molFOB is a count variable measured per individual over a specific exposure time (time at risk), and as chosen in which the exposure time at risk is

used as offset. If we define μj as the log of the number of genotypes at visit j, then for each infection pattern j (0-0-0 or 0-0-1) we have μj = exp(βxj + offsetj + vj), where β is a vector of regression coefficients, offsetj=log(exposure time) and vj follows a gamma distribution (to give a negative binomial distribution). Incidence rate ratios (IRR) and adjusted IRR (aIRR) were calculated with their respective 95% confidence intervals. Because using the collapsed data to model molFOB for each individual does not allow for the analysis of time-changing covariates, factors influencing frequency of parasite positivity and frequency of clinical episodes within the study period were explored using multiple failure time models allowing for time-changing covariates [29]. For multiple failure time models, hazard rate ratios (HRR) are calculated with 95% confidence intervals. In these models, parasite positivity and clinical episodes were equivalent to a 'failed' outcome, respectively. In addition to the adjusted statistical models presented in the main manuscript, univariate analyses and multivariate analyses with backward selection are provided as Supplemental Materials. Versions of each model analysis were implemented with backwards selection to eliminate non-significant covariates resulting in the most parsimonious models. Statistical analyses were conducted using R v3.1.1 or STATA v14.

Results

Study population

A total of 1,274 individuals were enrolled at the beginning of the study. A total of 51.1% (651) were males and approximately half were aged between 18 and 59 years old (634; 49.7%). Nearly 40% (504) of the subjects claimed to have had at least 3 malaria episodes during their lifetime. A total of 17 individuals (1.3%) had experienced a malaria infection in the preceding two weeks and 40 (3.2%) had taken antimalarial drugs in the past two months. A total of 1,201 subjects (94.9%) had resided for more than 2 months in the study area. The number of individuals enrolled was similar in the three communities, Brasileirinho (n=430), Ipiranga (n=416) and Puraquequara (n=428). Characteristics of the study population are summarized in Table 1.

Prevalence by active case detection

Monthly *P. vivax* prevalence by qPCR ranged from 2.5% in June 2013 to 6.5% in November 2013 (Figure 2A). *P. falciparum* was not detected in August and September 2013, with the highest prevalence (~1.0%) occurring in March 2014 (Figure 2B). Both species presented a similar seasonality profile, with a higher prevalence of *P. vivax* from October to February, and of *P. falciparum* from November to March, coinciding with the rainy season (Figure 2D). *P. vivax* asymptomatic infections predominated in Puraquequara community, although clinical cases were mostly seen in Ipiranga. For *P. falciparum*, asymptomatic infections and clinical cases were both predominant in the Ipiranga community.

Risk factors associated with *P. vivax* positivity

In the multivariate analysis shown in Table 2, *P. vivax* infection was associated with the rainy season [illegible] nity of residence (P=0.002), with individuals

living in Puraquequara having aHR = 2.4 (1.1- 2.3) compared to Ipiranga. Age was associated with *P. vivax* infection (P=0.001), with individuals aged 20-60 years having aHR = 2.2 (1.1, 2.5) compared to children under 10 years. Bed net usage was significantly associated with higher *P. vivax* positivity [aHR = 1.06 (1.01-1.08), P=0.005]. Indoor residual spraying (IRS) in the house was associated with lower risk of *P. vivax* infection.

Risk factors associated with *P. vivax* clinical disease

P. vivax clinical disease was associated with the rainy season [(aHR: 10.6 (2.4-47.2), P=0.002)] (Table 2). Living in the Brasileirinho [(aHR: 0.11 (0.05-0.23), P<0.001)] and Puraquequara [(aHR: 0.26 (0.14-0.50), P<0.001)] communities, working in agriculture [(aHR: 0.43 (0.25-0.74), P=0.002)], and living in a house treated with IRS [(aHR: 0.92 (0.86-0.98), P=0.008)] were all variables associated with a reduced risk of clinical malaria disease.

Risk factors associated with *P. falciparum* positivity

P. falciparum infection was associated with community (P<0.001), with individuals living in Brasilerinho [aHR = 0.04 (0.01-0.26)] and Puraquequara [aHR = 0.23 (0.07-0.71)] having lower positivity than individuals in Ipiranga. Individuals with previous malarial episodes had significantly higher *P. falciparum* positivity [(aHR: 9.7 (4.5-20.9), P<0.001)].

Genetic Diversity and Multiplicity of Infections

The heterogeneity in the incidence of malaria infections is shown in Figure 3A. Both *P. vivax* and *P. falciparum* infections were restricted to a small proportion of the study population. Overall, 77.8% of the population remained free of *P. vivax* infection and 97.2% of the population remained free of *P. falciparum* infection over the course of the study period. Based on the two markers (MSP1 F3 and MS2), expected heterozygosity for *P. vivax* was 0.69 (MSP1_F3) and 0.86 (MS2), respectively. Overall, the multiplicity of infection was close to the value of 1 as determined with both markers (1.06 for MSP1_F3 and 1.04 for MS2, respectively), indicating that infections were mostly observed as monoclonal. Figure 3B shows the *P. vivax* molFOB over the entire year of follow-up. Similarly, new *P. vivax* infections were restricted to approximately 20% of the study population, whereas 80% did not receive new infections. Of individuals who had any *P. vivax* infection, the majority had molFOB = 1 to 2. The maximum number of genetically distinct infections/individual/year was molFOB = 5.

Factors associated with *P. vivax* molFOB

Table 3 shows the results of the multivariate negative binomial model applied to the molecular force of blood-stage infection of *P. vivax*. molFOB was significantly associated with community (P <0.001). *P. vivax* molFOB was lowest in the Brasileirinho community with aIRR = 0.47 (0.31-0.71) compared to Ipiranga. No significant difference in *P. vivax* molFOB was observed between Ipiranga and Puraquequara communities. Increased *P. vivax* molFOB was significantly associated with reported bed net possession

isodes [(aIRR:3.02 (2.02-4.53), P<0.001)].

Discussion

The present study highlights well-known differences in the epidemiology of *P. vivax* and *P. falciparum*, evaluating the incidence of Plasmodium spp. and clinical cases of malaria in peri-urban communities in the Brazilian Amazon and estimate the *P. vivax* molFOB in low transmission settings in the Americas. Previous studies have measured molFOB in Papua New Guinean children in observational and randomized clinical trial cohorts, and have related incidence of clinical infection and other factors to this measure [30,31]. The main factors related to *P. falciparum* molFOB in these earlier studies were seasonality, village of residence and age [30–33]. As for *P. vivax*, molFOB was strongly associated with incidence of clinical episodes and a high molFOB likely resulted in rapid acquisition of immunity against *P. vivax* in children [31].

Recent studies in the Amazon region and other malaria endemic areas in the world have shown a high proportion of submicroscopic *P. vivax* infections [7,17,34]. In the present study, factors associated with *P. vivax* positivity were age (20-60 years), seasonality, use of mosquito nets and IRS. In the Brasileirinho, Ipiranga and Puraquequara communities, the distribution of mosquito nets and IRS is mainly focused on areas where there are many cases of malaria (ascertained by active and passive case detection, registered by the SIVEP-Malaria platform). However, this measure may not have been effective in preventing infection in these areas, or it may not be sufficient to decrease *P. vivax* positivity, since many infections may derive from hypnozoites rather than new infections, as suggested by a study in Papua New Guinea [32].

A higher prevalence of malaria infection in the rainy season was also shown in previous studies, corroborating what has already been described for other regions of the Amazon [34,35]. The predictors of *P. vivax* clinical disease found in our study were seasonality, and marginally, frequency of travel and use of mosquito nets. Koepfli [31] also found the seasonality to be a predisposing factor in clinical disease by P. vivax. Protective factors of clinical disease such as being over 60 years old and working in agriculture may be associated to prolonged exposure to *P. vivax* infection during lifetime. Due to age- and exposure-dependent acquired immunity, clinical presentation of malaria becomes rarer in relation to age, thus increasing the number of asymptomatic carriers [31,36].

We also observed that *P. vivax* prevalence and molFOB in the study area were higher and less affected by seasonality as compared to *P. falciparum*. Whereas *P. vivax* prevalence and molFOB peaked in November in Ipiranga community, transmission indicators remained more stable throughout the entire observational period in the other two communities, with almost no seasonality observed in Brasileirinho. In contrast, the annual *P. falciparum* prevalence profile was characterized by a very sharp peak in January in Ipiranga community during which the observed prevalence increased by almost 10-fold alongside a sharp rise of clinical malaria cases. In fact, nearly all clinical cases observed in the present study occurred during this period and in Ipiranga community. The outbreak caused by *P. falciparum* subsided by April and the remainder of the observational period was characterized by very low *P. falciparum* prevalence in all three

communities. These observed differences in the seasonality profiles of the two species indicate a more stable transmission of *P. vivax* in contrast to in contrast to a more unstable transmission of *P. falciparum*.

The overall lower *P. vivax* parasite densities are close to the threshold of detection of even molecular diagnostic tests, thus hampering the characterization of the true incidence of new infections detected by genotyping, as reported elsewhere [37,38]. Therefore, estimation of molFOB can be dominated by clones with higher parasite densities, while low density, sub-dominant clones may not be detected, especially if they have arisen from relapses. As such, the molFOB reported in this study is likely an underestimation of the true force of blood-stage infection.

In conclusion, *P. vivax* infection prevailed in the area and infections were mostly observed as monoclonal. High proportions of symptomatic and submicroscopic infections were also found. Previous malaria episodes were associated with significantly higher *P. vivax* molFOB, likely indicating that effective radical cure is an important strategy to be addressed in these endemic communities. Asymptomatic and submicroscopic infections pose substantial challenges for *P. vivax* malaria control, hampering accurate surveillance efforts needed to pursue elimination, especially in low transmission settings.

Declarations

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Author contributions

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Tables

Table 1.

Baseline characteristics of the population enrolled in the study.

Variable	Participants	
	Number	%
Total	1,274	100.0
Gender		
Male	651	51.1
Female	623	48.9
Age group (years)		
<10	368	28.9
10-20	634	49.6
21-60	128	10.0
≥61	160	12.5
Occupation		
Agriculture	179	14.1
Office worker	170	13.3
House wife	218	17.1
Pre-school children	175	13.7
School children	331	26.0
Retired	63	5.0
Unemployed/Other	138	10.8
Previous infection (number) in the last two months		
0	406	31.9
1-3	364	28.6
>3	504	39.6
Infection in past two weeks		
Yes	17	1.3
No	1,251	98.7
Antimalarial in past two months		
	40	3.2

No	1,229	96.8
More than 2 months of residency		
Yes	1,201	94.9
No	65	5.1
Community		
Ipiranga	430	33.8
Brasileirinho	416	32.7
Puraquequara	428	33.6

Table 2.

Risk factors associated with *P. vivax* positivity, *P. vivax* clinical disease, and *P. falciparum* positivity. Adjusted hazards ratio (aHR) was calculated using a multiple failure time model.

Risk factor	P. vivax positivity		P. vivax clinical disease		P. falciparum positivity	
	aHR	p	aHR	p	aHR	p
Community (ref. Ipiranga)						
Season (Ref: Jun-Nov)	2.56 (1.89-5.74)	<0.001	10.56 (2.36-47.19)	0.002	0.08 (0.01-0.51)	0.008
Community (ref. Ipiranga)						
Brasileirinho	1.01 (0.4-1.08)	0.002	0.11 (0.05-0.23)	<0.001	0.04 (0.01-0.26)	<0.001
Puraquequara	2.39 (1.11-2.31)		0.26 (0.14-0.5)		0.23 (0.07-0.71)	
Age group (ref. 1-10)						
10-20	1.36 (0.78-1.95)		1.01 (0.54-1.88)	0.02	0.78 (0.19-3.27)	
21-60	2.22 (1.1-2.45)	0.001	0.63 (0.36-1.13)		1.33 (0.39-4.5)	0.300
≥61	1.9 (0.71-2.15)		0.15 (0.04-0.63)		2.15 (0.47-9.94)	
Employed in agriculture[1]	0.82 (0.52-1.12)	0.162	0.43 (0.25-0.74)	0.002	1.48 (0.41-5.29)	0.546
Male	1.21 (0.91-1.62)	0.187	1.16 (0.77-1.75)	0.482	1 (0.4-2.47)	0.996
Bednet usage[2,3]	1.06 (1.01-1.08)	0.005	1.06 (1.01-1.11)	0.018	0.95 (0.85-1.06)	0.322
Travel frequency[2]	0.99 (0.99-1.01)	0.516	1.01 (1-1.01)	0.02	1 (0.95-1.05)	0.934
House treated with IRS[2]	0.96 (0.92-0.99)	0.022	0.92 (0.86-0.98)	0.008	1.08 (0.94-1.23)	0.278
Windows protected by screen[1]	0.78 (0.72-1.37)	0.958	1.56 (0.87-2.8)	0.135	1.05 (0.27-4.08)	0.946
Reported previous malaria	1.11 (0.56-1.58)	0.814	0.66 (0.17-2.64)	0.558	9.65 (4.45-20.92)	<0.001

1: status at enrolment; 2: as time-changing covariate (average observed at time of outcome); 3: average bednet usage was defined as the proportion of times a person had answered 'yes' to the question: `Did you sleep under a bednet last night' during ACD; IRS, indoor residual spraying.

Table 3.
Factors associated with P. vivax molecular force of infection (molFOB). Adjusted incidence rate ratio (aIRR) was calculated using a Negative Binomial regression model.

Risk factor	**P. vivax $_{mol}$FOB**	
	aHR	**P**
Community (ref. Ipiranga)		
Brasileirinho	0.47 (0.31-0.71)	<0.001
Puraquequara	0.98 (0.69-1.39)	
Age group (ref. 1-10)		
10-20	1.15 (0.73-1.82)	
21-60	1.06 (0.71-1.60)	0.420
≥61	0.75 (0.43-1.29)	
Employed in agriculture[1]	1.22 (0.81-1.83)	0.350
Male	1.20 (0.91-1.58)	0.200
Bednet usage[2,3]	1.63 (1.24-2.15)	<0.001
Travel frequency[2]	1.00 (0.92-1.10)	0.950
House treated with IRS[2]	0.88 (0.66-1.16)	0.360
Windows protected by screen[1]	1.04 (0.74-1.45)	0.840
Reported previous malaria	3.02 (2.02-4.53)	<0.001

1: status at enrolment; 2: as time-changing covariate (average observed at time of outcome); 3: average bednet usage was defined as the proportion of times a person had answered $yes' \rightarrow thequestion$: Did you sleep under a bednet last night' during ACD; IRS, indoor residual spraying.

Figures

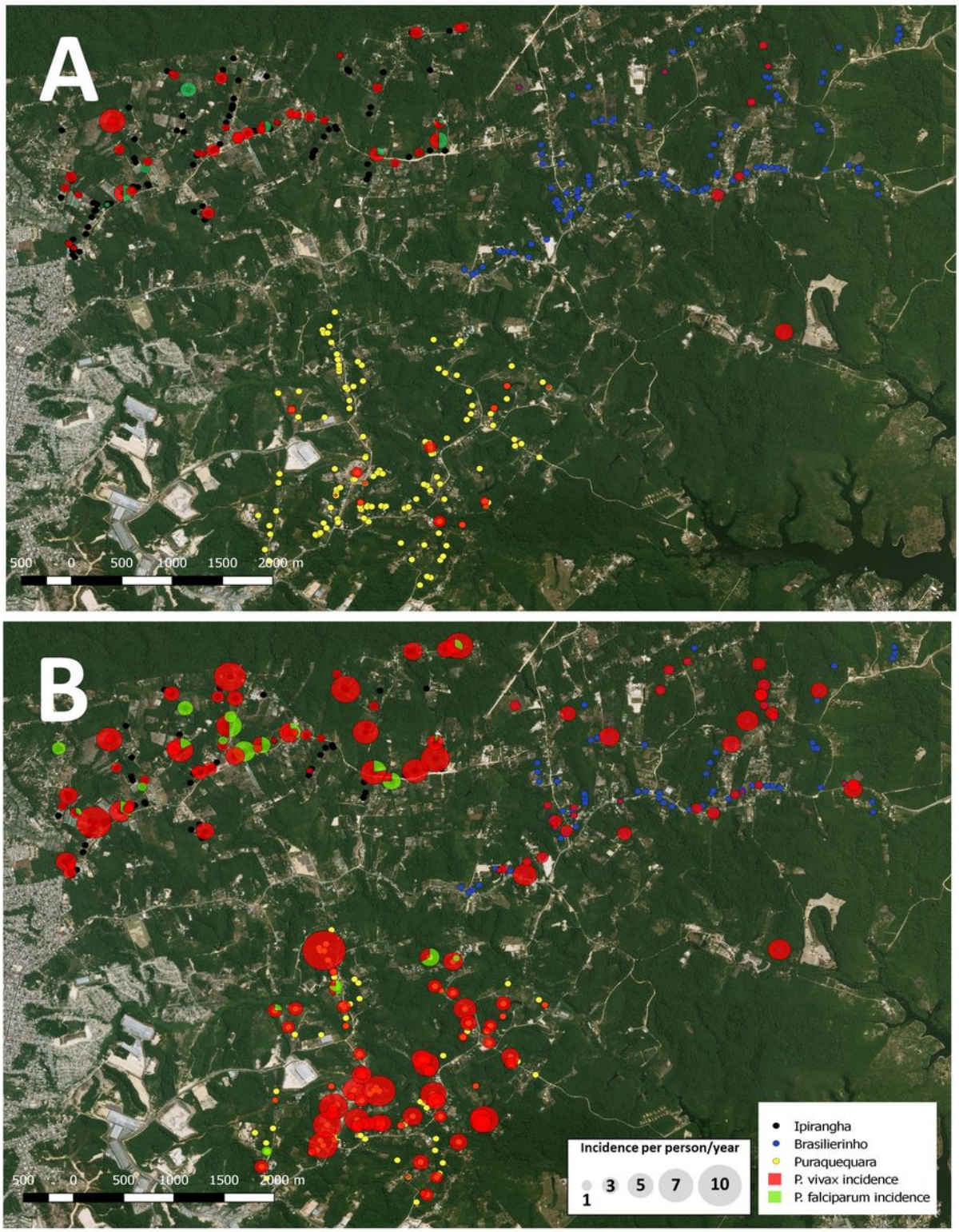

Figure 1

Spatial representation of clinical P. vivax and P. falciparum cases (Panel A) and qPCR detected P. vivax and P. falciparum infections (Panel B). Data are shown as incidence (cases/detections per person per year, aggregated to the household level). Increased diameter of the circles represents increased incidence. Maps were created using QGIS 2.18, with geodata collected for this study.

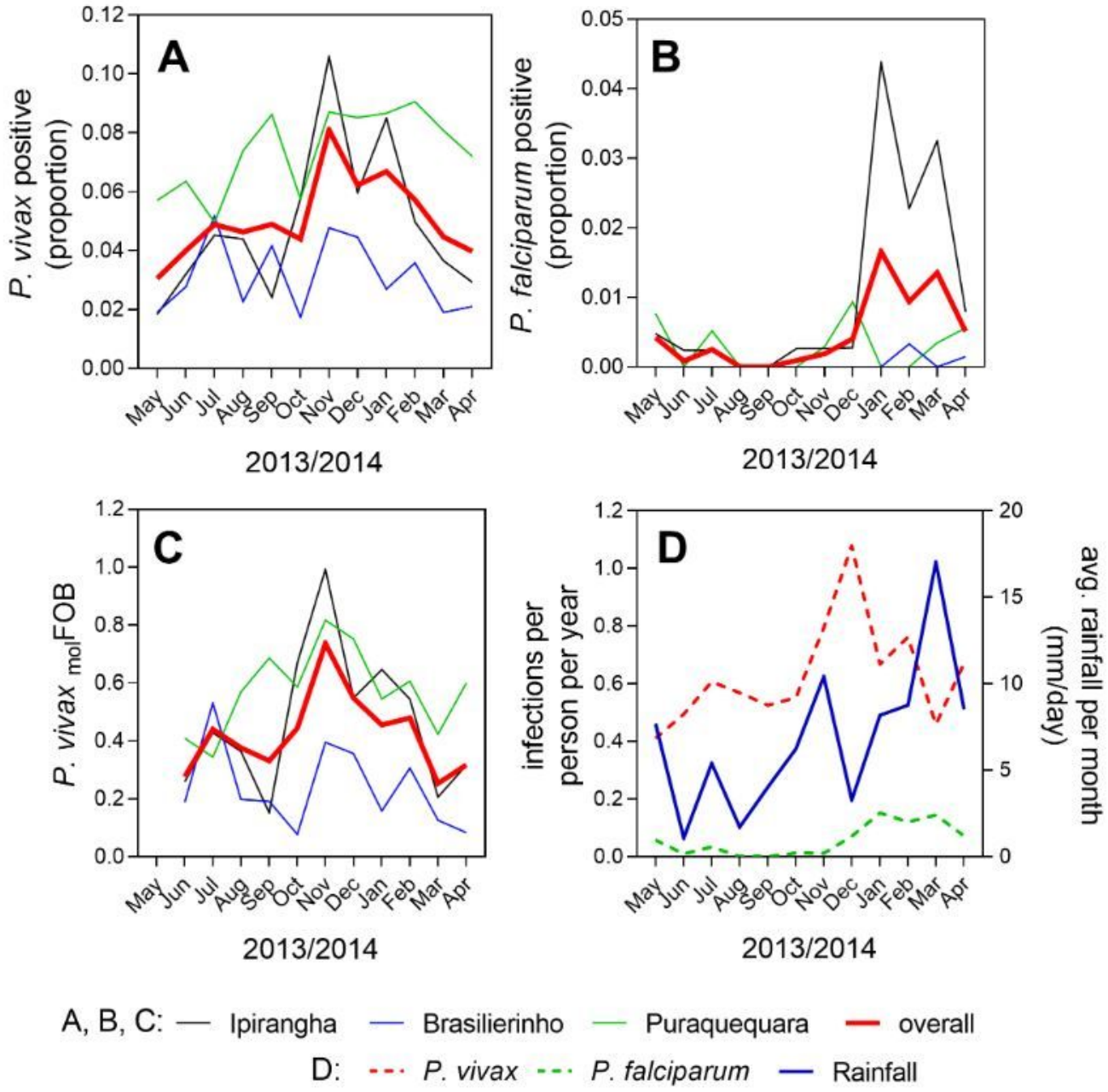

Figure 2

Infection prevalence, molecular force of P. vivax infection and rainfall within the three communities. (A) P. vivax infection prevalence in the three communities, and in in the entire study population. (B) P. falciparum infection prevalence in the three communities, and in in the entire study population. (C) P. vivax molecular force of blood stage infection (molFOB) in the three communities and in the entire population. (D) Rainfall and incidence of P. vivax and P. falciparum infections detected by PCR in the entire study area.

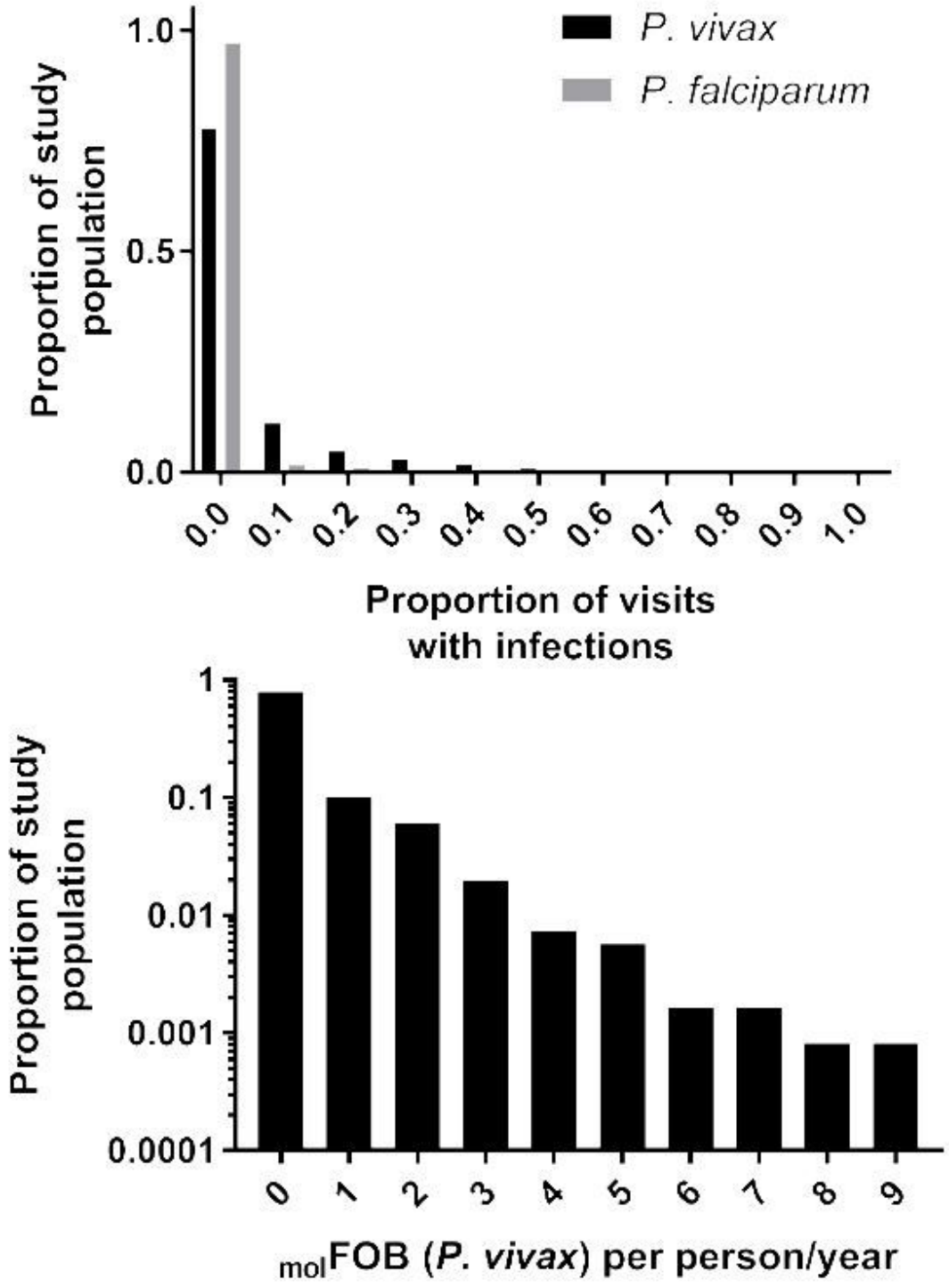

Figure 3

Distribution of Plasmodium infections in the study population. (A) heterogeneity in the incidence of malaria infections. (B) P. vivax molFOB over the entire year of follow-up.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTable2.docx
- SupplementaryTable5.docx
- SupplementaryTable1.Studydatabase.xlsx
- SupplementaryTable3.docx