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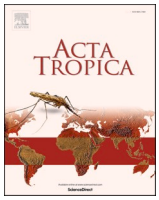

Human parvovirus B19 infection in a *Plasmodium vivax* endemic area on the Brazil-French Guiana border

Ester dos Santos Motta [a], Arthur Daniel Rocha Alves [b], Luciane Almeida Amado Leon [b], José Rodrigo Santos Silva [c], Marcelo Cerilo-Filho [a], Margarete do Socorro Mendonça Gomes [d], Rubens Alex de Oliveira Menezes [e], Ricardo Luiz Dantas Machado [a,f,*], Rita de Cássia Nasser Cubel Garcia [f,*]

[a] *Programa de Pós-Graduação em Microbiologia e Parasitologia Aplicadas, Instituto Biomédico, Universidade Federal Fluminense, Niterói, RJ, Brazil*
[b] *Laboratório de Desenvolvimento Tecnológico em Virologia, Instituto Oswaldo Cruz, Fiocruz, Rio de Janeiro, RJ, Brazil*
[c] *Departamento de Estatística e Ciências Atuariais, Universidade Federal de Sergipe, Brazil*
[d] *Superintendência de Vigilância em Saúde do Estado do Amapá, Brazil*
[e] *Coordenação do Curso de Enfermagem, Fundação Universidade Federal do Amapá, Brazil*
[f] *Departamento de Microbiologia e Parasitologia, Instituto Biomédico, Universidade Federal Fluminense, Alameda Barros Terra S/N, São Domingos, Niterói, RJ, 24020-150, Brazil*

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ABSTRACT

While human parvovirus B19 (B19V) infection is widespread in Brazil, over 99 % of reported malaria cases occur in the Amazon region, mainly by *Plasmodium vivax*. As B19V infection may contribute to anemia in children living in *P. falciparum* endemic areas, this study aimed to investigate the impact of B19V/*P. vivax* coinfection in residents of the municipality of Oiapoque, Amapá, Brazil. A total of 300 serum samples collected in 2014–2015, from individuals infected by *P. vivax* ($n = 148$) and non-infected ($n = 152$), were tested for B19V by serologic and molecular methods. Hemoglobin dosage and cytokine levels were evaluated by automatic method and flow cytometry/ELISA, respectively. Acute B19V infection was diagnosed in 56.8 % (84/148) of infected with *P. vivax* and 38.2 % (58/152) of non-infected individuals ($p < 0.01$), and *P. vivax* was considered a risk factor for B19V infection (OR=2.19; 95 % CI; $p = 0.001$). Participants were grouped into: B19V/*P. vivax* coinfected (CO), *P. vivax* monoinfected (M), B19V monoinfected (B19V), and endemic control (EC) who were negative for both agents. A significant association was found between the CO group and lower hemoglobin levels (RRR= 0.66; 95 % CI; $p = 0.0019$), but no link was found between anemia and coinfection. It was found that higher gametocyte counts (OR=1.002; 95 % CI; $p = 0.0164$), IL-5 (RRR=1.74, 95 % CI; $p = 0.025$) and IL-10 (RRR=1.45; 95 % CI; $p = 0.004$) levels were strongly associated with the CO group. No difference in viral load was observed between the CO and B19V groups. Our study highlights the importance of monitoring the circulation of B19V in *P. vivax* endemic areas.

1. Introduction

Human parvovirus B19 (B19V) is a common pathogen worldwide, classified as belonging to the *Parvoviridae* family, *Erythroparvovirus* genus, and *Erythroparvovirus primate1* species, due to its tropism for erythroid progenitor cells (Penzes et al., 2020; Bua et al., 2016). Currently, B19V has been grouped into three distinct genotypes (1, 2, 3), which are subdivided into subtypes (1a and 1b; 3a and 3b) that form a single serotype (Qiu et al., 2017). All three genotypes have been identified in Brazil (Cnc Garcia and Leon, 2021).

Infection is most commonly acquired during childhood and adolescence, but can occur at any age, usually causing a self-limiting rash known as erythema infectiosum in children and arthropathy in adults, particularly women (Qiu et al., 2017). Because B19V infects and replicates in human erythroid progenitor cells causing a temporary block in erythropoiesis (Bua et al., 2016), viral infection can lead to a marked decrease in hemoglobin in patients with either increased red cell destruction or impaired erythropoiesis, usually manifesting as an acute

* Corresponding authors.
E-mail addresses: ricardomachado@id.uff.br (R.L.D. Machado), ritacubel@id.uff.br (R.C.N. Cubel Garcia).

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episode of severe anemia (Algwaiz et al., 2023; Manaresi and Gallinela, 2019).

One such risk group is children living in malaria-endemic areas. Given the hemolytic potential of *Plasmodium falciparum* infection, a link between malaria and B19V has long been speculated, but the results of studies remain controversial. While several studies have shown that coinfection with B19V and *P. falciparum* may contribute to the worsening of anemia despite effective anti-malarial therapy (Scarlata et al., 2002; Wildig et al., 2006; Manning et al., 2012; Duedu et al., 2013; Toan et al., 2013; Tizeba et al., 2018; Lavrentyeva et al., 2018), others suggest that *P. falciparum* and B19V are independent risk factors for anemia (Wildig et al., 2010; Moses-Otutu et al., 2019; Herr et al., 2020).

Although Brazil has made significant progress in malaria control, this remains a serious public health problem in the Amazon region where >99 % of cases are reported (Carlos et al., 2019), and is predominantly caused by *Plasmodium vivax*, the most widely distributed type of this protozoan. The benign clinical course of this parasite has now been completely reconsidered in the light of evidence that *P. vivax* has the capacity to cause severe disease. However, pathogenesis remains poorly understood (Albrecht et al., 2020; Silva-Filho et al., 2021; Mehkri and Arahalli, 2025). Serologic studies indicate that B19V is also prevalent in an urban area of the Amazon region (Freitas et al., 2008; Figueiredo et al., 2005; Cnc Garcia and Leon, 2021), but the impact of B19V/*P. vivax* coinfection has not been elucidated.

P. vivax is known to induce a strong inflammatory response with robust production of immune effectors, including cytokines, which can lead to anemia and sepsis-like disease manifestations (Castro-Gomes et al., 2014; Menezes et al., 2018; Antonelli et al., 2020; de Jesus et al., 2022; 2023; Ramírez et al., 2023). The main cytokine response to both *Plasmodium* sp. and B19V acute infection is the Th1 profile (mainly IL-6, TNF-α, IFN-γ), which acts to clear these intracellular pathogens from the human body (Menezes et al., 2018; de Jesus et al., 2022; Kerr et al., 2001; 2004; Isa et al., 2007).

It is known that malaria coinfection with other tropical diseases, such as arboviruses and enteroparasitoses, may lead to a distinct cytokine production in infected patients that may affect disease prognosis (Menezes et al., 2018; Kotepui et al., 2023; Cerilo-Filho et al., 2024). Considering these factors, the aim of this study was to perform laboratory diagnosis of B19V infection in individuals living in an endemic area for *P. vivax* infection on the border of Brazil and French Guiana, in order to access the impact of B19V/*P. vivax* coinfection in this population.

2. Materials and methods

2.1. Study population and sample collection

A subset of samples previously evaluated by Menezes et al. (2018) was used. Samples were collected during 2014- 2015 and had been kept at −20 °C in the Center for Microorganisms' Investigation of the Fluminense Federal University until use. Among them, (i) 148 were from patients with acute febrile illness who tested positive for *P. vivax* infection and were collected between the second and sixth day of symptom onset and (ii) 152 were from participants who tested negative for *P. vivax* infection at the time of sample collection. The patients enrolled in this study complied with the following criteria: they sought medical assistance for clinical malaria symptoms, were over seven years old, had either a positive or negative malaria diagnosis by thick blood film or molecular techniques, and lived in Oiapoque, Amapá State, Northern Brazil. Patients who had received antimalarial treatment in the previous 30 days and pregnant women were excluded. All of the non-malaria subjects were genetically independent. Clinical and epidemiological data such as age, gender and number of past malaria attacks were obtained during a specific interview conducted by the physicians and from medical records. Only 2.3 % (7/300) of the participants had no previous contact with *P. vivax,* all of them under 10 years of age.

The quantification of parasitaemia and gametocytaemia was performed using a semi-quantitative evaluation by following the Walker technique according to the protocol described by the World Health Organization (*https://www.who.int/publications/i/item/HTM-GMP-MM-SOP-08*). The microscopic diagnosis was further confirmed by nested-PCR using a previously described protocol (Snounou et al., 1993).

2.2. Serologic and molecular diagnosis of B19V infection

Serum samples were tested for anti-B19V IgM and IgG using commercial enzyme immunoassays (EIA) (SERION ELISA classic Parvovirus, Brazil). Cutoffs were estimated from the optical density (OD) readings of samples and control standards according to the manufacturer's instructions. For IgM, samples were considered negative if the cutoff was < 0.25 IU/mL; borderline: between 0.25 and 0.35 IU/mL; and positive: > 0.35 IU/mL. For IgG, samples were considered negative if < 0.25 IU/mL; borderline: between 0.25 and 0.39 IU/mL; and positive: > 0.39 IU/mL. All the samples with inconclusive results were retested.

For molecular detection and quantification of B19V, DNA was extracted from serum (200μL) using a High Pure PCR Template Preparation Kit (Roche, Germany) according to the manufacturer's instructions. Real-time PCR was carried out using TaqMan system (Applied Biosystems™ 7500 Real-Time PCR System, Applied Biosystems, Waltham, MA, USA), according to Alves et al. (2019). For absolute quantification, a synthetic standard curve of B19V NS1 region (custom synthesized by IDT®, Coralville, USA) was designed (nt 1905–1987, Genbank:NC_000883.2). Primers for the NS1 region (nt 1905F and 1987R) (Nguyen et al., 2002) and a single labeled 5′ FAM probe (6FAM-ACCTCCAAA CCACCCCAATTGTCACA-MGBNFQ) were used. Viral load was expressed as international units/mL (IU/mL) that correspond to number of copies/mL × 3.34 (Koppelman et al., 2004).

Serologic and molecular tests were employed to accurately diagnose B19V infection. Both, IgM and/or qPCR positivity, were used as indicators of recent infection (Maple et al., 2014; Alves et al., 2022).

2.3. B19V genotyping

To genotype B19V strains, a semi-nested PCR to amplify a 476 bp fragment (4214–4689) of the VP1/VP2 capsid gene was performed as described previoulsy (Cubel Garcia et al., 2017). The 476-bp amplicons were purified using the PureLink™ Quick Gel Extraction Kit (Invitrogen, Life Techologies, Carlsbad, CA, USA) and then sequenced directly using the Big Dye Terminator@ kit and an ABI Prism1 3730 DNA analyzer (Applied Biosystems, CA, USA). Both strands of each amplicon were sequenced (Cubel Garcia et al., 2017).

For phylogenetic analysis, the sequences were aligned with the BioEdit Sequence Alignment Editor v7.2.5 software (https://bioedit.software.informer.com/download/) and a maximum likelihood phylogenetic tree was constructed with the Tamura 3-parameters plus gamma (T92 + γ) model and 1000 bootstrapping using the MEGA v. 11.0 software (Tamura et al., 2021). The B19V genotype was determined by including reference sequences of genotypes 1, 2, and 3 available in GenBank.

2.4. Assessment of the hemoglobin and cytokine dosage

Hemoglobin (Hb) concentration was measured in the venous blood using automated equipment (Mindray-BC-3000plus) by Menezes et al. (2018). Anemia was defined according to the World Health Organization (WHO) criteria as a hemoglobin concentration below 13 g/dL in men, below 12 g/dL in women, and below 11g/dL in children (WHO, 2011).

Serum levels of the cytokines IL-2, INFγ and TNF (Th1) and IL-4, IL-5, and IL-10 (Th2) were measured by Menezes et al. (2018) by using flow cytometry using a CBA (cytometric bead array) kit BD (Becton Dickinson, San Jose, CA, USA), they were read on a FACSCanto II-type flow cytometer (BD), while IL-6 levels were measured by de Jesus et al.

(2022) using an IL-6 Human Uncoated ELISA kit (Thermo Fisher Scientific, Waltham, MA USA).

2.5. Data analysis

Statistical analysis were performed with the program R v4.3.3 (R Core Team, 2024). The Shapiro-Wilk test was used to examine the normal distribution of the data. Categorical data (age group, gender, anemia and parasitaemia degree) were described as percentages (numbers). Continuous data (age, residence time in Oiapoque, number of previous malaria episodes, period since last malaria, hemoglobin levels, parasitaemia and gametocytes) were described as median and interquartile range (IQR). Viral load was described as mean and minimum and maximum values. Comparisons of categorical data between groups were performed using Chi-squared or Fisher's Exact Test. Kruskal-Wallis (DUNN or Games Howel post hoc) and ANOVA (Tukey HDS post hoc) were used to compare continuous data between groups. Odds Ratio (OR) was used to evaluate the risk of B19V infection between malarious and non-malarious individuals. Binominal logistic regression analyses were used to determine whether B19V/*P. vivax* coinfection increased parasite or gametocyte levels in the peripheral blood of individuals. Multinomial logistic regression analyses were used to observe the epidemiologic and clinical factors associated with *P. vivax* or B19V monoinfection and coinfection between these two agents. For all these analyses, the significance level was set at 5 % (95 % confidence interval - 95 % CI).

2.6. Ethical considerations

The samples included in this study were collected with the approval of the Research Ethics Committee of the Federal University of Amapa (protocol no 18740413.7.0000.0003). The participants who agreed to participate signed a written informed consent form. For minor participants, their parents or guardians signed the consent forms. The present work was approved (CAAE: 40790120.7.0000.0003) to reuse the samples, waiving the consent of the participants and the parents or guardians of the minors for this new analysis.

3. Results

3.1. Serologic and molecular diagnosis of B19V infection

As summarized in Table 1, among the 300 sera evaluated from individuals residing in a *P. vivax* endemic area, anti-B19V IgM was detected in 44 % (132/300) and anti-B19V IgG in 78 % (234/300) of them. Overlapping anti-B19V IgM and IgG were detected in 106/300 samples, while only IgM was detected in 20/300 samples and only IgG in 110/300 samples. Overall, anti-B19V IgM was detected in 53 % (79/148) of individuals with *P. vivax* infection and in 34.9 % (53/152) of individuals without *P. vivax* infection ($p = 0.0156$). No difference was found in the distribution of the IgM index ($p = 0.9205$) or in the prevalence of IgG ($p = 0.9024$) in individuals testing positive or negative for *P. vivax*.

B19V DNA was detected in 21.7 % (65/300) of the samples, of which 33/65 were from individuals with *P. vivax* infection (Table 1). Viral load ranged from 1.1×10^4 to 5.5×10^6 IU/mL (mean: 3.1×10^5 IU/mL). B19V DNA was detected in 28 % (37/132) of IgM positive samples, but viral load did not change with IgM positivity ($p = 0.2225$). Additionally, 10 B19V DNA positive samples were negative for both anti-B19V IgM and IgG.

The B19V nucleotide sequences obtained from 17/65 serum samples had satisfactory quality for phylogenetic analysis, and all sequences were classified as genotype 1A (Fig. 1). The nucleotide sequences generated in this study were deposited in GenBank under accession numbers PQ425442-PQ425458.

Using both serologic and molecular results, recent B19V infection (anti-B19V IgM positive and/or B19V DNA positive) was found in 56.8 % (84/148) of infected and 38.2 % (58/152) of non-infected individuals with *P. vivax*, and this difference was statistically significant ($p = 0.0015$). Therefore, odds ratio (OR) analyses showed that *P. vivax* infection is a risk factor for B19V infection (OR=2.19; 95 % CI: 1.38 – 3.49; $p = 0.001$).

Approximatelly, 10 % (27/300) of the individuals were found to be susceptible to B19V infection (anti-B19V IgM/IgG and B19V DNA negative). B19V infection status could not be determined in 1 % (3/300) of the participants whose samples tested negative for B19V DNA and had inconclusive results for anti-B19V IgM or IgG (Table 1). Thus, they were excluded from this study and subsequent analyses were performed on 297 samples.

Based on the temporal distribution of B19V cases, a peak occurrence was observed in two periods in 2015, April and July to October (Fig. 2).

3.2. B19V and P. vivax coinfection

The 297 individuals of this study were divided into four groups: (1) coinfected (CO): 84 individuals infected with *P. vivax* and B19V; (2) malaria (M): 62 individuals infected only with *P. vivax*; (3) B19V: 59 individuals infected only with B19V; (4) endemic control (EC): 93 individuals that tested negative for *P. vivax* and B19V infection, as summarized in Table 2. The majority of individuals infected with *P. vivax* were male, 70.9 % in the M group and nearly 60 % in the CO group. Regarding age, residence time in Oiapoque and previous malaria episodes, there was no statistical difference between the groups ($p > 0.05$). The period since last malaria was higher in the B19V and EC groups than in the CO and M groups ($p < 0.05$) (Table 2).

Multinomial regression analyses showed that male gender was a risk factor for CO group, while age, residence time and previous malaria episodes were not associated with coinfection. In addition, period of last malaria was considered as a protective factor for both CO and M groups. The previous malaria episodes were considered as a protective factor for B19V group (Table 3).

Analyses of some hematological data showed that hemoglobin levels were higher in the M group than in the others ($p < 0.05$) (Table 2), which was confirmed by multinomial regression analyses (RRR: 1.50; 95 % CI:

Table 1
Serologic and molecular diagnosis of human parvovirus B19 (B19V) infection in samples from individuals infected or not infected with *P. vivax*.

Laboratory Testing			*P. vivax* infected ($n = 148$)	*P. vivax* non-infected ($n = 152$)	Total number ($n = 300$)
DNA positive	IgM negative	IgG negative	5	5	10
DNA positive	IgM positive	IgG negative	4	2	6
DNA positive	IgM positive	IgG positive	16	15	31
DNA negative	IgM positive	IgG positive	45	30	75
DNA negative	IgM positive	IgG negative	14	6	20
DNA posative	IgM negative	IgG positive	8	10	18
DNA negative	IgM negative	IgG positive	46	64	110
DNA negative	IgM negative	IgG negative	8	19	27
DNA negative	IgM negative	IgG borderline	1	0	1
DNA negative	IgM borderline	IgG negative	1	1	2

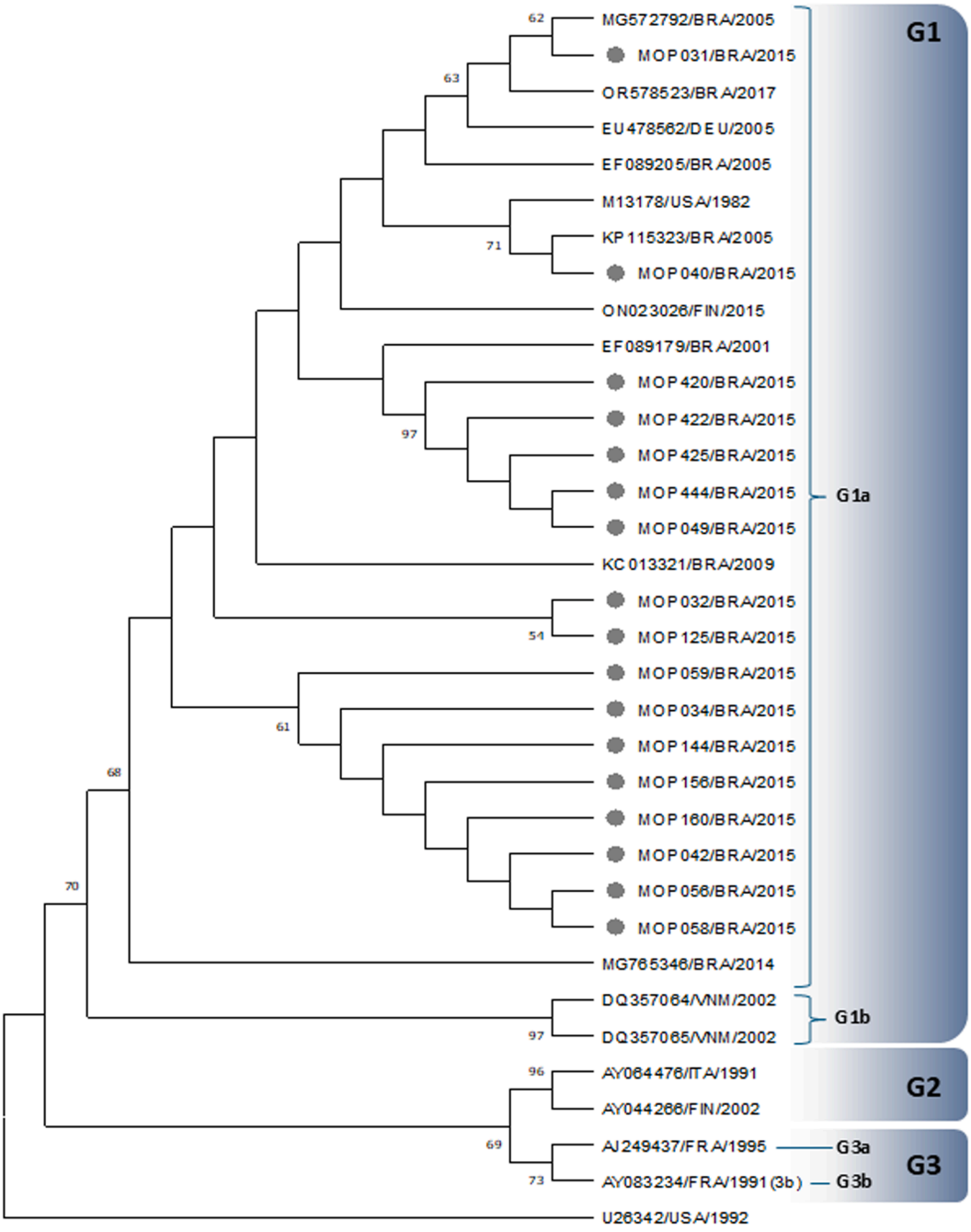

Fig. 1. Maximum likelihood phylogenetic tree of 427 bp fragment of the VP1/VP2 capsid gene of B19V based on Tamura 3-parameters plus gamma (T92+ γ) evolution model. Bootstraps values (> 50 %) based on 1000 replicates are shown. The reference sequences are indicated with accession numbers, country, and year of collection/submission. Sequences of serum analyzed in this study are indicated by gray circle. Simian parvovirus was used as the outgroup.

1.02 – 2.19; $p = 0.037$) (Table 3). In contrast, the B19V group was not associated with changes in hemoglobin levels. Additionally, no association was found between monoinfection (*P. vivax* or B19V) and anemia (Table 3).

An association was found between the CO group and lower hemoglobin levels (RRR: 0.66; 95 % CI: 0.47 - 0.94; $p = 0.019$) (Table 3). However, coinfection was not associated with anemia, considering the lower proportion of anemic individuals in this group compared to the EC (RRR: 0.29; 95 % CI: 0.11 – 0.76; $p = 0.012$) (Tables 2 and 3). Only two females in the CO group had severe anemia (Hb = 7.7 g/dL) (Table 2).

The median of parasitaemia and gametocyte count were higher in the CO group than in the M group, and these differences were statistically significant ($p = 0.0003$ and $p = 0.0008$, respectively) (Table 2). Binomial regression analyses showed a significant association between coinfection and higher peripheral blood gametocyte counts in these individuals (OR:1.002, 95 % CI: 1.001 – 1.004, $p = 0.0164$). No such association was observed for parasitaemia (OR:1.000, 95 % CI: 1.000 – 1.000, $p = 0.2614$).

Regarding to viral load, no difference was observed between the CO and B19V groups (mean: 3.7×10^5 IU/mL and 4.4×10^5 IU/mL, respectively) (Table 2).

3.3. Cytokine levels in the plasma

The circulating cytokine levels in the plasma were available for 138 individuals from groups CO (58/84), M (23/62), B19V (28/58) and EC

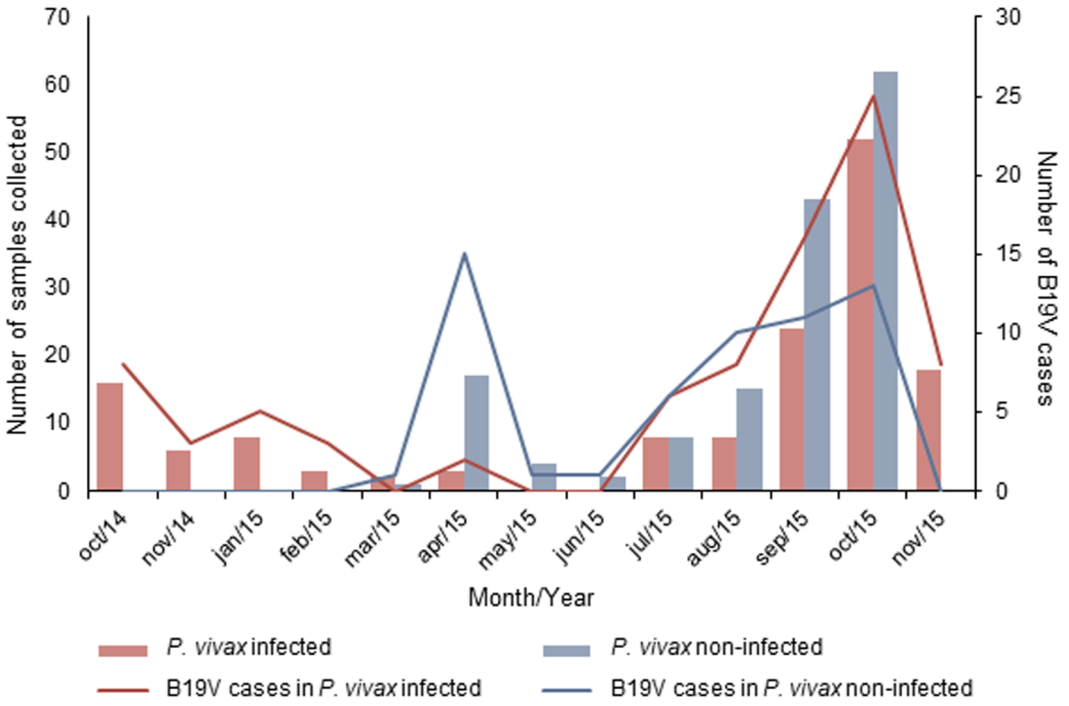

Fig. 2. Temporal distribution of 142 cases of acute B19V infection in individuals residing in the municipality of Oiapoque, Amapá, during the study period (October 2014-November 2015). Bars represent the number of samples collected from *P. vivax* infected (red) and non-infected (blue) individuals over the months; lines represent the number of B19V cases diagnosed in *P. vivax* infected (red) and non-infected (blue) individuals over the months.

Table 2
Epidemiologic and hematologic data for the studied groups (CO, M, B19V and EC).

	P. vivax (+)		*P. vivax* (-)		
	B19V (+) ($n = 84$)	B19V (-) ($n = 62$)	B19V (+) ($n = 58$)	B19V (-) ($n = 93$)	p-value
Groups	Coinfected (CO)	Malaria (M)	B19V	Endemic Control (EC)	
Sex					
Female	36 (42.9 %)	18 (29.%)	29 (50.%)	47 (50.5 %)	0.0525
Male	48 (57.1 %)	44 (70.9 %)	29 (50 %)	46 (49.5 %)	
Age (years)	25.5 (18 – 38)	30.5 (24.3–39.8)	24.5 (17 – 33.8)	23 (17 – 37)	0.1454
Residence time in Oiapoque (years)	24 (18 – 35.5)	30 (22.5– 37.8)	24.5 (17 – 33.8)	23 (17 – 36)	0.1981
Previous malaria episodes	4 (2 – 5)	6 (3.3 – 9)	4 (2 – 6)	5 (3 – 8)	0.0585
Period since last malaria (months)	8 (6 – 9) [b]	7.5 (6 – 9) [b]	9 (7 – 10) [a]	9 (8 – 10) [a]	**0.0026**
Hb levels (g/dL)	13.0 (11.9–12.8) [d]	13.7 (12.7–14.7) [c]	12.9 (11.9–14.0) [d]	12.7 (12–13.6) [d]	**0.0016**
Anemia	25 (29.8 %)	13 (20.9 %)	19 (32.8 %)	37 (39.8 %)	0.1107
Anemia degree					
Mild	18 (21.4 %)	13 (20.9 %)	14 (24.1 %)	28 (30.1 %)	0.1603
Moderate/Severe	7 (8.3 %)	0 (0.0 %)	5 (8.6 %)	9 (9.7 %)	
No anemia	59 (70.2 %)	49 (79 %)	39 (67.2 %)	56 (60.2 %)	
Parasitaemia (par./μL)	2000 (600–4000)	600 (300–2000)	–	–	**0.0003**
Gametocytes (G/μL)	275 (23.8–650)	40 (11.3 – 200)	–	–	**0.0008**
Viral load (UI/mL)	3.7×10^5 (1.4×10^4 – $5{,}5 \times 10^6$)		4.4×10^5 (4.2×10^3 – 4.6×10^6)		0.3110

Values expressed as medians (25 – 75 %): Age, Residence time in Oipoque, Previous malaria episodes, Period since last malaria, Hb levels, Parasitaemia and Gametocytes.
Values expressed as mean (minimum and maximum values): Viral Load.
Anemia was considered as Hb<13g/dL for men, Hb<12g/dL for women and Hb<11g/dL for children (<12 years) according to WHO, 2011.
[a] Difference between the indicated group and the Coinfected and Malaria groups.
[b] Difference between the indicated group and the B19V and Endemic control groups.
[c] Difference between the indicated group and the Coinfected, B19V and Endemic control groups.
[d] Difference between the indicated group and the Malaria group.

Table 3
Association of epidemiological, hematological and cytokine profiles between coinfected, malaria and B19V groups.

	Coinfected		Malaria		B19V	
	RRR (CI95 %)	p-value	RRR (CI95 %)	p-value	RRR (CI95 %)	p-value
Epidemiological data						
Age	1.00 (0.93–1.08)	0.997	1.00 (0.93–1.08)	0.946	0.98 (0.89–1.07)	0.614
Sex | M	2.08 (1.00–4.32)	**0.049**	1.52 (0.66–3.48)	0.325	0.89 (0.41–1.95)	0.771
Residence time in Oiapoque	1.02 (0.94–1.11)	0.672	1.01 (0.92–1.10)	0.877	1.04 (0.94–1.16)	0.430
Period since last malaria	0.86 (0.77–0.98)	**0.020**	0.76 (0.66–0.89)	**0.000**	0.97 (0.85–1.11)	0.675
Number of previous episodes of malaria	0.94 (0.82–1.07)	0.348	1.06 (0.92–1.21)	0.404	0.85 (0.72–0.99)	**0.040**
Hematological data						
Hemoglobin	0.66 (0.47–0.94)	**0.019**	1.50 (1.02–2.19)	**0.037**	1.05 (0.73–1.52)	0.794
Anemia	0.29 (0.11–0.76)	**0.012**	1.14 (0.37–3.55)	0.821	0.84 (0.30–2.36)	0.739
Cytokines profile						
IL-2	0.67 (0.38–1.18)	0.165	0.99 (0.56–1.75)	0.965	0.89 (0.51–1.56)	0.685
IL-4	0.74 (0.50–1.08)	0.116	0.72 (0.48–1.08)	0.112	0.69 (0.47–1.01)	0.054
IL-5	1.74 (1.07–2.83)	**0.025**	1.30 (0.79–2.14)	0.305	1.98 (1.18–3.31)	**0.010**
IL-6	1.02 (0.99–1.05)	0.124	1.01 (0.98–1.04)	0.634	0.99 (0.96–1.03)	0.627
IL-10	1.45 (1.13–1.86)	**0.004**	1.45 (1.13–1.86)	**0.004**	1.45 (1.13–1.86)	**0.004**
TNF-α	0.86 (0.69–1.07)	0.182	0.96 (0.78–1.17)	0.671	0.65 (0.50–0.86)	**0.003**
IFN-γ	1.03 (0.90–1.18)	0.643	1.10 (0.96–1.26)	0.155	0.97 (0.84–1.13)	0.714

RRR: Relative risk ratio; CI: Confidence interval; IL: Interleukin; TNF: Tumor necrose fator; IFN: Interferon.

(35/93).

Although increased IL-2 concentrations were observed in the M group, no significant differences were observed among all the groups studied (Fig. 3**A**). IL-4 concentrations were higher in the EC than CO group ($p < 0.001$). Comparisons between the other groups were not statistically significant (Fig. 3**B**). IL-5 showed higher concentrations in all groups compared to EC ($p = 0.006$). (Fig. 3**C**). Regarding the IL-10 cytokine profile, an increased concentration was observed in the CO group. Statistical differences were found for CO-EC ($p < 0.001$), CO-B19V ($p < 0.001$), CO-M ($p < 0.05$), M-EC ($p < 0.05$) and M-B19V ($p < 0.05$) groups (Fig. 3**D**). The concentration of TNF-α was higher in the CO group than in the B19V ($p < 0.05$) and EC ($p < 0.001$) groups. (Fig. 3**E**). Increased IFN-γ levels were found in CO and M groups, and there was a statistical difference for CO-EC ($p < 0.001$), CO-B19V ($p < 0.05$), M-EC ($p < 0.05$), M-B19V ($p < 0.05$) groups (Fig. 3**F**). Similar to what was observed for IL-10 levels, increased IL-6 levels were found in the CO group. Statistical differences were observed in CO-EC ($p < 0.001$), CO-M ($p < 0.001$), CO-B19V ($p < 0.001$), M-EC ($p < 0.05$), M-B19V ($p < 0.05$) groups (Fig. 3**G**).

Multinomial regression analyses showed that all groups (CO, M, B19V) were associated with increased plasma concentrations of IL-10, while higher IL-5 levels were associated with the CO and B19V groups. No associations were found between IL-6, IFN-γ, IL2 and IL4 concentrations with M, B19V and CO groups (Table 3).

4. Discussion

Although malaria is preventable and treatable, it remains a major public health problem, responsible for high rates of morbidity and mortality (WHO, 2023). Studies of the clinical impact of B19V infection in malaria-endemic areas have only been conducted in regions where *P. falciparum* is the most common etiologic agent of the disease, primarily in African countries (Scarlata et al., 2002; Manning et al., 2012; Duedu et al., 2013; Toan et al., 2013; Tizeba et al., 2018; Lavrentyeva et al., 2018). Similar to what has been reported in these areas, our results showed an increased risk of B19V infection in *P. vivax*-infected individuals, a surprising finding given that African studies have predominantly been conducted in children < 6 years of age, whereas 60 % of our population consisted of young adults aged 17–40 years.

This was probably due to the fact that Oiapoque is a municipality of the Cape Orange National Park (03° 50′ 33″ N; 51° 50′ 06″ W), with a geographical area of 23,034,392 km^2, and a population of 27,482 inhabitants, according to the Brazilian Institute of Geography and Statistics (IBGE, 2022). As B19V is mainly transmitted by the respiratory route, the lower population density (1.19 inhabitants/km^2) of this area makes transmission during childhood more difficult.

Another explanation is the sampling period, since there is evidence that 2014–2015 was an epidemic period for B19V, due to the increasing number of cases in patients with acute febrile illness (Di Paola et al., 2019; Figueiredo et al., 2019), in chronic kidney disease under hemodyalisis (Alves et al., 2020), spontaneous abortion (Oliveira et al., 2019), as well as in asymptomatic blood donors (Alves et al., 2020) in different regions of the country.

Accurate diagnosis using both serologic and molecular methods may also have contributed to the positivity rates found in this population. As the methodology used to define acute B19V infection (IgM and/or DNA detection) varies between different studies conducted in *P. falciparum* endemic areas (Wildig et al., 2006; Manning et al., 2012; Duedu et al., 2013; Toan et al., 2013; Tizeba et al., 2018), it is difficult to compare the results and properly assess the impact of the association of both pathogens. This concern was also addressed by Herr et al. (2020).

Phylogenetic analysis revealed that all the sequences in this study were classified as genotype 1a, which is the most common found in Brazil and worldwide (Qiu et al., 2017; Cnc Garcia and Leon, 2021). To date, no association has been established between genotype and clinical presentation, but Toan et al. (2013) found that genotype 2 was detected

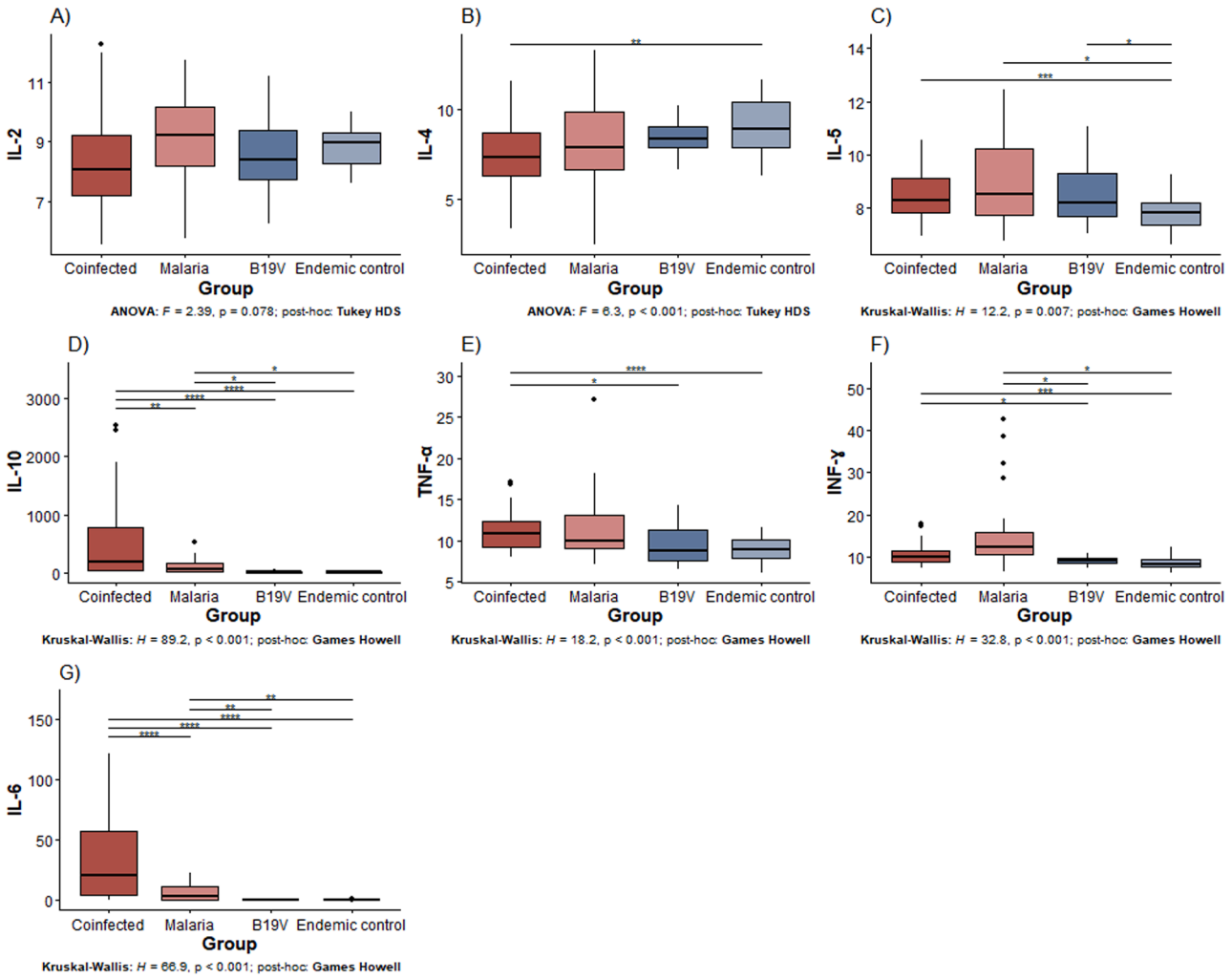

Fig. 3. Serum levels of IL-2 (a), IL-4 (b), IL-5 (c), IL-10 (d), TNF-α (e), INF-γ (f) and IL-6 (g) cytoklnes in pg/mL among malaria, B19V, coinfected and endemic control groups. Significant differences were estimated using the median values for each group, with $p < 0.05$ being considered significant. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and ***$p < 0.0001$.

significantly more often in children with severe malaria than in those with mild malaria. Therefore, monitoring circulating genotypes is important, especially in Brazil where B19V infection is not subject to mandatory reporting.

The seasonal pattern of B19V infection may differ across the country. In a previous study conducted in another state in the Amazon region, B19V was diagnosed between March and July (Figueiredo et al., 2019), whereas in the present study, an increase in B19V cases was observed mainly in the second half of the year, consistent with the seasonality observed in the southern, southeastern and midwestern regions of Brazil (Cnc Garcia and Leon AA, 2021; Almada et al., 2022; Lichs et al., 2024).

Although *P. vivax* malaria affects both genders, cases are known to be more prevalent in adult males, as they make up the majority of the economically active population, particularly in gold mining and agricultural activities in the border regions of Oiapoque (Gomes et al., 2020). Consistent with this, we found that male gender was a risk factor for B19V/*P. vivax* coinfection, but this association was not strong enough for *P. vivax* monoinfection, when males represented slightly >50 % of the malaria group.

The hemolytic potential of *P. falciparum*, coupled with the lyctic B19V infection in the erythroid progenitors, results in failure to restore hemoglobin levels even with antimalarial treatment (Scarlata et al., 2002; Bönsch et al., 2010). In this study, an association between B19V/*P. vivax* coinfection and lower hemoglobin levels was observed. However, no association was found between coinfection and anemia.

As also discussed by Menezes et al. (2018; 2019), the development of anemia in the study population is multifactorial and factors such as other coinfections, particularly enteroparasite infections, genetic alterations such as hemoglobinopathies, and nutritional deficiencies are complicating factors for this clinical picture. Unfortunately, the assessment of anemia severity was hampered by the fact that only two coinfected patients had severe anemia.

Unexpectedly, *P. vivax* monoinfection was associated with higher hemoglobin levels and consequently no association with anemia was found. Since *P. vivax* exhibits a strict tropism for reticulocytes, it may not affect hemoglobin availability as much as *P. falciparum*, which can infect red blood cells at different stages of the erythrocyte cycle (Antonelli et al., 2020). No association was found between B19V monoinfection and altered hemoglobin levels or anemia.

Most of the individuals in the present study had more than one episode of malaria. The data presented here indicate that shorter periods since last malaria may be a protective factor for both B19V/*P. vivax* coinfection and for *P. vivax* monoinfection. Surprisingly, we found a protective association between lower number of previous malaria episodes and B19V monoinfection, supporting the finding that *P. vivax* infection is a risk factor for B19V. It should be pointed out that individuals in the control group also had a history of malaria episodes, as they also live in this endemic area.

Both *Plasmodium* sp. and B19V acute infections induce the release of Th1-type cytokines (Menezes et al., 2018; de Jesus et al., 2022; Kerr et al., 2001; 2004; Isa et al., 2007). As expected, our univariate analysis showed that both coinfected and *P. vivax* monoinfected individuals had higher levels of proinflammatory cytokines, such as IFN-γ, TNF-α and IL-6. Curiously, the same was not observed for acute B19V monoinfection. However, these associations (coinfection and *P. vivax* monoinfection) were not strong enough as these data were not confirmed by multivariate analyses, suggesting that other possible factors, such as the individuals having a history of multiple malaria episodes, may be associated with the increase in this cytokine profile (Antonelli et al., 2020; Varo et al., 2020).

In terms of anti-inflammatory mechanisms, coinfected individuals had the highest IL-10 levels, followed by *P. vivax* monoinfected individuals. This finding was confirmed by multivariate analyses, which indicated that both coinfection and *P. vivax* monoinfection were strongly associated with high IL-10 levels. Unexpectedly, B19V monoinfection was also associated with increased levels of this regulatory cytokine but only in multivariate analyses. However, our findings suggest that coinfection of these two pathogens affects the IL-10-mediated anti-inflammatory response as much as *P. vivax* or B19V monoinfection, since the relative risk was the same for all these three groups.

Despite the anti-inflammatory effects of the Th2 profile during infection, it has an effector side against extracellular pathogens, such as helminths, mainly mediated by IL-4 and IL-5 cytokines (Butcher and Zhu, 2021). Furthermore, it is widely known that border regions, such as Oiapoque, are endemic for multiple pathogens, due to intense population flows that contribute to the spread of infectious diseases (Menezes et al., 2018; 2019; Gomes et al., 2020). Menezes et al. (2019) showed that approximately 60 % of the individuals in this study population were infected with some type of enteroparasite. Therefore, the strong association between high IL-5 levels and the B19V and CO groups found here is not surprising but highlights the importance of understanding the dynamics of the circulation of different pathogens in malaria-endemic areas.

It is known that high peripheral parasite load could be associated with severe malaria and inflammation, while high gametocyte counts are associated with increased transmissibility (Varo et al., 2020). Interestingly, we found higher parasite biomass and gametocyte counts in coinfected individuals. Furthermore, the multivariate analyzes only confirmed a strong association between B19V/*P. vivax* coinfection and high gametocyte counts.

While Wildig et al. (2010) found no association between parasitaemia levels and anti-B19V IgM positivity, Toan et al. (2013) found an association between B19V infection and slowly high parasitaemia in individuals with mild malaria. However, none of the articles on B19V and *P. falciparum* coinfection include an analysis of gametocyte counts (Newton et al., 1997; Duedu et al., 2013; Tizeba et al., 2018; Moses-Otutu et al., 2019; Herr et al., 2020).

Overall, coinfection with *Plasmodium* spp. and other pathogens such as helminths (Nacher et al., 2001) and HIV-1 (Roberds et al., 2021) has been shown to have an effect on high gametocyte counts, even in asymptomatic individuals. In concordance with these data, we show for the first time an association between increased gametocyte carriage and B19V/*P. vivax* coinfection, suggesting an impact of B19V on malaria transmissibility.

Regarding viral load, studies conducted in Kenya and Gabon showed that B19V load in children living in these *P. falciparum* malariaendemic areas ranged from 10^1 to 10^4 IU/mL and that coinfection between these two pathogens did not affect viral load (Wildig et al., 2010; Toan et al., 2013). We also find no association between B19V/*P. vivax* coinfection and B19V load and most of the acutely B19V infected individuals in the present study had lower viral loads (mean: 10^5 IU/mL).

During primary B19V infection, viral load in sera can reach 10^{14} IU/mL. As viremia precedes the seroconversion period, a decrease in B19V load is expected with the appearance of specific antibodies (Maple et al., 2014; Gallinella, 2018). Low viral loads (10^4 to 10^5 IU/mL) in acutely B19V infected patients have also been reported by others (Alves et al., 2020; 2022; Almada et al., 2022).

This study had the limitations inherent to retrospective studies: (i) it was designed to evaluate the effect of enteroparasite coinfection on malaria caused by *P. vivax* in the Brazilian Amazon, so only individuals over seven years of age with suspected of malaria infection, who were admitted to the municipality's Basic Health Units, were asked to participate; (ii) the long storage time of the samples which may have contributed to the lower B19V load, thus affecting the number of samples of satisfactory quality for B19V genotyping; (iii) the lack of homogeneity in sample collection over the study period hampered any association between the increase in B19V cases and the malaria season.

Nevertheless, our study has shown for the first time the effect of B19V infection in a *P. vivax* endemic area and highlights the importance of monitoring the circulation of this virus in the Amazon region, an area with broadly circulation of arboviruses and other pathogens associated with acute febrile illness. Further studies in children are needed to determine whether the coexistence of *P. vivax* malaria and B19V impacts the development of severe anemia.

5. Conclusions

This study is the first report of B19V/*P. vivax* coinfection in a malaria-endemic area. Our data show a significant risk of B19V infection in individuals with *P. vivax* malaria living on the border of Brazil and French Guiana. In addition, an association between high gametocyte counts and coinfection was found, suggesting an impact of B19V on malaria transmissibility. Although B19V/*P. vivax* coinfection was associated with lower hemoglobin levels, no link with anemia was found. However, changes in the cytokine profile were observed in coinfected individuals. Such information may guide further studies to clarify the effect of B19V/*P. vivax* coinfection in the Brazilian Amazon region.

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CRediT authorship contribution statement

Ester dos Santos Motta: Writing – review & editing, Writing – original draft, Methodology, Investigation. **Arthur Daniel Rocha Alves:** Writing – review & editing, Methodology. **Luciane Almeida Amado Leon:** Writing – review & editing, Methodology. **José Rodrigo Santos Silva:** Writing – review & editing, Formal analysis. **Marcelo Cerilo-Filho:** Writing – review & editing, Formal analysis. **Margarete do Socorro Mendonça Gomes:** Writing – review & editing, Investigation. **Rubens Alex de Oliveira Menezes:** Writing – review & editing, Investigation. **Ricardo Luiz Dantas Machado:** Writing – review & editing, Investigation, Funding acquisition, Conceptualization. **Rita de Cássia Nasser Cubel Garcia:** Writing – review & editing, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

All data are presented in the main text, tables and figures

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