Epidemiological characterization and sensitivity to traditional antimalarials of Plasmodium spp. isolates from Porto Velho, western Amazon, Brazil

Caracterização epidemiológica e sensibilidade a antimaláricos tradicionais de isolados de Plasmodium spp. de Porto Velho, Amazônia ocidental, Brasil

Caracterización epidemiológica y sensibilidad a los antipalúdicos tradicionales de Plasmodium spp. de Porto Velho, Amazonía occidental, Brasil

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ABSTRACT

Objective: To describe the epidemiological profile of patients infected with malaria in Porto Velho and the susceptibility of *Plasmodium* spp. to different classes of drugs used in current treatment methods. **Methods:** 708 patients infected with malaria were treated at the Center of Tropical Medicine in Porto Velho, where they answered standard epidemiological questionnaires. Maps were built using place of residence information of 199 infected patients. In chemosensitivity assay, dihydroartemisinin, chloroquine, mefloquine and lumefantrine half maximal inhibitory concentration (IC_{50}) was assessed by schizont maturation assay. **Results:** A higher prevalence of *Plasmodium* spp. infection among men, young people, with recurrent events of malaria and residents of urban area can be observed. The most prevalent malaria species was *P. vivax* followed by *P. falciparum*. Regarding treatment, it was observed that *P. falciparum* demonstrated resistance only to chloroquine (CQ) with a median of IC_{50} (MD of IC_{50}) = 119.8 nM, whereas *P. vivax* demonstrated sensitivity to all drugs (MD IC_{50} < 10; 100; 30; and 150 nM for dihydroartemisinin, CQ, mefloquine and lumefantrine, respectively). **Conclusion:** This study contributes to the understanding of the disease by the city's public health organs, to *Plasmodium* chemosensitivity literature and vigilance, and to decision making processes of city officials.

Key words: *Plasmodium falciparum*, *Plasmodium vivax*, Drug monitoring, Antimalarials, Epidemiology.

RESUMO

Objetivo: Descrever o perfil epidemiológico dos pacientes infectados com malária em Porto Velho e a suscetibilidade do *Plasmodium* spp. a diferentes classes de medicamentos usados nos métodos de tratamento atuais. **Métodos:** 708 pacientes infectados com malária foram atendidos no Centro de Medicina Tropical de Porto Velho, onde responderam a questionários epidemiológicos padronizados. Mapas foram

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construídos usando informações do local de residência de 199 pacientes infectados. No ensaio de quimiossensibilidade, a concentração inibitória (CI_{50}) de diidroartemisinina, cloroquina, mefloquina e lumefantrina foi avaliada pelo ensaio de maturação de esquizonte. **Resultados:** Maior prevalência de infecção por *Plasmodium* spp. entre homens, jovens, com episódios recorrentes de malária e moradores de área urbana. A espécie mais prevalente foi *P. vivax* seguida por *P. falciparum*. Quanto ao tratamento, observou-se que *P. falciparum* demonstrou resistência apenas à cloroquina (CQ) com mediana de IC_{50} (MD de CI_{50}) = 119,8 nM, enquanto *P. vivax* demonstrou sensibilidade a todas as drogas (MD de CI_{50} < 10; 100; 30 ; e 150 nM para diidroartemisinina, CQ, mefloquina e lumefantrina, respectivamente). **Conclusão:** Este estudo contribui na compreensão da doença pelos órgãos públicos de saúde do município, na literatura e vigilância sobre quimiossensibilidade ao *Plasmodium* e para os processos decisórios dos gestores municipais.

Palavras-chave: *Plasmodium falciparum*, *Plasmodium vivax*, Vigilância de medicamentos, Antimaláricos, Epidemiologia.

RESUMEN

Objetivo: Describir el perfil epidemiológico de los pacientes infectados con malaria en Porto Velho y la susceptibilidad de *Plasmodium* spp. a diferentes clases de fármacos utilizados en los métodos de tratamiento actuales. **Métodos:** 708 pacientes infectados con malaria fueron atendidos en el Centro de Medicina Tropical de Porto Velho, donde respondieron cuestionarios epidemiológicos estandarizados. Construyeronse mapas utilizando la información de residencia de 199 pacientes. La concentración inhibidora (CI_{50}) de dihidroartemisinina, cloroquina, mefloquina y lumefantrina se evaluó mediante el ensayo de maduración de esquizontes. **Resultados:** Mayor prevalencia de infección por *Plasmodium* spp. entre hombres, jóvenes, con episodios recurrentes de malaria y residentes de áreas urbanas. La especie más prevalente fue *P. vivax* seguida de *P. falciparum*. *P. falciparum* mostró resistencia solo a la cloroquina (CQ) con mediana IC_{50} (MD de CI_{50}) = 119,8 nM, mientras que *P. vivax* mostró sensibilidad a todas las drogas (MD de CI_{50} < 10; 100; 30; y 150 nM para dihidroartemisinina, CQ, mefloquina y lumefantrina). **Conclusión:** Este estudio contribuye a la comprensión de la enfermedad por parte de las agencias de salud pública del municipio, en la literatura y vigilancia sobre quimiosensibilidad a *Plasmodium* y en los procesos de toma de decisiones de los gestores municipales.

Palabras clave: *Plasmodium falciparum*, *Plasmodium vivax*, Vigilancia de medicamentos, Antipalúdicos, Epidemiología.

INTRODUCTION

Understanding malaria epidemiology in areas of transmission, including the Amazon region, should be a continuous effort due to the parasite being high mutable and because of epidemiological factors related to the disease (WORLD HEALTH ORGANIZATION, 2019). Besides this, malaria causes high morbidity rates in the affected population and, therefore, generates damages, including economic ones, since a large portion of the health sector's budget must be spent on malaria control and treatment (INSTITUTE OF MEDICINE, 2004). Under these circumstances, epidemiological knowledge can be useful in order to plan possible forms of intervention, aiming to control malaria.

Treatment of patients infected with *Plasmodium* is an important intervention strategy for the control and elimination of malaria. The Brazilian government recommends, aiming for the blood schizonticide effect, a fixed combination of artemether (160 mg) and lumefantrine (960 mg) for 3 days in patients > 35 kg; or, for adult patients > 50 kg, artesunate (200 mg) and mefloquine (400 mg) for 3 days, as a second choice for *P. falciparum* chemotherapy (BRASIL, 2021).

3-day treatment plans against *P. vivax* schizonts are mainly based on chloroquine (CQ) (10 mg/kg on day 1 and 7.5 mg/kg on days 2 and 3). In the case of treatment failure, with the presence of parasites above Day 4, an anti-*P. falciparum*-like protocol may be administrated to the patient. A primaquine protocol (0.25 mg/kg

for 7 days) is used in all *P. vivax* treatments since it possesses activity against hypnozoites and gametocytes and due to its synergic effect with CQ (BRASIL, 2021).

Despite the arsenal of medications available, recurrence of *P. falciparum* malaria in patients treated with Artemisinin-based Combination Therapy (ACT) have been reported near the northern border of Brazil (SCHLOSSER AR, et al., 2020). Although there is no evidence in the literature that these treatment failures are directly related to ACT resistance in Brazil, attention to parasite response to this treatment is necessary.

Surveillance studies must also take into consideration the fact that *P. falciparum* malaria may be imported from South American Amazonian countries, like Guyana, with the confirmed *Pfk13* gene mutation C580Y (MATHIEU LC, et al., 2020). This is important since entry control of the population along this part of the Brazilian border is impaired and may favor the entry of possible resistant strains that can spread throughout the country (FERREIRA MU e CASTRO MC, 2016).

P. vivax with reduced sensitivity to CQ has been described in endemic regions, including Brazil, and requires special care the infection it causes is considered benign, but the occurrence of severe malaria and death caused by this agent has been reported (OLIVEIRA-FERREIRA J, et al., 2010; ALEXANDRE MA, et al., 2010; LANÇA EF, et al., 2012).

In this study, we approach the epidemiological characteristics of Malaria cases in Porto Velho, a city in the Amazon basin, as well as the chemosensitivity profile of *P. falciparum* and *P. vivax* strains using the antimalarials recommended by the Brazilian Ministry of Health.

METHODS

Study site and data collection

Porto Velho, capital of Rondônia, has a territorial extension of approximately 34 thousand km^2. It borders the state of Amazonas to the north, the state of Acre to the northwest, and the Plurinational State of Bolivia to the southwest. The urban perimeter of the Porto Velho has an area of approximately 86 km^2 divided into 71 districts (PORTO VELHO, 2020).

The Research Center of Tropical Medicine (CEPEM-SESAU/RO) is a public health, teaching and research unit under the responsibility of the Rondônia State Health Department. CEPEM is the reference center for the diagnosis and treatment of malaria both for Porto Velho and for the surrounding cities.

This was a descriptive and cross-sectional study, carried out between January 2017 and October 2018, with convenience samples. The estimated population at the time of the survey was 518 thousand inhabitants according to the Brazilian Institute of Geography and Statistics (2020).

For Malaria epidemiology, symptomatic patients who spontaneously sought out treatment at the CEPEM were asked to answer a standard epidemiologic questionnaire. The questionnaires were accessed through the National Epidemiological Surveillance Information System and data regarding age, gender, malaria occurrence and dwelling place were obtained.

Malaria patients were approached and invited to participate in the chemoresistance study if they had ≥ 2,000 parasites/mm^3 as confirmed by microscopy counted in thick droplets (TD). Inclusion criteria used were minimum age of 18 years old, monoinfection, uncomplicated malaria and absence of treatment with antimalarials in the last 20 days. Patients with severe malaria, as defined by Ashley EA, et al. (2018), including any of the following symptoms: prostration, confusion or agitation, coma, respiratory distress, convulsions, shock, pulmonary edema, abnormal bleeding, jaundice, anuria and repeated vomiting; people with comorbidities or disabilities along with pregnant and indigenous individuals were excluded.

The epidemiologic results were subdivided into: (i) a group in which all the patients were consulted at CEPEM between January 2017 and October 2018 and (ii) a group of blood donors submitted to a schizont maturation assay (SMA) (referring to samples that acquired 40% maturation of *Plasmodium ex vivo*).

Based on information from these groups, the frequencies of *Plasmodium* spp., gender, age, malaria occurrence and dwelling place of patients were calculated with a confidence interval of 95% (CI 95%). The calculation of sample size for the SMA group was accomplished with 90% CI and 10% margin of error representing the patients seen at CEPEM between January 2017 and August 2018.

Spatial analysis

Based on the patient dwelling location data, *Google Earth software* was used to obtain the exact geographic coordinates.

The addresses were grouped by districts, which are grouped in this study as North, South, East and West zones. The north zone comprises all neighborhoods north of the Imigrantes Avenue polygon (-8.743342387628193, -63.915847241839096). The South zone comprises all the districts south of the polygon formed by the urban section of the BR-364 (Campo Sales Avenue, -8.80049440650604, -63.799932897284535).

The West zone comprises all districts west of the polygon formed by the urban stretch of BR-319 and BR-364 (Governador Jorge Teixeira Avenue, -8.770922781084762, -63.88363927607835) up to the limits of the north zone. The East zone comprises all districts to the east of the polygon formed by BR-319 and BR-364 (Governador Jorge Teixeira Avenue, -8.770466835832607, -63.882673680755886).

Using the Microsoft 3D Maps module from *Excel software*, a *Kernel Map* was created, aiming to identify hotspot regions of patients with malaria in circulation throughout the city.

***Ex vivo* drug susceptibility assay**

The parasitic samples were collected using venipuncture (5 mL) at CEPEM; the species of *Plasmodium* was confirmed by our group using TD stained with GIEMSA. Only samples > 50% of young trophozoites (rings) were used in the assay. CF 11 cellulose method was applied to remove host white blood cells and packed infected red blood cells alone were used for the *ex vivo* drug sensitivity assay. *P. vivax* was maintained in McCoys medium, with 25 mM HEPES, 2 mM L-glutamine, 40 mg/mL gentamycin and 20% AB plasma. *P. falciparum* were maintained in RPMI, with 25 mM HEPES, 2 mM L-glutamine and 40 mg/mL gentamicin and 20% AB plasma (MARFURT J, et al., 2011).

A 96-well plate containing the tested compounds was used to carry out the antimalarial assay. Next, a 2% solution of hematocrit was added (with blood from patients containing more than 2000 parasites/mm^3) using the medium for each species of *Plasmodium*. The antimalarials evaluated were dihydroartemisinin (DHART), CQ, mefloquine (MEF) and lumefantrine (LUM) in 1:4 dilutions from 1000-0.01 nM. The plates were incubated at 37 °C.

From 24-42 h (at intervals of 6 hours), TD were prepared to verify the minimum maturation of 40% of schizonts in the non-treated control group. Upon maturation, the sensitivity of the parasites to the compounds was evaluated by TD of the entire plate and stained with GIEMSA. Differential counts of 200 asexual parasites on the slides were classified as ring stage, trophozoites or schizonts. The schizont classification was selected if at least four well-defined chromatin dots could be seen in the parasite. Free merozoites and gametocytes were not included in the count (MARFURT J, et al., 2011).

Inhibition of 50% parasite growth (IC_{50}) was determined using dose response curves with the program Origin, comparing this data with the growth of the control with no treatment. The IC_{50} values for CQ above 100 nM were considered resistant if > 30nM for MEF, > 10 nM for DHART and > 150 nM for LUM (PADRINES B, et al., 2066; MARFURT J, et al., 2011; PRATT-RICCIO LR, et al., 2013).

Statistics

Measures of central tendency (mean, Md = median and standard deviation) were used for descriptive analyses and to calculate the proportions and the confidence intervals (CI 95%) for each variable. GraphPad Prism 8.4.3 was used to build scatter plots with the IC_{50} observed in the SMA group for each of the tested

antimalarials. The graph represents the probability density function estimated via the kernel (violin graph), to observe where the IC_{50} values are most concentrated.

Ethical Statement

The study protocol was approved (certificate no. 1.993.517) by the Research Center of Tropical Medicine ethics committee.

RESULTS

708 patients answered the questionnaire; 71.5% (71.0-71.9%) of them were males and 28.5% (28.1-29.0%) were females. The most frequent age of infection was 28-37 years old. A high frequency (25.9-36.3%) of patients who had been infected more than 5 times was observed. Most of the patients included in the study live in Porto Velho (70.0-70.9%).

Similar profiles were observed when the group was subdivided by species, although *P. falciparum* infection was most frequent in patients aged 48-57 years old (26.9-27.7%) and *P. vivax* infection was most prevalent in patients aged 28-37 (27.5-27.8%) years old.

Also, 78.1% were diagnosed with malaria caused by *P. vivax* (n = 553), making it more frequent than *P. falciparum* (n = 132, 18.6% of cases). 24 patients (3.3%) were not diagnosed at the species level or had a mixed infection (*P. falciparum* and *P. vivax*), were excluded from any “species” subset and were not blood donors. No *P. malarie*, *P. ovale* nor *P. knowlesi* was diagnosed during the period of this study at CEPEM (**Table 1**).

In relation to chemosensitivity studies, from the 199 blood donors of both species, 63 (32%) reached the minimal 40% maturation of forms (trophozoite to schizont stage). They were divided by species: 42 (66%) patients had *P. vivax* and 21 (24%) had *P. falciparum*. In the SMA group’s epidemiology, we observed that gender, age, malaria occurrence and dwelling place characteristics were the same for each species, indicating that our sample size represent the malaria patients attended at CEPEM (**Table 2**).

Table 1 – Epidemiological profile of malaria patients consulted at CEPEM between 2017 and 2018.

Variables		Total Malaria cases			Malaria cases divided by species					
					P. falciparum			*P. vivax*		
		N	%	CI (95%)	N	%	CI (95%)	N	%	CI (95%)
Gender	Male	506	71,5	(71,0-71,9)	80	60,6	(60,1-61,1)	412	74,5	(73,9-75,1)
	Female	202	28,5	(28,1-29,0)	52	39,4	(38,9-39,9)	141	25,5	(24,9-26,1)
Age	1-17	11	1,6	(1,4-1,7)	3	2,3	(1,9-2,7)	8	1,4	(1,3-1,6)
	18-27	125	17,7	(17,5-17,8)	22	16,7	(16,3-17,0)	101	18,3	(18,1-18,4)
	28-37	188	26,6	(26,4-26,7)	29	22,0	(21,6-22,4)	153	27,7	(27,5-27,8)
	38-47	145	20,5	(20,3-20,6)	32	24,2	(23,9-24,6)	111	20,1	(19,9-20,2)
	48-57	149	21,0	(20,9-21,2)	36	27,3	(26,9-27,7)	107	19,3	(19,2-19,5)
	58-65	57	8,1	(7,9-8,2)	4	3,0	(2,6-3,4)	49	8,9	(8,7-9,0)
	66-100	25	3,5	(3,4-3,7)	4	3,0	(2,6-3,4)	20	3,6	(3,4-3,8)
	No answer	8	1,1	(1,0-1,3)	2	1,5	(1,1-1,9)	4	0,7	(0,5-0,9)
Malaria occurrence	First time	3	0,4	(0,2-0,6)	0	0,0	(-0,5-0,5)	3	0,5	(0,3-0,8)
	Once	103	14,5	(14,3-14,7)	20	15,2	(14,6-15,7)	78	14,1	(13,9-14,3)
	2-4x	160	22,6	(22,4-22,8)	23	17,4	(16,9-17,9)	136	24,6	(24,4-24,8)
	More than 5x	185	26,1	(25,9-26,3)	38	28,8	(28,3-29,3)	139	25,1	(24,9-25,4)
	No answer	257	36,3	(36,1-36,5)	51	38,6	(38,1-39,1)	197	35,6	(35,4-35,8)
Dwelling place	Porto Velho	499	70,5	(70,0-70,9)	102	77,3	(76,1-78,5)	388	70,2	(69,6-70,7)
	Countryside	61	8,6	(8,2-9,1)	10	7,6	(6,4-8,8)	43	7,8	(7,3-8,3)
	Other state	40	5,6	(5,2-6,11)	6	4,5	(3,3-5,7)	33	6,0	(5,4-6,5)
	No answer	108	15,3	(14,8-15,7)	14	10,6	(9,4-11,8)	89	16,1	(15,6-16,6)

Note: Some questions remain as “no answer” due to the difficulty in retrieving information from the health system. 23 patients were not diagnosed at the species level or had a mixed infection and therefore were not included in any “species” subset nor were they blood donors. n = Absolute frequency. % = Percent frequency. CI (95%) = 95% confidence interval. 2-4x = Patients who reported having malaria between 2 up to 4 times. More than 5x = Patients who reported having malaria more than 5 times.

Source: Medeiros DSS, et al., 2022.

Table 2 – Epidemiological profile of the SMA group.

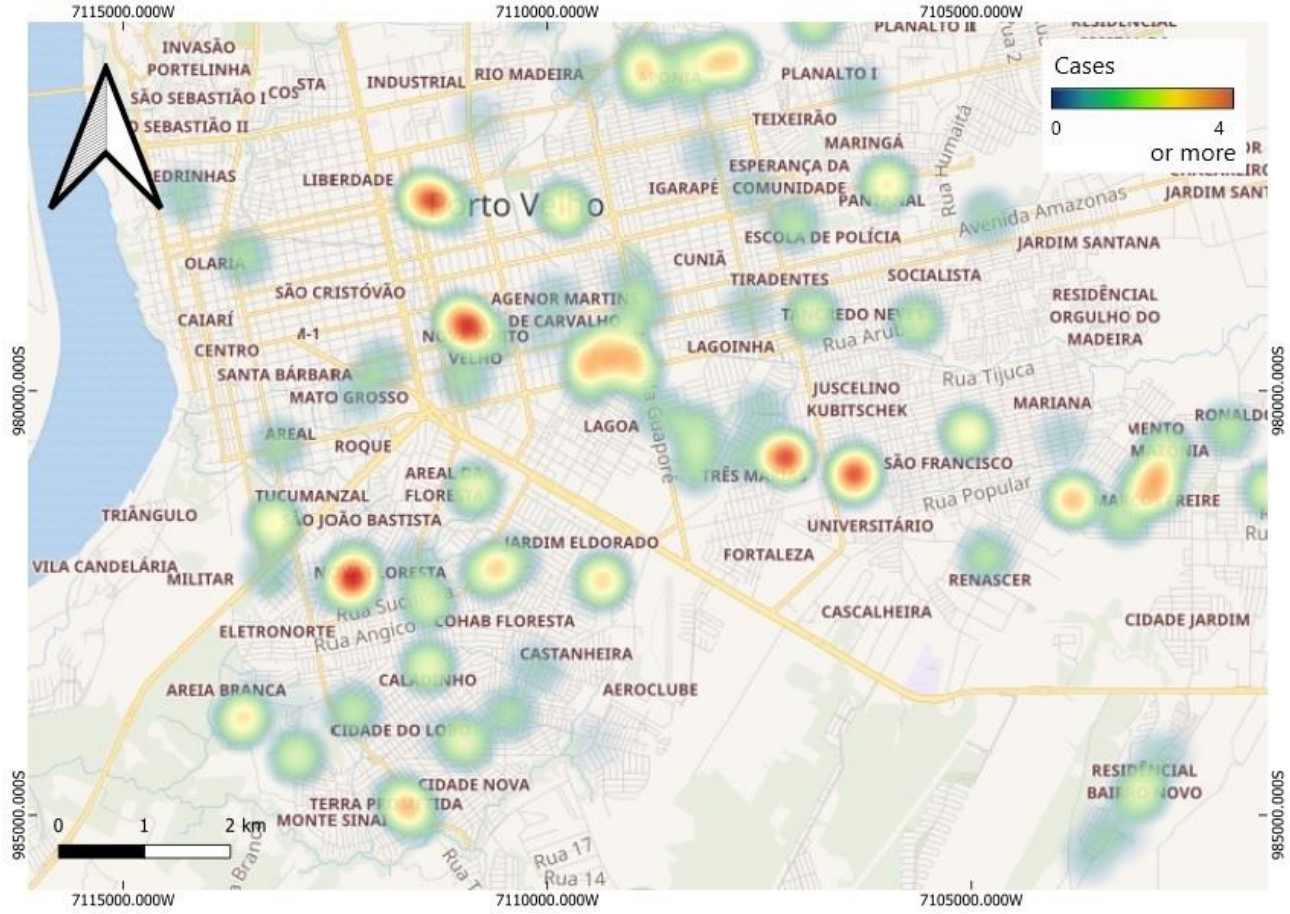
Variables		Total *Plasmodium* samples			*Plasmodium* samples divided by species					
					P. falciparum			*P. vivax*		
		n	%	CI (95%)	n	%	CI (95%)	n	%	CI (95%)
Gender	Male	47	74.60	(72.86-76.34)	13	61.90	(60.46-63.35)	34	80.95	(78.28-83.63)
	Female	16	25.40	(23.66-27.14)	8	38.10	(36.65-39.54)	8	19.05	(16.37-21.72)
Age	1-17	0	0.00	(-0.60-0.60)	0	0.00	(-1.20-1.20)	0	0.00	(-0.70-0.70)
	18-27	15	23.81	(23.21-24.41)	5	23.81	(22.61-25.01)	10	23.81	(23.11-24.51)
	28-37	16	25.40	(24.79-26.00)	5	23.81	(22.61-25.01)	11	26.19	(25.49-26.89)
	38-47	13	20.63	(20.03-22001.24)	4	19.05	(17.84-20.25)	9	21.43	(20.73-22.13)
	48-57	16	25.40	(24.79-26.00)	7	33.33	(32.13-34.54)	9	21.43	(20.73-22.13)
	58-65	3	4.76	(4.16-5.37)	0	0.00	(-1.20-1.20)	3	7.14	(6.44-7.85)
	66-100	0	0.00	(-0.60-0.60)	0	0.00	(-1.20-1.20)	0	0.00	(-0.70-0.70)
Malaria occurrence	First time	2	3.17	(2.57-3.78)	0	0.00	(-1.37-1.37)	2	4.76	(4.03-5.49)
	Once	8	12.70	(12.10-13.30)	4	19.05	(17.68-20.42)	4	9.52	(8.79-10.26)
	2-4x	14	22.22	(21.62-22.83)	2	9.52	(8.16-10.89)	12	28.57	(27.84-29.30)
	More than 5x	19	30.16	(29.56-30.76)	8	38.10	(36.73-39.46)	11	26.19	(25.46-26.92)
	No answer	20	31.75	(31.14-32.35)	7	33.33	(31.96-34.70)	13	30.95	(30.22-31.68)
Dwelling place	Porto Velho	50	79.37	-	17	80.95	-	33	78.57	-
	Countryside	5	7.94	-	3	14.29	-	2	4.76	-
	Other state	2	3.17	-	0	0.00	-	2	4.76	-
	No answer	6	9.52	-	1	4.76	-	5	11.90	-

Note: Some questions remain as “no answer” due to the difficulty in retrieving information from the health system. n = Absolute frequency. % = Percent frequency. CI (95%) = 95% confidence interval. 2-4x = Patients who reported having malaria between 2 up to 4 times. More than 5x = Patients who reported having malaria more than 5 times.

Source: Medeiros DSS, et al., 2022.

As observed in the heatmap, malaria patients' residences are mostly located in the south and east zones of Porto Velho including Bairro Novo, Agenor de Carvalho, Aponiã, Três Marias and Caladinho districts (**Figure 1**).

Figure 1 – Heatmap of malaria patients' residences consulted at CEPEM between 2017 and 2018.



Source: Medeiros DSS, et al., 2022. Microsoft 3D Maps module from *Excel software*, a *Kernel Map.*

After the chemosensitivity evaluation for the circulating strain, *P. vivax* exhibited high sensitivity for all the antimalarials. Medians (MD) in nM were 4.8 for DHART, 16.6 for CQ, 19.8 for MEF and 7.5 for LUM. On the other hand, *P. falciparum* presented a resistance to CQ (MD = 119.8 nM) and sensitivity to the other antimalarials with MD = 1.7 nM for DHART, 15.1 nM for MEF and 17.7 nM for LUM (**Table 3**).

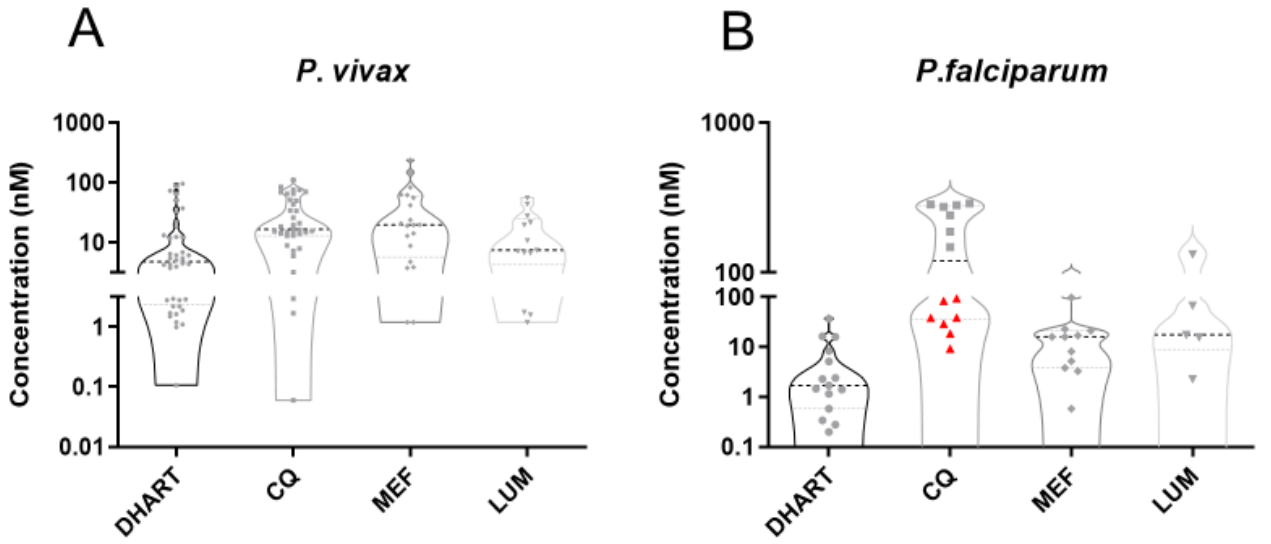
Table 3 – Plasmodium sensitivity to antimalarials, divided by species.

Drug	*P. vivax*		*P. falciparum*	
	n	MD (range) nM	n	MD (range) nM
DHART	40	4.8 (0.1-96.4)	15	1.7 (0.2-36.8)
CQ	36	16.6 (0.06-113)	14	119.8 (9.1-289.4)
MEF	20	19.8 (1.2-240.5)	11	15.1 (0.58-98.8)
LUM	13	7.5 (1.2-56.0)	5	17.7 (2.3-132.3)

Note: n = sample that acquired 40% of schizont maturation and dose-response in the non-parametrical regression. MD = Median. nM = Nanomolar. DHART = Dihydroartemisinin. CQ = Chloroquine. MEF = Mefloquine. LUM = Lumefantrine. **Source:** Medeiros DSS, et al., 2022.

Interestingly, although the profile remains sensitive to traditional antimalarials, it was observed that some patients exhibited IC_{50} above or close to 100 nM for CQ, which is an indication that *Plasmodium* spp. lost sensitivity; > 30 nM for MEF and > 10.5 nM for DHART (**Figure 2**).

Figure 2 – *Ex vivo* susceptibility of *Plasmodium* spp. to antimalarials (dispersion of IC_{50} means in nM with median from malaria patients).



Note: A – *Plasmodium vivax*. B –*Plasmodium falciparum*. Red triangles = IC_{50} of CQ < 100 nM for *P. falciparum*. nM = Nanomolar. DHART = Dihydroartemisinin. CQ = Chloroquine. MEF = Mefloquine. LUM = Lumefantrine. **Source:** Medeiros DSS, et al., 2022.

For *P. vivax*, this phenomenon was observed against CQ (n =1, IC_{50} = 113.0 nM), MEF (n = 7, IC_{50} ≥ 42 nM) and DHART (n = 12, IC_{50} ≥ 11.8 nM) (**Figure 2a**). For *P. falciparum*, this was observed for MEF (n = 1, IC_{50} = 98.8 nM) and DHART (n = 3, IC_{50} ≥ 15.9 nM) (**Figure 2b**). It's worth mentioning that this variation in the IC_{50} could be caused by factors other than loss of sensitivity, such as high parasitemia of the sample and/or an unsynchronized culture.

The inverse phenomenon could be observed in *P. falciparum* IC_{50} for CQ (**Figure 2b – red triangles**). We observed that even with a resistant phenotype (MD IC_{50} > 100 nM), half of the samples presented a sensitive response to CQ (n = 7, IC_{50} ≤ 91.9 nM).

DISCUSSION

Porto Velho is the capital of the state of Rondônia, in the Brazilian legal amazon. It is located in the Northern part of the state (8° 45′ 43″ S 63° 54′ 7″ W) and has a tropical, humid, hyperthermic climate and rainforest-like vegetation (COCHRANE TT and COCHRANE TA, 2006). The city registered 6-7 thousand cases of malaria in recent years, which places it as the main city responsible for malaria cases in the state (about 31%). When this study was carried out, 5,068 cases of *P. vivax* and 933 cases of *P. falciparum* malaria were reported in the city (BRASIL, 2020).

The main population type affected by malaria in the municipality of Porto Velho is, according to the literature and our observations, that of young-adult males and residents of rural areas. They are more frequently affected by *P. vivax* infection than *P. falciparum* (**Table 1**) (BRASIL, 2020).

Gender-related (males = 503 cases) and age-related (27-38 years old = 188 cases) infection in the Amazon can be explained by population habits and occupational activities like vegetal product extraction, illegal mining, road and dam construction and agriculture, since this labor-population is more affected by social determinants that may reflect gender-age asymmetry in malaria incidence (**Table 1**) (SOUZA PF, et al., 2019).

Katsuragawa TH, et al. (2010) commented that the high prevalence of infected persons, and proportionally higher infection by *P. vivax* (78%), is due to asymptomatic infections in Porto Velho, especially near the riverside. In addition, relapses caused by *P. vivax* contribute to a higher proportion of these parasites in the population.

It was observed that part of the population reported having an episode of *P. vivax* malaria more than once (> 63% in both general and SMA groups) (**Table 1**). Simoes LS, et al. (2014) argued that in the Porto Velho population, a higher rate of relapse can be observed in men, with less symptomatic time and with a higher initial level of parasitemia prior to treatment. In addition, the literature exhaustively shows that hypnozoite activation is responsible for relapses in *P. vivax* infection.

In relation to the distribution of malaria cases in Porto Velho and the prevalence of *Plasmodium* spp. infection, something that should be taken into account is the population increase in Porto Velho in recent years which forces the population to live in the peri-urban area; this corroborates our results which demonstrate that many cases are localized in peri-urban areas including neighborhoods with higher vegetation and hydrographic density, such as Bairro Novo, Agenor de Carvalho, Aponiã, Três Marias and Caladinho (**Figure 1**). These features contribute to *Anopheles* vector breeding and development, making the area a permanent site of infection (GIL LHS, et al., 2015).

Porto Velho's population flow between residential areas and the Jirau hydropower dam reservoir, a large undertaking carried out practically inside the Amazon rainforest, and between the urban area and farms or resorts contributes to the occurrence of infected patients in the city (ANGELO JR, et al., 2017). Even if infection does not occur at the patient's place of residence, *P. falciparum*, for example, is present in non-human primate reservoirs in the state and end up maintaining its life cycle (ARAÚJO MS, et al., 2013).

Malaria treatment in Porto Velho follows the guidelines of the Brazilian Ministry of Health (PORTO VELHO, 2018). Artemether is used with lumefantrine for *P. falciparum* and chloroquine with primaquine for *P. vivax*, as first choice of treatment, respectively. In relation to the susceptibility of *Plasmodium* to the class of artemisinins, both species are sensitive to artemisinin, similar to that observed in previous studies carried out in this city (DAHER A, et al., 2018).

Once identified the localities in the city that have the highest number of malaria cases and their cause, a closer integration between the State Malaria Control Program and the Porto Velho Municipal Health Plan is necessary. At the State level the responsibility is, in addition to technical assistance in health actions, to give conditions for the municipality to develop its basic sanitation plans. Providing conditions conducive to human life, especially in peri-urban/expanding regions.

As for the municipal manager, it is up to the reassessment of the current Porto Velho Municipal Health Plan, since it has only one objective related to Malaria, to reduce 10% of cases each year. Note that this objective refers to the year 2016 where the number of indigenous cases was 2,870 (PORTO VELHO, 2018).

In the following year, which is the beginning of this study, the number of cases in the municipality was 2,704 (- 5.8%) while in 2018, when this study was completed, the number of cases was 3,350 (+ 23.9%) (SIVEP, 2021). The increase of indigenous cases plus the observed results in this study reveals the complexity that involves the transmission of *Plasmodium* spp. in the city and the need for more specific goals and proposals for more energetic action plans by the manager.

As a cross-sectional study, malaria cases were not considered taking time into account in our study, however, it can be observed that in the last year there are approximately 12 thousand new cases of malaria in the state of Rondônia, with almost 6 thousand cases in Porto Velho, which demonstrate fails in the search for the elimination of the disease that is a global objective in public health (BRASIL, 2020).

Regarding *P. vivax* susceptibility to quinoline derivatives, the samples obtained were sensitive (IC_{50} <100 nM), agreeing with the current literature (AGUIAR ACC, et al., 2014). Despite this, we observed IC_{50} > 100 nM (n = 1 for QC and n = 7 for MEF) in 8 samples (12%) from patients infected with *P. vivax* with values ranging from 113 to 240 nM. This should be observed with caution for comparisons in future studies of the constancy, increase or decrease of this proportion of cases.

Studies on the Chinese border with Myanmar, which until then were believed to have *P. vivax* CQ-sensitive strains concluded the emergence of a resistant circulating strain based on 64 patient's ex vivo assays, where they found 18.8% of parasites with IC_{50} above the established cut-off of 220 nM in addition to molecular evidence such as mutations in the *Pvmdr1* gene and Pvcrt-o protein (LI J, et al., 2020).

In this study, it was observed that 7 samples of the 20 (35%) tested for MEF with *P. vivax* presented IC_{50} above the admitted cut-off (> 30 nM) however only one sample presented IC_{50} > 100 nM for CQ (2.7%). This factor seems to be due to the treatment, sometimes incomplete, using antimalarial drugs to treat mixed malaria (*P. falciparum* + *P. vivax*). A study conducted with North Cambodian strains showed similar results since *P. vivax* isolates had an average IC_{50} almost 8 times higher for MEF compared to CQ (162.2 nM vs 22.1 nM, respectively (CHAORATTANAKAWEE S, et al., 2017).

A study carried out in Manaus with isolates collected 10 years before the samples of this study, identified 10.7% (n = 11) resistance to CQ and 6.4% resistance to MEF (n = 3) for *P. vivax* (CHEHUAN YF, et al., 2013). This study came to confirm the therapeutic failure observed some years earlier by the same research group, where it is possible to observe 10.1% failure (n = 11) among volunteers who completed the scheme with CQ + PQ. These same patients had acceptable plasma concentrations of CQ (≥ 10 ng / mL) eliminating the possibility of failure to absorb the medication (SANTANA-FILHO FS, et al., 2007).

The circulating strain in Porto Velho, by comparison, in this historical context has already shown an absence of *P. vivax* recrudescence after treatment with CQ + PQ [30]. However, in 9 years, 5.2% (n = 186) of recrudescence was observed after the same treatment scheme (SIMOES LS, et al., 2014).

More recent studies have shown therapeutic failure in 1.2% of volunteers infected with P. vivax in Porto Velho (n = 1) on the 29th day after complete treatment with CQ + PQ (PEREIRA D, et al., 2016). This phenomenon cannot alone confirm the emergence of the circulating strain in the city, but it can correlate what was observed in this clinical study with the findings of this study with the presence of samples with IC_{50} for CQ and MEF > 100 nM and 30 nM, respectively.

Most of the samples obtained from *P. falciparum* were resistant to CQ (MD IC_{50} = 119.8 nM), however almost half of the samples presented a sensitive profile which is not expected nor described in the literature for the city with IC_{50} < 100 nM (sensitive profile). In strains in Senegal this phenomenon was observed with an increase in the sensitivity for CQ (mean IC_{50} = 39.44 nM), probably due to the lack of pressure of this drug in the country since it stopped being used in that country in 2003 (LU F, et al., 2017).

These African patients have been described as having mutations at positions 72-76 and 220 of the *Pfcrt* gene which are responsible for the natural reversal of *P. falciparum* resistance to CQ (LU F, et al., 2017). For further studies, this group intends to analyze the aforementioned codons of *P. falciparum* samples considered sensitive to chloroquine and, if confirmed, it will be the first description of this phenomenon in the region.

This study was carried out in a sectional way with samples from the years 2017 to 2018, however in 2020 there was the worldwide spread of COVID-19 disease caused by the Sars-coV-2 virus and it was believed that quinolinic derivatives such as chloroquine could act against the virus (MEO SA, et al., 2020). However, later the scientific community refuted the efficacy and safety, in the doses administered, for the treatment of this disease (TAKLA M and JEEVARATNAM K, 2020).

Especially in Brazil, treatment with CQ and hydroxychloroquine is still used in the treatment of COVID-19 and stimulated by public figures such as the Minister of Health Eduardo Pazuello and the President of the Republic Jair Messias Bolsonaro (HALLAL PC, 2021). As the effect of the pressure of these antimalarials on *Plasmodium* spp. is not yet known, especially in endemic areas such as Porto Velho, constant surveillance is suggested, especially regarding the response to CQ, of the strains circulating in the city.

CONCLUSION

Based on the results obtained, it can be concluded that the patients affected by malaria treated at CEPEM/RO are mostly men of working age and residents of Porto Velho. Many of them report occurrences of malaria more than once during their lifetime, most of these cases being caused by *P. vivax* infection. Regarding the chemosensitivity of the circulating strain to antimalarials recommended by the Ministry of Health and used in the treatment of the disease in the city, the sensitivity profile of *P. vivax* for all antimalarials tested and resistance of *P. falciparum* only to CQ can be seen. This work is the beginning of a surveillance campaign of

the response to antimalarials in the city and this author suggests monitoring over time both the epidemiological profile of patients and the response to treatment, as recommended by the WHO.

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