

High Prevalence of viral and bacterial coinfections in malaria in Venezuela

David Alberto Forero-Peña (✉ vacter.cv@gmail.com)

Biomedical Research and Therapeutic Vaccines Institute, Ciudad Bolívar

Marisol Sandoval de Mora

Department of Internal Medicine, "Complejo Hospitalario Universitario Ruiz y Páez" Ciudad Bolívar

Iván D. Amaya Rodríguez

Department of Parasitology And Microbiology, School of Health Sciences, Universidad de Oriente, Núcleo Bolívar

Ángel F. Gamardo

Biomedical Research and Therapeutic Vaccines Institute

Melynar Chavero

Biomedical Research and Therapeutic Vaccine Institute, Ciudad Bolívar

Luisamy Figuera

Biomedical Research and Therapeutic Vaccine Institute, Ciudad Bolívar

Natasha A. Camejo-Avila

Biomedical Research and Therapeutic Vaccine Institute, Ciudad Bolívar

María V. Marciano

Biomedical Research and Therapeutic Vaccine Institute, Ciudad Bolívar

Mariana Hidalgo

Immunoparasitology Laboratory, Center for Microbiology and Cell Biology, Venexuelan Institute for Scientific Research, Caracas

Cariagne J. Arenas

Biomedical Research and Therapeutic Vaccines Institute, Ciudad Bolívar

Myriam Arévalo-Herrera

Caucaseco Scientific Research Center: Centro de Investigacion Cientifica Caucaseco

Sócrates Herrera

Caucaseco Scientific Research Center: Centro de Investigacion Cientifica Caucaseco

Research

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Abstract

Background

Malaria remains a significant public health problem worldwide. Simultaneous infections with other pathogens complicate its diagnosis and can also change the clinical course of the disease. The similarities in the clinical presentation of malaria and other infections and the superimposed endemicity result in underdiagnosis of coinfections and increase mortality. No studies have focused on the presence of coinfections in patients with malaria in Venezuela.

Methods

Between June and November 2018, we conducted a cross-sectional study in patients with malaria who presented to any of the three reference medical centers in Ciudad Bolívar, Venezuela. A clinical and laboratory evaluation searching for coinfections with Dengue (DENV), Chikungunya (CHIKV), Viral Hepatitis (VH) (A, B, and C), and Leptospirosis (LEP) was performed using ELISA to test each patient.

Results

We studied a total of 161 patients of whom 106 (65.8%) presented *P. vivax* infection, 43 (26.7%) *P. falciparum*, and 12 (7.5%) had mixed malaria infections. Coinfections were found in 55/161 (34.2%) patients and were more frequent in patients with *P. falciparum* (48.8%) than in those with *P. vivax* (29.2%), or mixed infection (25%) [OR = 2.43; 95% CI = 1.39–4.25; $p = 0.018$]. The most prevalent coinfection was with DENV (14.9%), followed by HAV (11.8%), HBV (6.2%), CHIKV (5.5%), and LEP (3.7%). Coinfection with HCV was absent. Complicated malaria was significantly more frequent in coinfecting individuals (56.4%) than those without coinfection (35.8%) [OR: 2.31; 95% CI = 1.18–4.92; $p = 0.013$].

Conclusion

We found a high prevalence of coinfections in patients with malaria in this region, which was related to a worse outcome. Further prospective studies with samples at different points of infection and the use of molecular tools are needed.

Introduction

Malaria remains a significant public health problem worldwide, with an estimated 229 million malaria cases and 409,000 deaths in 87 malaria endemic countries in 2019 [1]. In the Region of the Americas, malaria cases reduced by 40%, but the region's progress in recent years has suffered from the major increase in malaria in Venezuela due to the recent worsening of its economic and political conditions [2], going from having about 35 500 cases in 2000 to over 467 000 with 403 deaths by 2019, representing more than half of the reported cases and over 70% of malaria deaths in the region [1]. The reported data from Venezuela in 2019 indicate that *Plasmodium vivax* accounted for 77.3% of cases in Venezuela, followed by *Plasmodium falciparum* (16.2%), and mixed (*P. vivax/P. falciparum*) malaria (6.5%) [1]. Historically the malaria incidence remained relatively focused on the Bolívar State, where 70–80% of the cases were reported [3].

Malaria is often associated with outdoor occupations, including mining and agriculture, that also expose people to other vector-borne diseases. Although in malaria-endemic countries, fever is often assumed to be due only to malaria, there is evidence of widespread overdiagnosis in people presenting with severe febrile illness, especially in those living in areas with low-to-moderate transmission and in adults [4]. In recent years, many tropical countries reported an unexpected increase and spread of Dengue (DENV) and Chikungunya (CHIKV) virus cases [5, 6]. Several of these studies describe the concurrent circulation of malaria and these arboviruses [7], and other febrile icteric illnesses like leptospirosis (LEP) [8] and viral hepatitis [9, 10]. It suggests a need for considering differential diagnoses in malaria or mixed infections, mainly in the presence of unexpected clinical findings or apparent inadequate responses to antimalarial treatment in patients suspected to have only malaria.

Coinfections with more than one pathogen complicate the diagnosis and change the clinical course of the diseases and the management; further, the similarities in the clinical presentation of malaria, added to superimposed endemicity, can result in underdiagnosis of coinfections. Limited information is available about the clinical outcome and the precise interactions of these pathogens in coinfections; however, it is assumed that multiple infections complicate the malaria course. Delay in either diagnosis or therapy initiation for any of these infections could have fatal outcomes. The diagnosis of non-malaria febrile illness in resource-limited rural areas remains challenging [11].

A recent decline of the Venezuelan health system capacity has led to the deterioration of the epidemiological surveillance and the malaria control program [2]. Additionally, Venezuela's tropical location favors the concomitant circulation of different zoonoses, including arboviruses, and, consequently, the presence of multiple coinfections in malaria patients. Here we conducted a cross-sectional study to determine the clinical-epidemiological characteristics in patients diagnosed with malaria presenting to the leading diagnostic centers in Ciudad Bolívar, Bolívar state.

Methods

Study site

Bolívar state is located in the south of Venezuela; the total area corresponds to 26.2% of the national territory almost entirely occupied by The Guayana Shield, with 1,837,485 inhabitants in 2018 [12]. This region's mean altitude is 220 meters above sea level, and the temperature range between 26°C and 30°C. Malaria

cases are clustered in the Sifontes municipality, an area where gold mining is the main economic activity [3,13].

Study design and participants

We conducted a cross-sectional study that included patients with malaria, confirmed by microscopic examination of thin and thick blood smears (TBS) and who presented to type II outpatient centers: "El Perú", "La Sabanita" and the "Complejo Hospitalario Universitario Ruiz y Páez", during the period June to November 2018. A trained clinician performed the standard clinical evaluation and a detailed physical examination of all study participants. All patients were treated by the local health provider as soon as the infections were confirmed, using the national antimalarial therapeutic protocol approved by the health authorities [14].

Coinfection evaluation

Two blood samples were collected by venipuncture from each study participant: 3 mL in a tube with EDTA, which was used for hematology evaluation, and 2 mL in a tube without anticoagulant, which was used for the analysis of blood chemistry (urea, creatinine, glycemia, electrolytes, transaminases, lactate dehydrogenase). Coinfections were diagnosed by the presence of specific antibodies detected in serum by enzyme-linked immunosorbent assay (ELISA). We used, Dengue (IgG/IgM, B.Q. Kits, Inc. USA), Chikungunya (IgM/IgG, Abcam USA), Hepatitis A (IgG/IgM, Abcam USA), Hepatitis B (Surface Ag/Anti-Core, Abcam USA), Hepatitis C (IgG/IgM, Abcam USA), and Leptospirosis (IgG/IgM Serion ELISA, USA) following the manufactures' guidelines. These serological tests were selected based on sensitivity and specificity, both higher than 92%.

Data analysis and interpretation

The data were processed using IBM SPSS Statistics for Windows v.25.0 (IBM Corp, NY), and the figures plotted in Microsoft Excel 2013 and Microsoft Power BI v.2.78 (Microsoft, Washington, USA). The data analysis considered descriptions of the characteristics of the studied sample by using descriptive statistics. The distribution of the parameters was statistically evaluated using Kolmogorov-Smirnov test and comparison tests were applied as required (Chi² Pearson, Chi² with Yates correction, Fisher's exact test and t-Student) The Odds Ratio (OR) for complications was determined according to the presence of coinfection with a 95% CI. A *p*-value < 0.05 was considered statistically significant.

Results

We included a total of 161 patients diagnosed with malaria, 106 (65.8%) presented *P. vivax* infection, 43 (26.7%) *P. falciparum*, and 12 (7.5%) mixed malaria (*P. vivax* / *P. falciparum*). Fifty-five (34.2%) patients presented coinfection with other pathogens. Whereas in most of them (44/55), a coinfection with a single pathogen was identified, in the remaining 11 patients harbored more than one pathogen. Coinfections were more frequent in patients with *P. falciparum* (n=21/43, 48.8%) than in those with *P. vivax* (n=31/106, 29.2%) and mixed infections (n=3/12, 25%) [OR=2.43; 95% CI= 1.39-4.25; p=0.018]. Among the coinfecting, DENV coinfection was the most frequent (24/55, 43.6%), followed by HAV coinfection (19/55, 34.6%) (Figure 1). No patients were found coinfecting with the hepatitis C virus.

The prevalence of coinfection in our study was 14.9%, 11.8%, 6.2%, 5.5%, and 3.7% for DENV, HAV, HBV, CHIKV, and LEP, respectively. In patients coinfecting with two or more pathogens, simultaneous coinfection with DENV/HAU was found in 4/11 (36.4%), while other coinfections (DENV/CHIKV, HAV/HBV, HAV/LEP, CHIKV/LEP, and DENV/LEP) were present in one patient each. We found two patients coinfecting by three different pathogens, one with DENV/CHIKV/HAU and the other with DENV/CHIKV/LEP.

As shown in Table 1, the groups' characteristics are homogenous, except for a higher frequency of coinfecting cases with HAV found in Heres municipality than other regions (p= 0.048). Of the 159 patients from the Bolívar state, most had been in the Sifontes municipality (63/159, 39.6%) in the last month, mainly from "Kilometro 88" (52.4%) and "El Dorado" (25.4%). The second most frequent municipality of origin was Heres (52/159, 32.7%), of whom 46/52 (88.5%) came from Ciudad Bolívar, followed by six other municipalities (Figure 2).

Clinical manifestations

The most frequent symptoms in malaria patients were fever (100%), chills (100%), and headache (98.1%) without significant differences between different coinfections. DENV coinfection was associated with asthenia (p= 0.025), cough (p= 0.033), splenomegaly (p= 0.011) and somnolence (p= 0.003). HBV coinfection was associated with lower paleness (p= 0.023) while HAV coinfection was associated with stupor (p= 0.038) and seizures (p= 0.038), (Tables 2 and 3).

Laboratory findings

Hemoglobin levels were similar between coinfecting and non-coinfecting patients. The patients coinfecting with CHKV had lower leucocyte counts (p= 0.010). Coinfecting patients in general, showed elevated aspartate aminotransferase (AST) levels, but, HAV coinfection was associated with higher AST levels (p=0.007). Interestingly, we found a significant association between LEP coinfection and low hematocrit (p= 0.047) and platelet (p=0.019) levels, elevation of AST (p=0.006) and alanine aminotransferase (ALT) (p=0.034) levels, low potassium levels (p=0.043) (Table 4).

Association between complicated malaria and coinfections

A total of 69/161 (42.9%) patients were classified as having complicated malaria according to the local and the WHO [14,15] guidelines. Eight of these patients (11.6%) presented with two or more complications simultaneously. Taken individually, the most frequent complications in patients were jaundice (plasma or serum bilirubin >3 mg/dl) (n=58; 84%), cerebral malaria (n=8; 11.5%), severe anemia (n=6, 8.6%), pulmonary edema (n=3; 4.34%) and renal

impairment (n=2; 2.89%). Of the complicated cases, 42/69 (60.8%) were caused by *P. vivax* infection, 24/60 (34.7%) by *P. falciparum*, and 3/69 (4.34%) by mixed *P. vivax/P. falciparum* malaria. However, the average proportion of complicated over total cases per parasite species was higher for *P. falciparum* than *P. vivax* cases and *P. vivax/P. falciparum* (55.8% vs. 39.6% vs. 25%, respectively; p= 0.045). Complicated malaria was significantly more frequent in the coinfecting group than in the not coinfecting group (56.4% vs. 35.8%) [OR: 2.31; 95% CI= 1.18-4.92; p= 0.013] (Figure 2).

Discussion

Several studies, mainly from sub-Saharan Africa and Southeast Asia, report malaria coinfections with other pathogens such as DENV [16], CHIKV [17,18], HAV [9], HBV [10,19], LEP [20,21], HIV [22], helminths [23] and other febrile illnesses [24]. In Latin America, however, reports of coinfections in malaria patients are limited [10,25,26,27,28]. To the best of our knowledge, there are no reports about the interactions of these pathogens in coinfections in Venezuela, despite multiple infections may complicate malaria and lead to failure when it comes to treatment responsiveness. High prevalence of malaria coinfection was found in this study (34.2%), even higher than reported in Brazil (20%) [25]; but lower than that found in a recent study in India (60%) [29]. Thus, physicians should be suspicious of coinfection in malaria cases with inadequate treatment response or atypical manifestations.

The prevalence of malaria coinfection with DENV (24/161; 14.9%) was much higher than the found in a cross-sectional study in hospitalized patients with the acute febrile syndrome in the Brazilian Amazon (44/1578; 2.8%) [25] or in another study in Mumbai (16/156; 10.25%) [16], or during a dengue outbreak in India (27/367; 7.4%) [30]. In contrast, in Pakistan, the prevalence found was higher (26/78; 33.3%) [31], as well as in India (29/66; 44%), [29]. Thus, the prevalence of coinfection may fluctuate, depending on local endemicity and the sensitivity of the diagnostic methods used. In these studies, the prevalence was estimated based on hospitalized and non-hospitalized patients; therefore, it could not be extrapolated to the community level. We found that DENV coinfection was significantly associated with somnolence and splenomegaly. In agreement with our findings, a study in French Guiana showed worse clinical outcome, with a higher risk of severe thrombocytopenia and anemia in DENV coinfection than in patients with only malaria [28]. Other studies have reported a markedly low platelet count [31] or high elevation of transaminases in the DENV coinfection group [16], however, we did not find these paraclinical alterations in our study. A study in Peru indicated *Plasmodium*/DENV coinfection was not associated with worse disease [26], similar to another study in India where the coinfection with DENV serotype 4 (DENV-4), even was associated with mild malaria. [29]. Differences in DENV serotypes or *Plasmodium spp* may explain the differences of the results.

The second most frequent coinfection was HAV (19/161, 11.8%), higher than that found by Klein *et al.* (10/222, 1.7%) in children from sub-Saharan Africa [9]. This high incidence could be due to a deteriorated water system in Venezuela [32] added to low vaccination rates [33]. In this study, neurological manifestations (stupor and seizures) were associated with HAV coinfection. In contrast, no significant alterations were found in the clinic and in the liver function of the coinfecting patients, as in the study carried out in sub-Saharan Africa [9]. The age group studied could explain this; however, limited information is available on this coinfection. On the other hand, the prevalence of coinfection with HBV (10/161, 6.2%) in our study was similar to that found in Nigeria (11/166, 6.6%) [19] but higher than documented by Braga *et al.* [27] in western Brazilian Amazon (4.2%). In the same study, patients with coinfection presented no clinical differences from those with malaria only, and similar to our findings, nor showed any association with classic signs of a hepatic disorder. In another study, HBV coinfection was more likely to be asymptomatic (OR: 120.13, p<0.0001), even *Plasmodium* parasitemia inversely correlated with plasma HBV DNA levels (R=-0.6; p=0.0003) [10]. In contrast, other studies revealed that coinfection amongst individuals significantly affected the hematological and liver parameters [34, 35]. Our result should be interpreted with caution due to the limited number of coinfecting patients evaluated. We found no malaria/HCV coinfection cases, although coinfection is possible [36], our finding may be explained by the low prevalence of HCV previously reported in Venezuela [37].

CHIKV coinfection was found in 9/161 patients (5.5%), a prevalence lower to that found in Tanzania (8/112, 7.14%) [17], and reported in Kenyan Children (15/158, 9.4%) [38]. In contrast, two extensive studies in India [39] and Senegal [40] found low coinfection prevalence (15/1564, 1.3%) and (3/13845, 0.02%), respectively. The observed variations in the prevalence of CHIKV between different studies may be attributed to study site location including seasonal variations, targeted age groups, agricultural activities, and time [41-43]. Our findings on LEP coinfection (6/161, 3.7%) contrasts with those reported in South India (48/222, 22%) [20] and Thailand (15/193, 7.7%) [44], incidence of LEP in these regions could explain these differences. Although a previous study of *Leptospira* found a high prevalence (80.6%) of Leptospirosis in Bolivar city, this was documented in a group of febrile patients highly suspected of leptospirosis [45]. Other LEP coinfection cases have been documented [21] and have even been associated with severe sepsis [46]. We found an association between LEP coinfection with aminotransferases elevation and thrombocytopenia previously described [47, 48]. In Latin America, LEP/malaria coinfections are rarely reported, but high clinical suspicion must prevail since a late diagnosis could increase morbidity and mortality. Thus, for patients with severe malaria presenting with fever, thrombocytopenia, and altered liver and kidney function diagnosis [49, 50] and empirical treatment for this coinfection should be considered.

Interestingly, simultaneous coinfection with DENV/HAH was found in 4/11 (36.4%) patients with malaria. To date, there are few case reports of this concurrent mixed infection [51]. Thus, we consider they likely occur more frequently than reported in the available literature mainly in developing countries. Other coinfections with two or three pathogens could be explained by overlapping breeding sites for mosquito vector species, especially in malaria, DENV, and CHIKV [52, 53]. Additionally, outbreaks of febrile illnesses are often associated with rainy seasons in the tropics [8].

A high frequency (42.9%) of complicated disease was found and complications were more likely in coinfecting patients compared to patients without coinfections, suggesting that coinfection with another pathogen could exacerbate the clinical course of malaria. Nevertheless, due to the small sample size, further investigation is needed to confirm this observation. Similar results have been found for patients with *P. vivax* and DENV coinfection who had a higher chance of presenting severe disease than those mono-infected with dengue [25]. In contrast, Andrade *et al* found (among 636 Brazilian patients) that HBV infection was associated with a decreased intensity of malaria infection among individuals in the study [10]. In order to determine an appropriate correlation between coinfections, prospective studies should be designed that include a larger number of patients, however controlling real life variables remains a challenge in Venezuela.

This study has some limitations. Although we evaluated both specific IgG and IgM antibodies of possible coinfections, cross-reactivity cannot be ruled out due to chemical similarities of the antibodies investigated as a consequence of the polyclonal activation induced by *Plasmodium spp* infection [54, 55], just as it happens with other highly prevalent infectious including that caused by Epstein Barr virus [56]. Another limitation was the absence of comparison between acute and convalescent sera from the same patient, and the inability to perform molecular tests to evaluate the coinfection. Nonetheless, this a frequent real-life situation regarding resources and poor settings where tests for follow-up of recovered patients are usually not collected and molecular diagnostic studies are limited. Another limitation is that all the enrolled individuals were febrile patients, then studies enrolling asymptomatic individuals should be performed in the future to evaluate the real burden of coinfections in malaria. Finally, the small number of coinfecting patients along with the even smaller frequencies for some coinfections and a number of highly prevalent diseases that were not explored (Chagas disease, Tuberculosis, leishmaniasis, HIV, Syphilis), also represented a limitation.

Conclusions

To the best of our knowledge, this is the first malaria coinfection study in Venezuela. The high prevalence of coinfections found in the main Venezuelan endemic state should contribute to the understanding of the clinical and paraclinical behavior, in order to subsequently develop guidelines and protocols aimed to optimize early diagnosis and target treatments in patients with acute febrile illness. Delay in either diagnosis or start of therapy for any of these infections could have fatal outcomes. Our results should be interpreted with caution due to the limited number of coinfecting patients and to the possibility of cross-reactivity due to polyclonal activation induced by malaria infection. Prospective studies with samples at different points of infection and the use of molecular tools are needed to clarify these findings.

Abbreviations

DENV: Dengue virus; CHIKV: Chikungunya virus; TBS: thick blood smear; HV: viral hepatitis; HAV: Hepatitis A virus; HBV: Hepatitis B virus; HCV: Hepatitis C virus; LEP: Leptospirosis; ELISA: enzyme-linked immunosorbent assay; CI: confidence interval; OR: odds ratio

Declarations

Ethics approval and consent to participate

The study protocol was approved by the Ethics Committee “Complejo Hospitalario Universitario Ruiz y Páez” (CHRRP-CBBS-001-2018). The written informed consents were administered to adult patients who met the recruitment criteria and were procuring treatment. Patients agreed on allowing genetic studies on the parasite.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare they have no competing interests.

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Authors' contributions

DAF-P, MS-DM, MA-H, and S.H. conceived and designed the study. DAF-P, MS-DM, IDAR, AFG, MC, LF., NAC-A, MVM, MH, CJA, and MH carried out the laboratory analyzes. DAFP, MVM and SH conducted data management and data analysis. DAF-P wrote the first draft of manuscript. All authors reviewed and approved the final manuscript.

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Tables

Table 1. Characteristics of patients infected with malaria according to coinfection.

	Coinfection		DENV n=24	HAV n=19	HBV n=10	CHIKV n=9	LEP n=6
	No n=106	Yes n=55					
Age (years)	34 (SD:135)	34 (SD :14.5)	32 (SD:14.9)	35 (SD:14.1)	38 (SD:10.3)	33 (SD:14.1)	38 (SD:17.5)
Sex							
Female	36 (34)	22 (40)	11 (45.8)	6 (31.6)	4 (40)	4 (44.4)	2 (33.3)
Male	70 (66)	33 (60)	13 (54.2)	13 (68.4)	6 (60)	5 (55.6)	4 (66.7)
Municipality*							
Heres	33 (31.1)	19 (34.5)	4 (16.7)	10 (52.6)	4 (40)	4 (44.4)	2 (33.3)
Sifontes	41 (38.7)	19 (34.5)	11 (45.8)	3 (15.8)	3 (30)	4 (44.4)	3 (50)
Angostura	13 (12.3)	5 (9.1)	3 (12.5)	1 (5.3)	1 (10)	-	-
Caroní	1 (0.9)	2 (3.6)	2 (8.3)	2 (10.5)	-	-	-
Cedeño	2 (1.9)	2 (3.6)	1 (4.2)	1 (5.3)	-	1 (11.1)	-
El Callao	7 (6.6)	2 (3.6)	1 (4.2)	1 (5.3)	-	-	-
Piar	-	2 (3.6)	1 (4.2)	-	1 (10)	-	-
Sucre	4 (3.8)	4 (7.3)	1 (4.2)	1 (5.3)	1 (10)	-	1 (16.7)
No Bolívar	6 (4.6)	-	-	-	-	-	-
Scholarship							
High school	43 (40.6)	32 (58.2)	16 (66.7)	10 (52.6)	4 (40)	4 (44.4)	1 (16.7)
Primary	42 (39.6)	16 (29.1)	7 (29.2)	6 (31.6)	3 (30)	4 (44.4)	4 (66.7)
Professional	8 (7.5)	2 (3.6)	1 (4.2)	-	1 (10)	-	-
Technical	5 (4.7)	2 (3.6)	-	2 (10.5)	1 (10)	-	-
Neither	8 (7.5)	3 (5.5)	-	1 (5.3)	1 (10)	1 (11.1)	1 (16.7)
Occupation†							
Miner	43 (40.6)	17 (30.9)	9 (37.5)	2 (10.5)	6 (60)	3 (33.3)	1 (16.7)
Housewife	19 (17.9)	11 (20)	5 (20.8)	6 (31.6)	1 (10)	3 (33.3)	1 (16.7)
Merchant	10 (9.4)	2 (3.6)	2 (8.3)	1 (5.3)	-	-	-
Student	4 (3.8)	2 (3.6)	1 (4.2)	1 (5.3)	1 (10)	-	-
Farmer	5 (4.7)	5 (9.1)	1 (4.2)	3 (15.8)	1 (10)	-	1 (16.7)
Other	22 (20.8)	17 (30.9)	6 (25)	5 (26.3)	1 (10)	3 (33.3)	3 (50)
Unemployed	3 (2.8)	1 (1.8)	-	1 (5.3)	-	-	-

Data are expressed as means and standard deviation (S.D.) or as numbers and percentages for discrete variables. Coinfected patients with more than one pathogen were independently added to each coinfection group.

*Statistically Significant differences in HAV patients (p=0.048; Fisher's exact test).

† Statistically Significant differences in HAV patients (p=0.038; Fisher's exact test) and in HBV patients (p=0.434; Fisher's exact test)

DENV: Dengue virus, HAV: Hepatitis A Virus, HBV: Hepatitis B virus, CHIKV: Chikungunya virus, LEP: Leptospira.

Table 2. Symptoms in malaria patients according to coinfection.

Symptoms	Coinfection		DENV n=24	HAV n=19	HBV n=10	CHKV n=9	LEP n=6
	No n=106	Yes n=55					
Fever	106 (100)	55 (100)	24 (100)	19 (100)	10 (100)	9 (100)	6 (100)
Chill	106 (100)	55 (100)	24 (100)	19 (100)	10 (100)	9 (100)	6 (100)
Headache	103 (97.2)	55 (100)	24 (100)	19 (100)	10 (100)	9 (100)	6 (100)
Lumbar pain	50 (47.2)	23 (41.8)	11 (45.8)	9 (47.4)	3 (30)	3 (33.3)	2 (33.3)
Asthenia*	47 (44.3)	33 (60)	17 (70.8)	9 (47.4)	3 (30)	8 (88.9)	4 (66.7)
Diaphoresis	41 (38.7)	24 (43.6)	10 (41.7)	7 (36.8)	4 (40)	6 (66.7)	3 (50)
Abdominal pain	38 (35.8)	23 (41.8)	11 (45.8)	9 (47.4)	3 (30)	3 (33.3)	3 (50)
Myalgias	43 (40.6)	23 (41.8)	13 (54.2)	9 (47.4)	2 (20)	3 (33.3)	2 (33.3)
Arthralgia	37 (34.9)	23 (41.8)	12 (50)	10 (52.6)	2 (20)	4 (44.4)	2 (33.3)
Othalgia†	2 (1.9)	5 (9.1)	3 (12.50)	1 (5.3)	-	-	1 (16.7)
Dyspnea	14 (13.2)	6 (10.9)	5 (20.8)	1 (5.3)	-	2 (22.2)	1 (16.7)
Bleeding	-	2 (3.6)	2 (0.3)	-	-	2 (22.2)	-
Cough‡	13 (12.3)	10 (18.2)	7 (29.2)	4 (21.1)	-	-	1 (16.67)
Diarrhea	14 (13.2)	7 (12.7)	4 (16.7)	2 (10.5)	1 (10)	-	-
Emesis	20 (18.9)	11 (20)	5 (20.8)	5 (26.3)	1 (10)	2 (22.2)	1 (16.7)

Data are expressed as numbers and percentages. Coinfected patients with more than one pathogen were independently added to each coinfection group.

*Significant association only with DENV (p=0.025; Chi2).

† Significant association only with non-coinfected (p=0.034; Chi2).

‡ Significant association only with DENV (p=0.033, Fisher exact test).

DENV: Dengue virus HAV: Hepatitis A Virus, HBV: Hepatitis B virus, CHIKV: Chikungunya virus, LEP: Leptospira.

Table 3. Signs in malaria patients according to coinfection.

Sing	Coinfection		DENV n=24	HAV n=19	HBV n=10	CHIKV n=9	LEP n=6
	No n=106	Yes n=55					
Jaundice	34 (32.1)	16 (29.1)	9 (37.5)	6 (31.6)	1 (10)	2 (22)	2 (33.3)
Pallor*	60 (56.6)	29 (52.7)	15 (62.5)	8 (42.1)	2 (20)	6 (66)	2 (33.3)
Splenomegaly [†]	5 (4.7)	9 (16.4)	4 (16.7)	2 (10.5)	-	2 (22)	1 (16.7)
Hepatomegaly	10 (9.4)	6 (10.9)	10 (9.43)	1 (5.3)	1 (10)	2 (22)	1 (16.7)
Oliguria	6 (5.7)	7 (12.7)	4 (16.7)	1 (5.3)	1 (10)	1 (11)	1 (16.7)
Somnolence [‡]	9 (8.5)	8 (14.5)	6 (25)	2 (10.5)	-	2 (22)	2 (33.3)
Stupor [§]	1 (0.9)	2 (3.6)	2 (8.3)	2 (10.5)	-	1 (11)	-
Seizures	1 (0.9)	2 (3.6)	2 (8.3)	2 (10.5)	-	1 (11)	-
Edema	3 (2.8)	3 (5.5)	2 (6.7)	1 (5.3)	-	-	-
Ascites	-	1 (1.8)	1 (4.8)	-	-	-	-

Data are expressed as numbers and percentages. Coinfected patients with more than one pathogen were independently added to each coinfection group.

*Significant association with Hepatitis B (p=0.023; Fisher's exact test).

[†]Significant association with Dengue (p=0.011; Chi² Yates' correction).

[‡]Significant association with Dengue (p=0.003; Chi² Yates' correction).

[§]Significant association with Hepatitis A (p=0.038; Chi² Yates' correction).

^{||}Significant association with Hepatitis A (p=0.038; Chi² Yates' correction).

DENV: Dengue virus HAV: Hepatitis A Virus, HBV: Hepatitis B virus, CHIKV: Chikungunya virus, LEP: Leptospira.

Table 4. Paraclinical findings of malaria patients according to coinfection.

Laboratory	Coinfection		DENV n=24	HAV n=19	HBV n=10	CHIKV n=9	LEP n=6
	No n=106	Yes n=55					
Hemoglobin (g/dL)	11 (2)	11 (2)	10 (3)	12 (2)	11 (2)	11 (2)	10 (2)
Hematocrit (%)	35 (5)	33 (7)	32 (9)	3 (7)	34 (4)	33 (9)	29 (7)*
Leucocytes (10 ⁹ /L)	6 (2)	6 (4)	7 (5)	6 (2)	6 (1)	5 (1) [†]	6 (2)
Platelets (10 ⁹ /L)	90 (44)	87 (55)	100 (76)	76 (29)	95 (24)	80 (25)	57 (24) [‡]
Glycemia (mg/dl)	78 (26)	81 (23)	90 (23) [§]	74 (15)	84 (24)	80 (24)	80 (36)
Urea (mg/dl)	32 (22)	40 (33)	22 (44)	32 (9)	38 (33)	37 (20)	38 (21)
Creatinine (mg/dl)	1 (0.6)	1.2 (1.2)	1.4 (1.7)	1.1 (0.3)	1 (0.2)	1.3(0.4)	1 (0.2)
TB (mg/dl)	3 (2.8)	3.7 (3)	3.6 (3.6)	4.1 (3.2)	4 (3.3)	2.3 (0.8)	3.2 (2.2)
AST (U/L)	90 (62)	121 (98)	118 (114)	118 (70) [¶]	89 (44)	136 (77)	190 (148)**
ALT (U/L)	93 (99)	136 (115)	84 (67)	98 (169)	85 (62)	117 (63)	227 (160) ^{††}
LDH (U/L)	541 (190)	530 (156)	457 (85)	546 (153)	583 (202)	530 (143)	530 (56)
Sodium (mEq)	140.2 (2.3)	140 (3.8)	139.8 (5.1)	140.5 (2.8)	140.1 (1.6)	139.8 (3.2)	139.5 (5.3)
Potassium (mEq)	4.2 (0.5)	4.2 (0.6)	4.4 (0.5)	4.4 (0.4)	4.4 (0.5)	4.3 (0.8)	3.5 (0.7) ^{‡‡}
Chlorine (mEq)	102 (3)	103 (3)	103 (2.96)	103.1 (2.7)	101.7 (1.73)	104.6 (3.4)	105.7 (5.69)

Data are expressed as means and standard deviation (S.D.), except for ALT in HAV which is expressed as median and interquartile range. Coinfected patients with more than one pathogen were independently added to each coinfection group.

*Statistically significant differences (p=0.047; t Student)

[†] Statistically significant differences (p=0.010; t Student)

[‡] Statistically significant differences (p=0.019; t Student)

[§] Statistically significant differences (p=0.016; t Student)

^{||} Statistically significant differences (p=0.023; t Student)

[¶] Statistically significant differences (p=0.007; t Student)

** Statistically significant differences (p=0.006; t Student)

^{††} Statistically Significant differences (p=0.034; t Student)

^{‡‡} Statistically significant differences (p= 0.043; t Student)

DENV: Dengue virus, HAV: Hepatitis A virus, HBV: Hepatitis B virus, CHIKV: Chikungunya virus, LEP: Leptospira, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, TB: Total bilirubin.

Figures

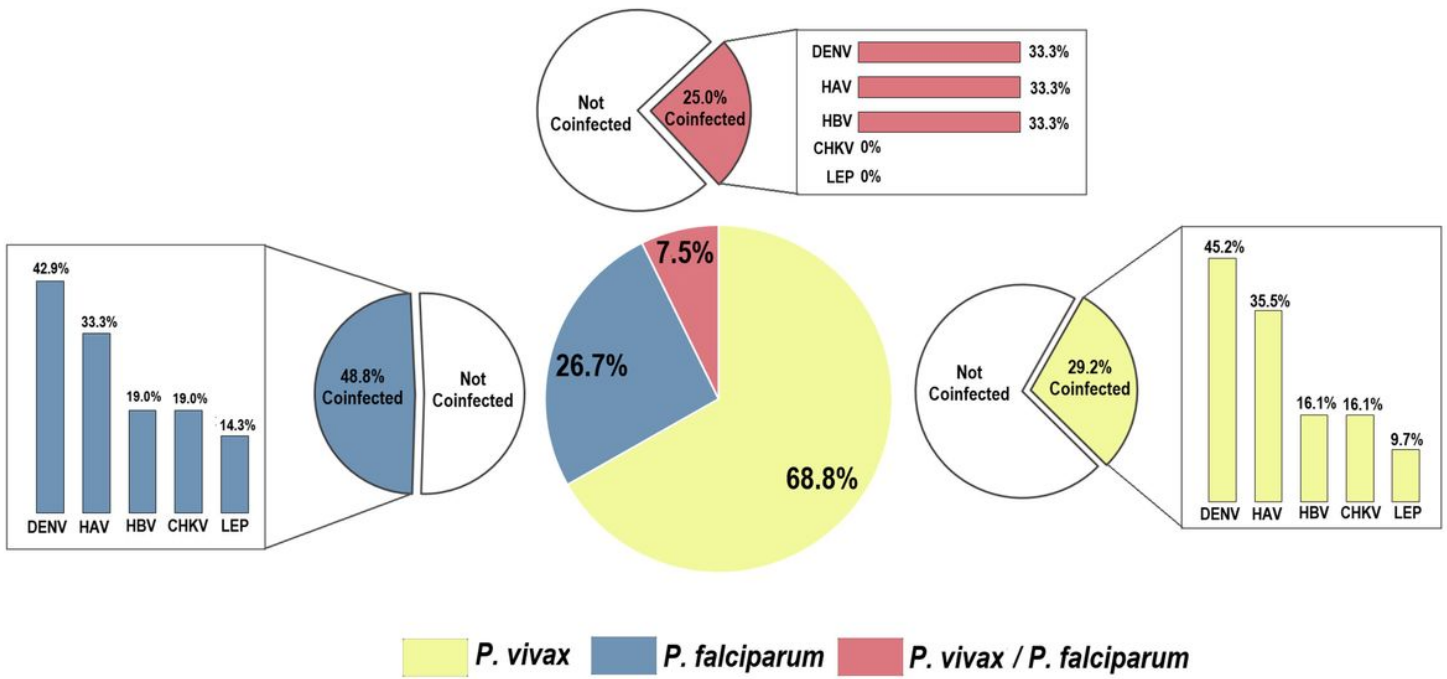


Figure 1
 Frequency of malaria coinfections according to the parasitic species. Central pie chart shows proportions of patients according to Plasmodium species. Small pie charts show proportions of coinfecting patients. Bars show proportions of patients according to coinfecting pathogen. Patients with more than one coinfection were added individually to each group. DENV: Dengue virus, HAV: Hepatitis A virus, HBV: Hepatitis B virus, CHKV: Chikungunya virus, LEP: Leptospira.

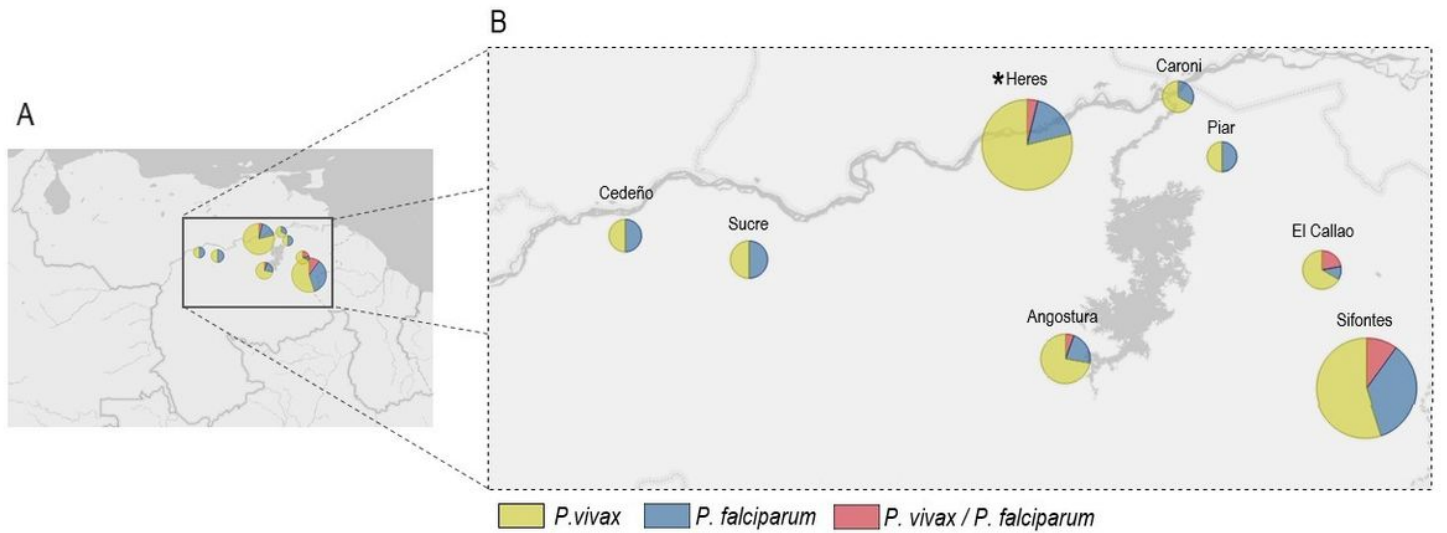


Figure 2
 Origin of malaria patients and their parasitic distribution. A. Map of Venezuela B. Location of the main studied municipalities in Bolivar state, to the south of Venezuela. Pie charts show the proportion of patients from that municipality according to the Plasmodium species. The map is also showing other relevant features of the landscape, including the localization of the capital (asterix), Ciudad Bolivar in Heres municipality. Map was created in Microsoft Power BI version 2.78.5740.861 (February, 2020). Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

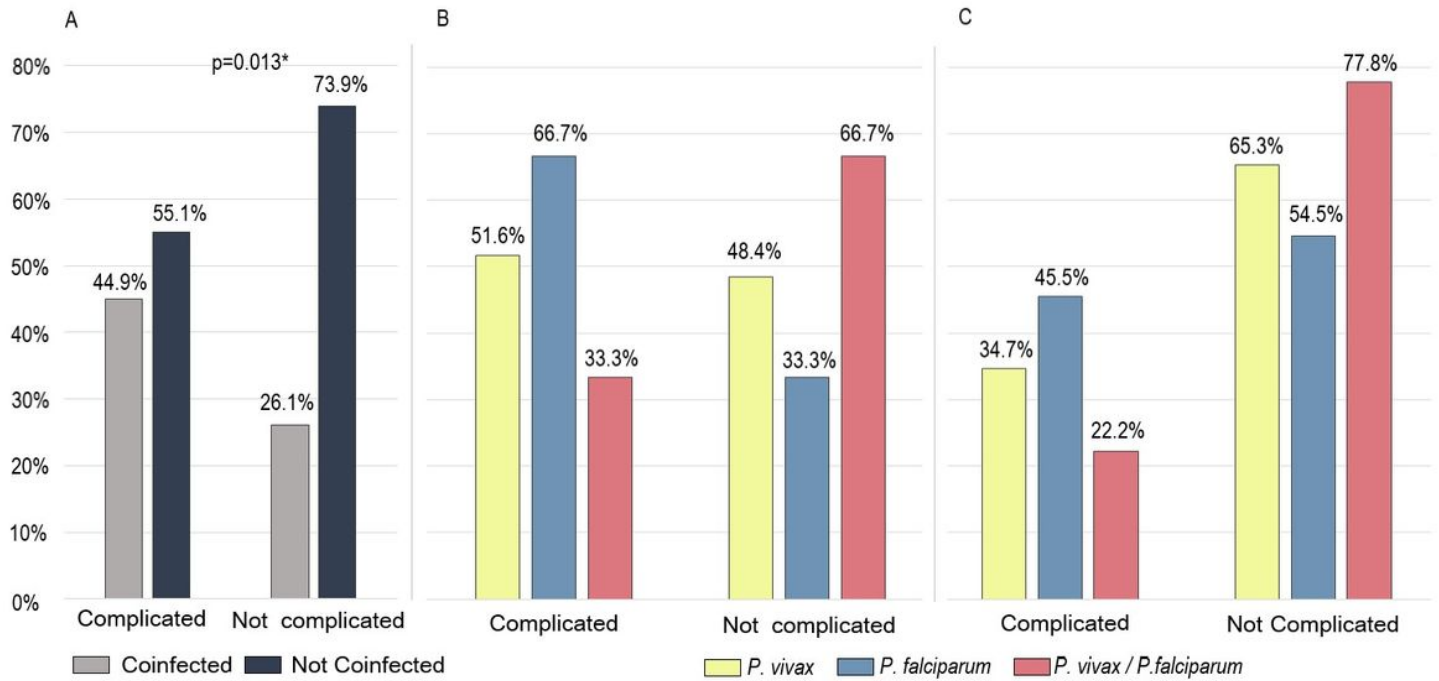


Figure 3
 Malaria infection according to parasite species and coinfections and their complications. Panel A: Malaria patients according to coinfection and complication states. Panel B: Coinfected patients. *P. vivax*: *p*=0.419 (Chi2); *P. falciparum*: *p*=0.226 (Chi2); *P. vivax/P. falciparum*: *p*=0.819 (Chi2 Yates correction). Panel C: Not coinfected patients. *P. vivax*: *p*=0.693 (Chi2); *P. falciparum*: *p*=0.291 (Chi2); *P. vivax/ P. falciparum* : *p*=0.598 (Chi2 Yates correction).