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Severe and benign *Plasmodium vivax* malaria in Emberá (Amerindian) children and adolescents from an endemic municipality in Western Colombia

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Summary Malaria in children is still an important public health problem in endemic areas of South-East Asia and Latin America. Certain forms of the disease, such as *Plasmodium vivax* severe malaria, are still neglected. This descriptive study assessed the frequency of severe and benign *P. vivax* infection in Emberá children (<14 years of age) from an endemic municipality in Colombia in 2013, using the WHO criteria. During 2013, 270 Emberá children presented 349 episodes of malaria. From them, 22 (8.1%) presented at least one of the criteria for severe malaria. Some patients with *P. vivax* presented with severe malaria (severe anemia, renal dysfunction,

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respiratory distress and seizure). Mixed malaria cases presented more complications than those with monoinfection (OR = 5.535; 95%CI 1.81—16.9). In Colombia, few data are available about severe *P. vivax* malaria in children, especially in the Amerindian ethnic groups. Mixed infections were associated with increased risk of severe malaria. At the same time, detailed and prospective studies are needed to measure the real impact of severe vivax malaria, as was evidenced in this paper.

Introduction

Despite significant reductions made during the last decade in the incidence of malaria in different regions of the world, this parasitic disease is estimated to kill 660,000—1,240,000 people annually, with children under five years of age representing >85% of malaria-related deaths [1—4]. In the specific case of the Americas, over the past decade, the number of confirmed malaria cases reported in the region decreased by almost 58%, from 1.1 million in 2000 to 469,000 in 2012 [1—3]. The reduction in children under five years of age was 48% [1—3]. The malaria mortality is projected to decrease by 52% in all of the age groups and by 60% in children younger than 5 years of age by 2015 [1—4]. This reduction represents substantial progress toward the World Health Assembly's target of reducing the malarial burden by 75% by 2015 [1—4].

This change in pattern in the Americas was observed in all of the endemic countries, with the exception of Venezuela, Haiti and Guyana [1,2]. In Venezuela, which is a country with a Human Development Index (HDI) of 0.748 and gross national income per head of US$11,475, the malaria incidence increased between 2000 and 2012. A similar increase in incidence was observed in Guyana and Haiti, but both of these countries have a much lower HDI than Venezuela, and a devastating earthquake hit Haiti during that period [2].

In Colombia, although there has been a significant reduction also, specifically, from 125,262 cases in 2007 to 60,179 in 2012 [1,5,6], there are many endemic areas that have high annual parasitic indexes (API) (>50 cases/1000 pop.) [7], especially in the western pacific coast area, where there are 32 departments of the country; Chocó has the highest number of cases and incidences [5,6]. In Colombia, approximately 12 million people are estimated to be at some risk for malaria. *Plasmodium falciparum* is responsible for <20% of the malaria cases overall, although the proportion is higher in some departments of the country [1,5,6].

Departments in Colombia are grouped by regions, and one of them, which is located in the Andean area, is the Coffee-Triangle. This area is a topographical region that includes three departments (Caldas, Quindío and Risaralda) with 53 municipalities and a total population of 2,484,345 for the year 2013. In this region, one of the municipalities, Pueblo Rico (Fig. 1), reported 43.7% of the malaria cases between 2007 and 2011 (2877 of 6582), with 59.5 cases/1000 pop. in 2009 (the highest incidence in this 53-municipalities region) [7].

These less developed municipalities included urban areas above 1560 m.a.s.l. and rural below that altitude, with a population of 12,966 inhabitants in 2013 [8,9]. This population includes three ethnic groups: mestizo (54%), afro-colombians (14%) and Amerindians (Emberá) (32%); the last of which is considered to be a high biological and behavioral malaria-risk group in the area [7].

The urban area of this municipality has the only hospital in the area, Hospital San Rafael, which is responsible to attend to the population that lives in Pueblo Rico, including patients with malaria, who, as occurs in Colombia and other countries, are freely attended to from diagnostics to treatment, which is covered and funded by the government through the Malaria Control Programme [5].

Although it has epidemiological relevance, there is a lack of studies on malaria in the municipality (Pueblo Rico) as well as in the department where it is located (Risaralda) [7]. Thus, our research groups were interested on developing studies to understand malaria epidemiology in risk groups as well as clinical and therapeutics issues in this area. In this study, our aim is to assess the frequency of severe and benign infection due to *Plasmodium vivax*, the predominant etiological species in Pueblo Rico and Colombia, in Emberá children (under 15 years of age) from the Pueblo Rico municipality, Risaralda, Western Colombia (Fig. 1), during the year 2013.

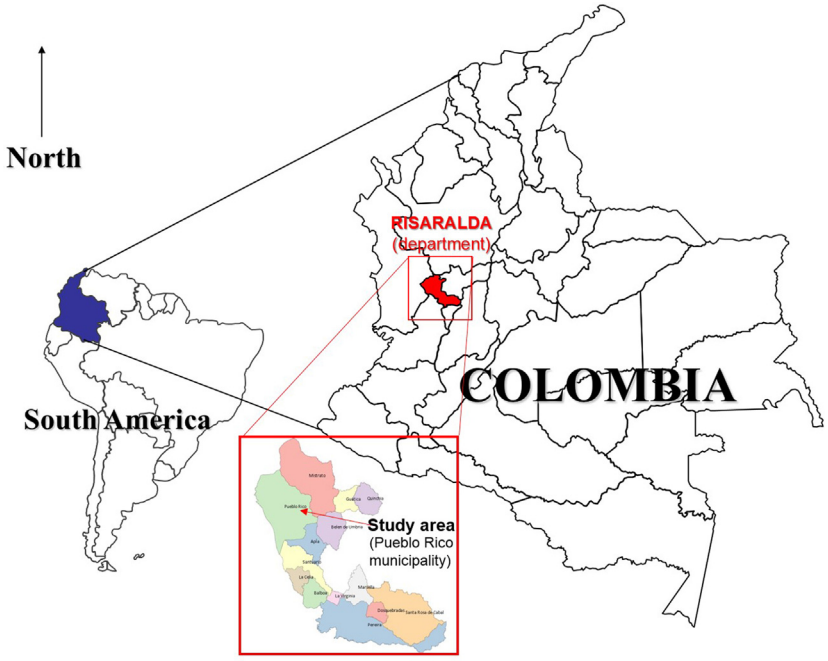

Figure 1 Study area, Pueblo Rico municipality, Risaralda department, Colombia, South America.

Methods

This research was a descriptive, cross-sectional study in which the discharge records of the Emberá children and adolescents (<14 years of age) who were admitted at a primary care hospital in Pueblo Rico municipality (Hospital San Rafael) (Fig. 1), Risaralda (Colombia) during January 2013 to December 2013, were examined retrospectively. All of the acutely sick children who were admitted with a febrile illness that was confirmed as malaria (by testing the peripheral smears) were included in this study. Malaria infection was confirmed in all of the cases with thick and thin blood smears, with external quality control. The different *Plasmodium* species were identified morphologically by laboratory experts who were dedicated to interpreting malaria smears at the Malariology Regional Offices in Colombia. In addition, all of the positive smears and 10% of those considered to be negative by the regional laboratories were reevaluated by a third national malaria reference microscopist, to confirm the diagnosis of malaria. However, all of the children who were admitted with a febrile illness but were negative for the malaria test and those who had other associated infections such as enteric fever, urinary tract infections or proven meningitis/encephalitis, were excluded from the study. The children from the other departments with a positive malaria diagnosis were also excluded from the study. The case definition of severe malaria was made according to the 2014 WHO criteria for *P. falciparum* and *P. vivax* [10] (the presence of one or more of the following clinical or laboratory features: impaired consciousness, respiratory distress [acidotic breathing], multiple convulsions, prostration, shock, pulmonary edema [radiological], abnormal bleeding, jaundice, severe anemia, hypoglycemia, acidosis, hyperlactatemia, renal impairment) [10].

The test results on malaria for the study period were collected as well as the case sheets and discharge summaries of the cases. The case sheets and discharge summary sheets were examined in detail, to identify the clinical manifestations and laboratory findings of the associated cases. The study was approved by the institutional committee as well as by the Committee of Bioethics of the Universidad Tecnológica de Pereira, Pereira, Risaralda, Colombia.

Data regarding the patients' age, sex, clinical presentation, time length for symptoms, investigations, reappearance (new episode in a patient who suffered malaria during the same year) and outcome were recorded. Patients were categorized into severe and non-severe groups based on WHO guidelines for the classification of severe malaria. Reappearance was defined as a patient who presented with symptoms and confirmed malaria 28 days after treatment. Relapse definition cannot be applied for *P. vivax* malaria in this setting given that patients reside in an endemic zone.

All of the data were recorded on a predesigned form; the results were tabulated and analyzed statistically by SPSS statistical software (version 22). The paired *t*-test, independent sample *t*-test and repeated measures analysis of variance were used for the analysis as well as the chi-square test for categorical variables; 2-sided *p*-values are reported at the 0.05 significance level. The reappearance times (the time between the first diagnosis and subsequent diagnosis) of *P. vivax* were calculated using survival analysis.

Results

During the study period, 270 Emberá children presented 349 episodes of malaria. Out of this total, 20.7% ($n = 56$) of the patients presented more than one episode of malaria during the year, which sums to 135 additional episodes. From the total number of children with malaria, 14.1% ($n = 38$) presented two episodes, 4.8% ($n = 13$) three episodes and 1.85% ($n = 5$) four episodes during the same year. The median number of episodes per patient per year was 1.0 (interquartile range [IQR] 1—1). Among those reappearance cases, 40.7% were less than 2 years of age.

The age of the subjects ranged from 6 days to 13.8 years, with a median of 2.64 years of age (IQR 1.29—5.08) (73.3% were <5 years of age) (Table 1). One case occurred in a newborn of 6 days (congenital malaria) and one case in a neonate of 24 days (neonatal malaria). The etiological agent of these cases corresponded to *P. vivax* in 89.7% of the cases (Table 1). All of the patients came from the rural areas of the municipality, and all of them were included in the government-subsidized health insurance.

Once diagnosed, all of the patients were treated with chloroquine, primaquine (*P. vivax* cases) and artemether-lumefantrine (*P. falciparum* with or without *P. vivax* coinfection). During the study period, no malaria-related deaths were reported at the hospital and none in Risaralda department.

All of the patients presented fever (100%). From the total, 22 patients (8.1%) presented at least one of the WHO criteria for severe malaria and, then, were hospitalized (Table 2). The hospitalization rate was significantly higher in mixed infections (26.3%) than in monoinfections (either due to *P. vivax* or *P. falciparum*) (6.1%) (crude OR = 5.535; 95%CI 1.81—16.9). Three patients were hospitalized two times (6 episodes, 5 due to *P. vivax* and one due to *P. vivax*/*P. falciparum*). Among them, one patient (7 months) presented with severe anemia and renal dysfunction (due to *P. vivax* infection). Among the hospitalization episodes, 32% presented with respiratory distress (6 due to *P. vivax* and 2 due to *P. vivax*/*P. falciparum*). Three hospitalized patients presented hepatosplenomegaly (12%); two were due to *P. vivax*/*P. falciparum*, and one was due to *P. vivax*. One patient who was hospitalized with *P. vivax* malaria presented with seizure (4%). Due to these complications (severe anemia in two cases, respiratory distress in two cases, seizure and jaundice with one case in each), six patients were referred to a tertiary hospital in Pereira (capital city of Risaralda department). No deaths were reported.

Median hemoglobin (Hb) was 10.0 g/dL (IQR: 8.3—11.2 g/dL, range 4.6—12.3, $n = 58$). In *P. vivax* median, Hb was 10.0 g/dL (IQR 8.3—11.3 g/dL, range 4.6—12.3, $n = 51$). In *P. vivax*/*P. falciparum*, the median Hb was 8.7 g/dL (IQR: 7.1—10 g/dL, range 7.0—10, $n = 4$). In *P. falciparum*, the median

Table 1 Demographic and clinical features of Emberá children with malaria, Pueblo Rico, Colombia, 2013.

Variables	($n = 270$ children/349 episodes)	%
Demography		
Age (year-old, median, IQR)	2.64 (1.29—5.08)	
Gender (female:male, %)	129:141	47.8:52.2
Clinical		
Symptoms length (days, median, IQR)	3.0 (2.0—6.0)	
Patients hospitalized	22	8.1
Reappearances	135	38.7
Median number of episodes/patient/year	1.0	
Etiology		
P. vivax	313	89.7
Mixed (*P. vivax*/*P. falciparum*)	19	5.4
P. falciparum	17	4.9

Table 2 Association between demographic and clinical variables regard hospitalization among those Emberá children with malaria, Pueblo Rico, Colombia, 2013.

Variables	(*n* = 22 children/25 hospitalizations)	%
Demography		
Age (year-old, median, IQR)	0.98 (0.53—2.29)	
Gender (female:male, %)	13/9	59.1/40.9
Clinical		
Hospitalization length (days, median, IQR)	2.0 (1.0—3.0)	
Etiology		
P. vivax (*n* = 313)	19	6.1
Mixed (*P. vivax*/*P. falciparum*) (*n* = 19)	5	26.3
P. falciparum (*n* = 17)	1	5.9

Complications	(*n* = 22 children/25 hospitalizations)	%	*P. vivax*	Mixed[a]
Respiratory distress	8	32.0	6	2
Hepatosplenomegaly	3	12.0	1	2
Severe anemia (<5 g/dL)	3	12.0	2	1
Jaundice	2	8.0	2	0
Seizure	1	4.0	1	0

[a] Mixed (*P. vivax*/*P. falciparum*).

Hb was 10.6 g/dL (range 10.0—11.6 g/dL) (*n* = 3). From all of these patients, 5.2% (3/58) presented with severe anemia (<5 g/dL), which corresponds to two due to *P. vivax* and one due to *P. vivax*/*P. falciparum*. No severe anemia was observed in patients with *P. falciparum*.

No cases of thrombocytopenia were recorded. The median platelet count was 207,900 (IQR 185,850—245,700 cells/μL; range: 153,300—333,900 cells/μL). No leucopenia cases were recorded. The median white blood cell count was 11,250 (IQR 8450—12,600 cells/μL; range: 4400—17,600 cells/μL).

Geometric mean parasitemia was 4858 parasites/μL (95%CI 4180—5646) in those with *P. vivax*; 4777 parasites/μL (95%IC 2873—7943) in those with *P. falciparum*; and 7604 parasites/μL in those with mixed infections (95%CI 4195—13,783). Parasitemia was significantly associated with hemoglobin levels in these children with malaria ($r^2 = 0.9593$; $p < 0.0001$) (Fig. 2).

The mean time for the malaria first reappearance among those who presented with more than one episode in a year was 75.9 ± 59.4 days (range: 12.8—291.9), while the mean time to a second reappearance was 128.8 ± 46.4 days (range: 52.3—209.1), and among those with three episodes (*n* = 6), it was 140.1 ± 54.9 days (range: 87.8—209.1). During the first 100 days of reappearance, 83.3% of the cases occurred, and between days 100 and 200, 68% for the second episode (Fig. 3).

Discussion

Malaria is still a significant public health problem in tropical countries in Africa, Asia and Latin America [2—4,11,12]. In the specific case of Latin America, significant achievements in the reduction of the burden and incidence of the disease have been reached during the last decade in all of the countries of this region, except for Venezuela, Guyana and Haiti [2—4]. Although this significant advance involves reductions of more than 50% in all of the age groups and close to 60% in children younger than five years, malaria still represents a significant burden in this age group, especially in rural and more underdeveloped areas of such countries, as occurs in certain areas of Colombia [7].

In South East Asia and Latin America, there is a common pattern of malaria etiology, in which >80% of the cases are due to *Plasmodium vivax*. The same pattern occurs in Colombia and in most of its departments, including Risaralda, where our study was performed [7]. Tropical areas below 1500 m.a.s.l. in the country are ecologically suitable for the presence of vector species of *Anopheles* [13]. In addition, the social conditions, especially domestic mobility, represent today important issues for clinical diagnosis and treatment of malaria in Colombia, as in other countries [14], especially in children [15].

The clinical profile that is exhibited in malaria between adults and children tends to be

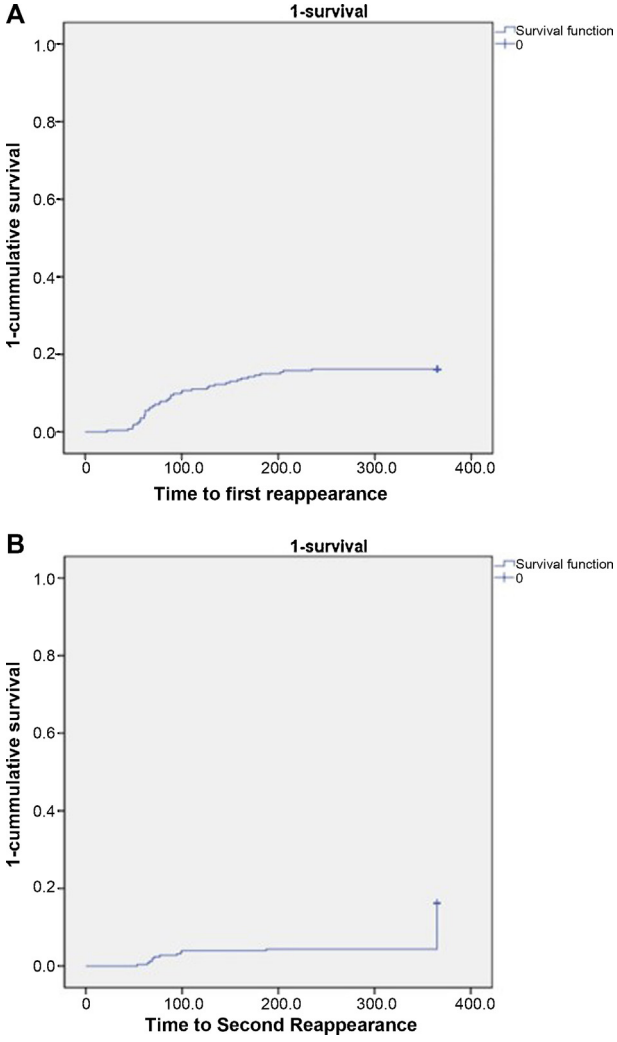

Figure 2 Relationship (modeled by non-linear regression) between parasitemia and hemoglobinemia in Emberá children with malaria.

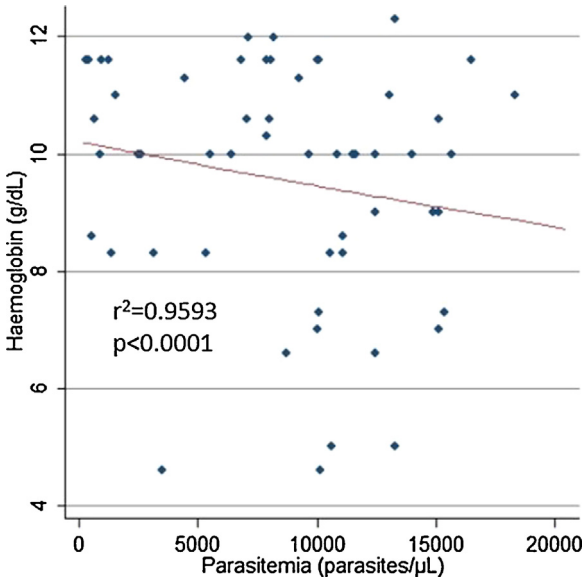

Figure 3 (A) Time to first reappearance among children with malaria, Pueblo Rico, Colombia, 2013 (Kaplan—Meier survival analysis). (B) Time to second reappearance among children with malaria, Pueblo Rico, Colombia, 2013 (Kaplan—Meier survival analysis).

different, especially in complicated cases [16]. In both settings, the complicated and severe cases were traditionally related to *P. falciparum*. However, in the last decade, a growing number of studies in Asia and the Americas have showed that *P. vivax* can also produce complicated and severe malaria in different population groups, including adults, pregnant women and children [17—19]. Then, this relatively new concept should be incorporated into medical education as well as the guidelines for the diagnosis and management of children with malaria, including *P. vivax* infection [19], but should also be addressed in clinical and epidemiological studies. Although a severe disease mechanism would be different between *P. vivax* and *P. falciparum*, it is clear that according to WHO, the research evidence [10—43] is that severe vivax malaria exists and occurred in our series by fulfilling the criteria of severe disease [10].

Although there is too much that remains to know about the pathogenesis of severe vivax disease, genetic polymorphisms have been associated with increased risk in patients with alpha- and beta-thalassemia and decreased risk in those with G6PD deficiency and ovalocytosis [10].

In Colombia, few data are yet available about severe *P. vivax* malaria, in either adults or children. As part of a retrospective analysis of the national program of malaria, a study found that during the malaria outbreak of 2010 in Colombia, hepatic (OR = 1.47; 95%CI 1.05—2.06) and pulmonary complications were higher in those patients with *P. vivax* (20.9% and 5.5%) compared with those with *P. falciparum* (15.9% and 5.0%) [21]. However, this study was in the general population, with 30% of them being children less than 15 years of age. Moreover, the complications were not compared at this age group between *P. vivax* and *P. falciparum* [21]. Another recent study, a 16 patient-case series, described observed complications in *P. vivax* infection in three municipalities of the Pacific coast departments of Nariño and Valle del Cauca, finding hyperbilirubinemia, thrombocytopenia, shock and severe anemia to be among the most frequent [22]. Nevertheless, from these 16 patients, only 2 of them were less than 15 years of age [22]. Thus, our study is the first in the country to include a larger series of cases that describe severe *P. vivax* malaria

in children. Furthermore, this study assessed children from one of the Amerindian ethnic groups that is present in Colombia, the Emberá. Among this ethnic group, there is a lack of studies of malaria [23], even though they are a large group (50,000 people living in Colombia) and present in malaria endemic areas at the departments Antioquia, Caldas, Risaralda, Quindío and Valle del Cauca.

Plasmodium vivax infection has been associated with severe and fatal disease in endemic areas, including Indonesia, Papua New Guinea, India, Brazil, Venezuela, Thailand, Malaysia and Sudan [10—43]. Severe manifestations associated with *P. vivax* infection in this series include severe anemia, respiratory distress and acute lung injury, acute kidney injury, splenic rupture, metabolic acidosis, jaundice, multi-organ dysfunction, shock and rarely coma. *P. vivax* also causes substantial morbidity, especially severe anemia and low birthweight [10—43].

Emberá children from Pueblo Rico, Risaralda, Colombia, presented a significant number of malaria episodes during the year (1/5 presented 2 episodes in a year, and some presented even 4 episodes in a year), which indicates a high transmission pattern in the area and a need for more efficient vector control by the health authorities. Although it is known that in Pueblo Rico, there are efficient vector species, such as *Anopheles darlingi* and *A. albimanus*, among others, no entomological studies on malaria have been published in this municipality and none in the department of Risaralda. Those *Anopheles* species are especially transmitting *P. vivax*, which represented almost 90% of the etiology in this study. Then, given the high transmission in the area, although in *P. vivax* malaria, the activation of latent liver-stage parasites (hypnozoites) can lead to new blood-stage infections (relapse); this aspect cannot be discriminated in this study, but the time between episodes in Pueblo Rico can be as short as 8 weeks.

As expected, most of the affected children (almost 3/4) were those who were less than 5 years of age, as was previously reported between 2007 and 2009 for the department of Risaralda [7], including one congenital and one neonatal case, using the corresponding definitions [24]. Then, is also raised the question as to assessing the impact of malaria during pregnancy, which is also important in a *P. vivax* infection [25], in this region of the country, where no studies about it have been reported.

In this series, although the frequency was low, the number of mixed malaria cases (due to *P. vivax* and *P. falciparum*, 5.4%) was quite high compared to other studies in Latin America, which usually have reported less than 2% [7,26—28]. However, the mixed malaria cases were those that most frequently presented complications and required the patient to be hospitalized in this study (crude OR = 5.535; 95%CI 1.81—16.9). Very similar recent reports in Bikaner found this, northwest India, which indicate that the risk of developing severe malaria, multiorgan dysfunction and mortality was higher in patients with mixed infection in comparison to *P. falciparum* or *P. vivax* monoinfection [29]. In our series, other variables, such as sex, geographic origin, health insurance and monoinfection, were not significantly associated with hospitalization risk.

The clinical research on the impact of mixed infection on human health is still controversial, while some authors believe it to be a beneficial situation whereas others consider it to be detrimental [29]. The vast difference in the results could be due to large-scale incorrect species diagnosis by microscopy, which is attributed to various factors. Previous studies on severe malaria with a larger number of cases have strongly indicated the limitations of the study with a note of concern that mixed infections might have been largely underestimated and the use of a more sensitive diagnostic method might have had different results [29]. Furthermore, as has been recently reported in the new review of the World Health Organization (WHO) on severe malaria, 2014 [10], in areas that have higher endemicity of both species (*P. falciparum* and *P. vivax*), mixed infections are associated with an increased risk of severe malaria, including severe anemia, coma and death [30,31]. However, as in *falciparum* malaria, host, parasite and socio-geographical factors likely contribute to the risk of severe disease and death in *vivax* malaria, as well as specific severe manifestations [10].

Despite this pattern, which is related to mixed malaria infections, in this study, in general, patients with *P. vivax* presented with severe malaria, including severe anemia, renal dysfunction, respiratory distress and seizure. These aspects not only have been reported in many studies elsewhere, in adults [32—35] and children [30,36,37] but also have been included recently in the review of WHO on severe malaria [29], where it was acknowledged that *P. vivax* can cause complicated and severe disease. In the WHO review, *P. vivax* severe malaria includes: severe anemia, acute lung injury (ALI) and respiratory distress, acute kidney injury, shock and multi-organ dysfunction, bacterial co-infection and bacteremia, coma and other vivax-associated neurological complications, splenic rupture and infarction, among other complications [29].

In this study, no deaths were reported, and some previously common reported manifestations of *P. falciparum* and *P. vivax* malaria, such as thrombocytopenia and leucopenia [38—40], have not been observed in these patients. Because this study is retrospective, we cannot further assess this aspect, but it will be carefully observed in the upcoming prospective studies that we are planning to perform on the same populations.

With respect to the limitations of this study, all of the patients were diagnosed by thick and thin blood smears, but none with PCR. However, as has been clearly stated in the methods section, all of the positive smears and 10% of those considered to be negative by our hospital laboratory were reevaluated by a regional second reference laboratory in Pereira and by a third national malaria reference microscopist in Bogotá, to confirm the diagnosis of malaria. Moreover, further studies in Risaralda will include PCR-based diagnosis techniques to prospectively study severe *P. vivax* malaria. In addition, the molecular techniques will allow us to assess the parasite burden and truly determine the parasitemia in patients with *P. vivax* and other species in these endemic areas.

Implications of these findings, as well as implications from other observational studies on severe vivax malaria, have clear benefits in patient care because they can suggest to the attending physicians in *P. vivax*-endemic zones that this parasite can produce severe forms; then, an assessment can be made of this evolution and the clinical alarm signs and the progression to conditions that fulfill the criteria of severe malaria, according to WHO, which now includes also *P. vivax* severe malaria infection [10]. Then, avoiding and/or reducing the risk of fatality can be accomplished by the early management of complications.

However, many epidemiological, clinical and pathogenic questions have been raised regarding severe *P. vivax* malaria [41—43], and there is growing evidence of its importance, including this study for Emberá (Amerindian) children from an endemic area in Colombia. Probably in other endemic areas of the Coffee-triangle region, which include 53 municipalities with variable levels of malaria transmission, mostly due to *P. vivax*, this aspect would also be occurring [44]. More detailed and prospective studies are warranted to measure the real impact of *P. vivax* infection in children with severe malaria as well as to reduce its impact as the residual burden of malaria in Southeast Asia and Latin America countries [37], such as Colombia.

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Competing interests

None declared.

Ethical approval

Committee of Bioethics of the Universidad Tecnológica de Pereira, Pereira, Risaralda, Colombia.

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