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Prevalence of asymptomatic *Plasmodium vivax* infections in the north-eastern focus of malaria of Venezuela

Prevalencia de infecciones asintomáticas por Plasmodium vivax *en el foco malárico oriental de Venezuela*

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SUMMARY

Malaria remains as a public health problem in Venezuela. In 2015 there were 136,402 cases reported by the Ministry of Popular Power for Health, being the parasite prevalence 73.95% for Plasmodium vivax, 17.6% for Plasmodium falciparum*, 0.0095% for* Plasmodium malariae *and 8.42% mixed infections* (P. vivax + P. falciparum)*. During the period 1999-2002 the number of cases in Venezuela ranged between 21,685 and 29,337, being the Sucre State with highest levels of malaria prevalence, with Plasmodium vivax as the unique specie in this region. In 2002 the Municipality of Cajigal had the highest Annual Parasite Incidence (API) of country, being 260 cases per 1000 inhabitants. In view of the difficulty in controlling malaria in this area, the prevalence of asymptomatic carriers was investigated as one of the epidemiological factors contributing to the persistence of malaria transmission. One hundred fifty people were included in the study, with no history of recent malaria infection, or any symptom and also, not having used antimalarial drugs during the 30 days prior to study entry. To do this, a malaria Rapid Diagnostic Test (mRDTs) was used for the determination of antigenemic (OptiMAL®) and PCR (polymerase chain reaction) in conjunction with the reference "Gold Standard", the conventional thick and thin blood smears (TTBS). It was found a prevalence of infection of 1.33% by mRDTs and TTBS and 8% by PCR which allowed the detection of 10 asymptomatic cases in addition, with a sensitivity and specificity of 100% and 93.4% respectively. The presence of asymptomatic carriers in this area reveals the difficulties that face the Malaria Control Program in the eventual elimination of this specific malaria foci. It is necessary reinforces the maintenance of the epidemiological surveillance using more sensitive diagnostic techniques, as well as to adapt the control measures based on the current findings.*

Key words: *Asymptomatic malaria,* Plasmodium vivax*, thick and thin blood smears (TTBS), mRDTs, OptiMAL®, PCR.*

RESUMEN

La malaria sigue siendo un problema de salud pública en Venezuela. Para el año 2015 el Ministerio del Poder Popular para la Salud reportó 136.402 casos, siendo la fórmula parasitaria 73,95% para Plasmodium vivax, 17,6% para Plasmodium falciparum, 0,0095 para *Plasmodium malariae* y 8,42% para infecciones mixtas (*P. vivax + P. falciparum*). Durante el período 1999-2002, el número de casos en Venezuela estuvo entre 21.685 y 29.337, siendo el Estado Sucre el que mostró los niveles más altos de prevalencia de malaria, con *P. vivax* como única especie en la región. En el año 2002 el Municipio Cajigal registró el Índice Parasitario Anual (IPA) más alto del país, siendo 260 casos por 1000 habitantes. En vista de las dificultades para controlar la malaria en esta área, se investigó la prevalencia de portadores asintomáticos como uno de los factores contribuyentes en la persistencia de la transmisión malárica. Ciento cincuenta personas fueron incluidas en el estudio sin historia de infección reciente por malaria o ningún síntoma, así como no haber consumido drogas antimaláricas durante los 30 días anteriores de ingresar al estudio. Para ello, se usó la prueba rápida de diagnóstico para malaria (PRDxm) para la determinación de antígenemia (OptiMAL®) y la técnica de biología molecular basada en la Reacción en Cadena de la Polimerasa (PCR), conjuntamente con la "prueba oro," como método convencional, la Gota Gruesa y Extendido de Sangre (GGES). Se encontró una prevalencia de infección de 1,33% por GGES y por prueba rápida de diagnóstico OptiMAL® y 8% mediante PCR. La técnica de PCR permitió la detección adicional de 10 casos asintomáticos con una sensibilidad y especificidad del 100% y 93,4% respectivamente. La presencia de portadores asintomáticos en esta área revela las dificultades que enfrenta el Programa de Control de la Malaria en la eliminación eventual de esta parasitosis en este foco. Es necesario reforzar el mantenimiento de la vigilancia epidemiológica usando técnicas de diagnóstico más sensibles, así como adoptar medidas de control basadas en estos hallazgos.

Palabras clave: Malaria asintomática, *Plasmodium vivax*, Gota Gruesa y Extendido de Sangre (GGES). Prueba rápida de diagnóstico OptiMAL®, PCR.

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INTRODUCTION

The clinical manifestations of malaria by *P. falciparum* have a wide spectrum comprising asymptomatic parasitemia, clinical malaria (parasitemia with febrile episodes), and severe malaria that can lead to death (WHO, 2000; Breman, 2001; Hommel & Gilles, 2005; Noya & Ossenkoff, 2011). In contrast, *P. vivax* infections in nonimmune people develop symptoms of clinical malaria with recurrent febrile paroxysms, which produces temporary disability and less severe complications (Breman, 2001; Mendis *et al.*, 2001; Sina, 2002). However, recurrent episodes induce higher morbidity and severe malaria cases by this species (Bruce *et al.*, 2000; Mendis *et al.*, 2001; Baird, 2013). Recently, there has been an increase in the number of studies related to severe malaria for *P. vivax* in endemic countries. The results of eight relatively high quality studies showed evidence on severe malaria in *P. vivax* infection compared with that in *P. falciparum* infection, identifying that the incidence of severe malaria in patients infected with *P. vivax* was considerable, indicating that *P. vivax* is a major cause of severe malaria (Nadkar *et al.*, 2012 ; Naing *et al.*, 2014).

For people living in malaria endemic areas, it has been reported asymptomatic *P. vivax* infection, although less than that recorded for *P. falciparum* infection (Prata, 1988; Alves *et al.*, 2002; Rodrigues *et al.*, 2006; Suárez-Mutis *et al.*, 2007; Cucunubá *et al.*, 2008). On the other hand, the earlier emission of gametocytes by *P. vivax* at the beginning of the erythrocyte cycle in comparison with *P. falciparum* is one of relevant advantages of this species to facilitate early transmission in populations. If it adds to this that this species has the ability to produce relapses by the activation of hypnozoites, together with a high frequency of asymptomatic carriers in the different regions studied, explain why it has been more difficult to control and eliminate this species in America and south-east Asian countries, than *P. falciparum*.

In general, asymptomatic infections have been associated with acquired immunity after repeated episodes of the disease and therefore would be more frequent in areas of stable and high malaria endemicity, where adults and some children may carry parasites in asymptomatic condition for both *P. malariae* (Gilles, 1986) and *P. falciparum* (Greenwood, 1987; Greenwood *et al.*, 1987; Achidi, 1995; Vounatsou *et al.*, 2000; Singer *et al.*, 2003). However, even in areas of unstable malaria, many individuals control the infection and remain asymptomatic, with very low parasitemia making it difficult for the clinical and parasitological diagnosis in *P. falciparum* infections (Roper *et al.*, 1996) and *P. vivax* (Barbosa *et al.*, 2014).

Latin American National Malaria Control Programs (NMCPs) are based on early diagnosis by microscopy and appropriate treatment mainly by passive case finding. The first reports of asymptomatic infections using the TTBS were conducted in Brazil (Prata *et al.*, 1988), which recorded less prevalence than in Africa. In Venezuela malaria morbidity is caused essentially by *P. vivax*. Of the 136,402 cases reported in 2015, 73.95% was caused by this species in addition of 8.42% of mixed infections (*P. falciparum*/*P. vivax*) (Bol. Integral Salud Amb., 2015). The main transmission areas in Venezuela are constituted by three foci located in Southern (Bolívar and Amazonas States), Western (Táchira, Mérida, Barinas and Apure States) and North-Eastern (Sucre, Monagas, Anzoátegui and Delta Amacuro States) (Noya, 2011). The last focus was reactivated in 1985, after 20 years of being eliminated by the Malaria Control Program (Barrera *et al.*, 1999).

The Sucre State originated the majority of cases in the focus North-Eastern of the country during the period 1999-2002, with two big epidemic episodes in the years 2000 and 2002, which shaped in turn the behavior of the disease in the country. The Cajigal Municipality was one of the municipalities of this State that contributed with the largest number of cases and API of 260 cases per 1000 inhabitants (Cáceres, 2004).

Due to this situation, the objective of the present study was to estimate the prevalence of asymptomatic carriers during the decline of transmission in 2003, using more sensitive alternative methods.

MATERIALS AND METHODS

Study Area and Population

It was performed a cross-sectional study based on the active search for asymptomatic malaria

cases in the Cajigal Municipality of the Sucre State, located northeast of Venezuela (Fig. 1) (10° 34' 6.9" north and 62º 49' 44" west), with an approximate area of 365 km^2 and a population of 25,722 inhabitants (OCEI., 2000). In 2002, the annual parasite index (API) of the municipality was the highest in the country (260 cases per 1000 inhabitants) Cáceres (2004). After the implementation of highly effective interventions, of control program, the API decreased to 68 API/1000 inhabitants in 2003 (Cáceres, 2004). This random sampling was conducted in the first two weeks of September 2003, corresponding to the end of the rainy season and based on the area where were reported malaria cases a month earlier (Bol. Integral Salud Amb., 2003).

Individuals were notified about the characteristics of the study and agreed to participate by signing the Informed Consent Form. Adults, as well as the parents of all children between 5 to 17 years old included in the study, were asked to sign an informed consent form signed.

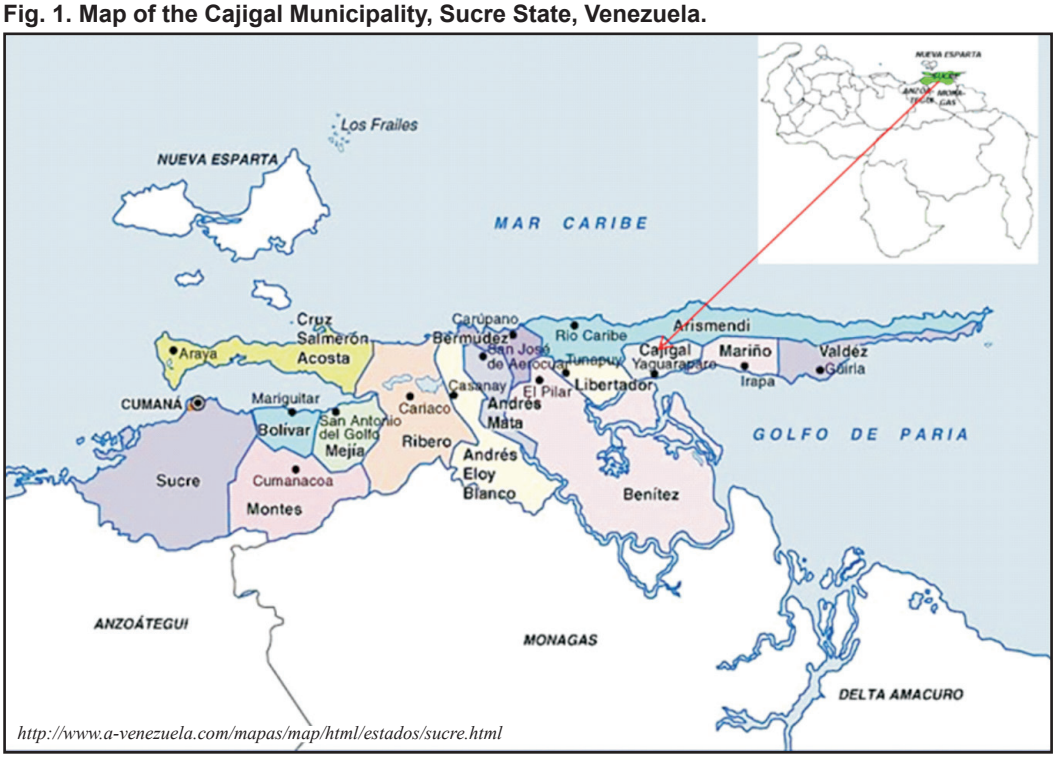
Asymptomatic were defined as those who had not in the previous 30 days: fever, chills, headache, musculoskeletal pain and nausea/vomiting. It was excluded in the study: persons under the influence of alcohol, under 5 years old and people with mental disorders or pregnant.

The sample size (n) was calculated based on the prevalence of the infection and the population size. Taking into account prior information, we assumed a prevalence of asymptomatic infections of 5%. The "n" sample obtained was 75, but it was decided to double the size calculated, because the results would approach more to reality.

Biological samples

Two types of blood samples were collected: capillary in the earlobe for parasitological diagnosis (TTBS) and venous (OptiMAL® and PCR tests), because the anticoagulant interferes with the quality of the parasitological method and therefore with the diagnosis of the infection. In addition, urine samples were collected for determination of antimalarial drugs. Sampling was carried out based on the area where they were reported malaria cases month earlier. This research was approved and conducted under the

Fig. 1. Map of the Cajigal Municipality, Sucre State, Venezuela.



supervision and support of the Venezuelan Ministry of Health and Social Development (MSDS).

Clinical and parasitological follow-up

Individuals in the study, who tested positive for malaria infection by microscopy were followed by clinical and parasitological examination for at least a week, before treatment was supply to each patient. By ethical reasons, it was not possible to further continue the follow-up.

Parasitological diagnosis was performed by examination by thick and thin blood smears (TTBS) stained with Giemsa. Slides were examined by two independent well trained observers. At least 100 fields of the thick blood film with 100X objective and 10X eyepiece were examined before considering a negative sample (Bruce-Chwatt, 1986; Ferreira & Avila, 2001). Parasitemia was determined in the thick blood film in all positive samples (OMS, 1992; Makler *et al.*, 1998).

Immunological diagnosis

Detection of parasite circulating was carried out by a malaria Rapid Diagnostic Test (mRDT) OptiMAL® (Flow Inc., Portland OR, USA) according to the manufacturer's instructions.

Diagnosis by Molecular Biology

It was based on the amplification of specific sequences of genus and species of ribosomal genes corresponding to the 18 S subunit of RNA of the parasites for the genus *Plasmodium*. The total genomic DNA extraction was performed from blood collected in EDTA tubes, with the modified method of phenol-chloroform and subsequent ethanol precipitation described by Snounou *et al.* (1993). The DNA obtained was quantified by spectrophotometry and run in 1% (p/v) agarose gel to assess the quality of DNA.

Sensitivity limit of the PCR

The sensitivity of the PCR test was performed using as a source of parasites, in vitro cultures of the *P. falciparum* FCB2 strain (Trager & Jensen, 1976). A sample with predominance of young trophozoites, was taken to a 1% parasitemia and diluted with uninfected blood for a range of parasites from 0.1% to 0.00001%.

Developed of the PCR

A nested PCR was carried out with two rounds, the first for detecting the *Plasmodium* and a second one specific for *P. falciparum* and *P. vivax*. (Singh *et al.*, 1999). The primer pairs for the detection and identification of genus were rPLU1/rPLU5; rPLU3/rPLU4. The final product was analyzed and only positive samples were examined with the oligonucleotides rFAL1-rFAL2 and rVIV1-rVIV2 specific for *P. falciparum* and *P. vivax* respectively (Singh *et al.*, 1999).

The amplified products of the second round were separated by size on 2% (p/v) agarose gels, in parallel with the appropriate molecular weight marker, stained with ethidium bromide and visualized under UV light (Singh *et al.*, 1999).

Detection of antimalarial drugs in urine

It was used the thin layer chromatography technique described by Betschart *et al.* (1986), for the determination of chloroquine (CQ) and its main metabolite desethylchloroquine (DCQ) as well as quinine (QUIN), amodiaquine (AMO) and mefloquine in urine. In each run were included drug controls, untreated individuals control and monitoring of patients who had received the previous day chloroquine and primaquine. The presence of chloroquine and other antimalaric drugs was evaluated in a dark room using a UV lamp.

Statistical analysis

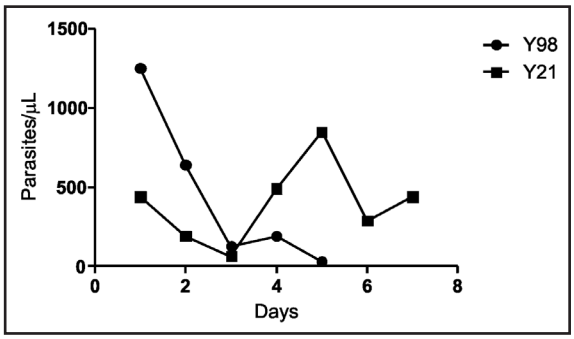
The sensitivity and specificity of the different diagnostic tests was analyzed through the Kappa index (Fleiss, 1981). The (kappa) statistic method was used to measure agreement between the tests. The prevalence was determined by means of the number of positive samples between the total of the taken samples multiplied by 100.

RESULTS

Prevalence of infection with Plasmodium *spp. through thick and thin blood smears*

A total of 2 asymptomatic people who represented the 1.33% (2/150) of the study group were positive for *P. vivax* (Table I), with densities

Fig. 2. Evolution of parasite burden of two asymptomatic persons, from the Cajigal Municipality, Sucre State, Venezuela.



between 440 and 1200 parasites/µL of blood. Both samples were from males, aged 11 and 20 years respectively. These people were followed up by clinical, parasitological and immunodiagnostic tests, demonstrating the persistence of parasitemia and positivity by OptiMAL® with the absence of symptoms. During follow-up, variations in parasitemia were observed in one of them and a downward trend in the other (Fig. 2, Table I). After 5 and 7 days of follow-up, patients received the conventional malarial treatment, achieving the clearance of parasites. The treatment antimalaric were based on Chloroquine 25 mg/Kg weight for 3 days distributed in 10, 10 and 5 mg/Kg weight and Primaquine (0,25 mg/Kg weight) for 14 days. It was approved and supervised for Malaria Control Program.

Prevalence of infection with Plasmodium *spp. by the rapid by diagnostic test OptiMAL®*

With OptiMAL® rapid test were positive 2.0% (3/150) for *Plasmodium*, of which 1.33% (2/150) corresponded to *P. vivax* and 0.66% (1/150) to *P. falciparum* (Table II), the latter showing a weak positive signal. This individual was monitored by PCR and TTBS being negative for both tests. The sensitivity limit of OptiMAL® was ≤ 300 parasites/µL of blood (Zerpa *et al.*, 2008).

Determining the sensitivity limit of the PCR

For determining the sensitivity limit of oligonucleotides for the detection of the genus *Plasmodium* and assay amplifying a 240 bp product was able to detect parasitemia between 1 and 0.00001%. Further dilutions only were able to show a faint band (Fig. 3).

Of the 150 samples evaluated, 12 were positive for the genus *Plasmodium*, and subsequently tested with oligonucleotides specific for *P. vivax* and *P. falciparum*, amplifying all a product of 117 bp corresponding to *P. vivax*, representing a prevalence

Table I. Comparison between microscopy, OptiMAL® and PCR in detecting asymptomatic infections.

Code	Thick and thin blood smear	Parasites/µL blood	OptiMAL®	PCR	Malarial history
					N° Episodes
Y-011	Negative	0	Negative	*P. vivax*	1
Y-013	Negative	0	Negative	*P. vivax*	4
Y-014	Negative	0	Negative	*P. vivax*	1
Y-018	Negative	0	Negative	*P. vivax*	6
Y-020	Negative	0	Negative	*P. vivax*	7
Y-021	*P. vivax*	440	*P. vivax*	*P. vivax*	3
Y-024	Negative	0	Negative	*P. vivax*	0
Y-049	Negative	0	*P. falciparum*	Negative	0
Y-071	Negative	0	Negative	*P. vivax*	1
Y-076	Negative	0	Negative	*P. vivax*	4
Y-081	Negative	0	Negative	*P. vivax*	4
Y-098	*P. vivax*	1250	*P. vivax*	*P. vivax*	15
Y-148	Negative	0	Negative	*P. vivax*	2
Mean (X=4.36); Median (X=4)					

Table II. Comparative prevalence of asymptomatic infections by *P. vivax* evaluated by microscopy, OptiMAL® and PCR.

	Microscopy		OptiMAL®		PCR	
Species	n/N	%	n/N	%	n/N	%
P. vivax	2/150	1.33	2/150	1.33	12/150	8
P. falciparum	0/150	0.0	1/150	0.66	0/150	0
Negative	148/150	98.60	147/150	98	138/150	92
Sensitivity					100	100
Specificity					99.33	93.24

Sensitivity: True positives/(True positives + false negatives). Specificity: True negatives/(True negatives + false positives). k: 0.796 (TTBS/OptiMAL®). k: 0.265 (TTBS/PCR).

Fig. 3. Sensitivity of PCR with oligonucleotides for the *Plasmodium* genus.

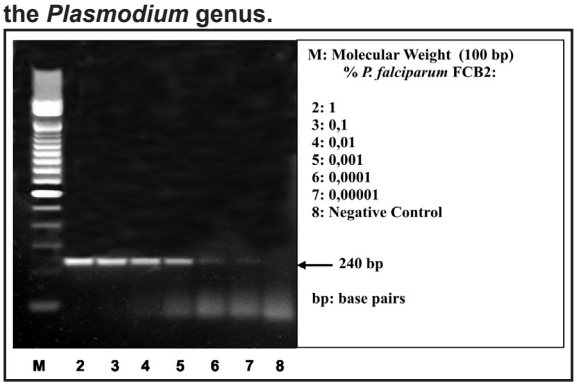
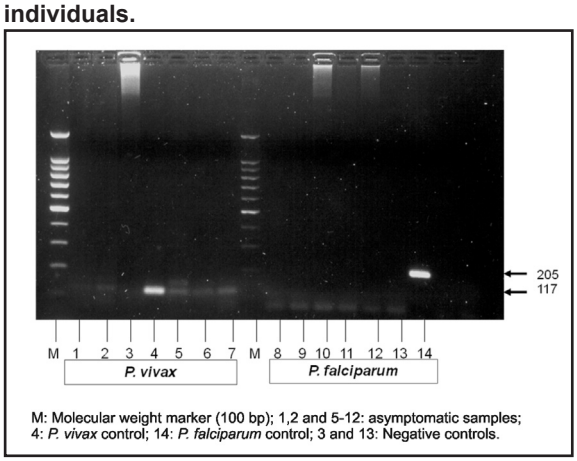


Fig. 4. Analysis of two steps PCR of total DNA from blood samples of ten asymptomatic malaria individuals.



of 8% of infection by this specie while all were negative for *P. falciparum* (Fig. 4).

Comparison between microscopy, OptiMAL® rapid test and PCR in detecting asymptomatic infections

There was concordance in the detection of 2 malaria infections by TTBS, PCR and OptiMAL® rapid test. A positive sample for OptiMAL® rapid test was negative by TTBS and PCR. On the other hand, ten samples were only positive by PCR. The kappa index between TTBS and OptiMAL® rapid test was 0.796 and the kappa index between TTBS and PCR was 0.265, considering the TTBS the Gold Standard test (Table II).

Determination of antimalarial drugs in urine

No metabolites of chloroquine, amodiaquine and quinine were detected in the 150 samples tested. The values of ratio of front (Rf) were 0.68 for amodiaquine, 0.64 chloroquine and 0.48 quinine metabolites (Fig. 5).

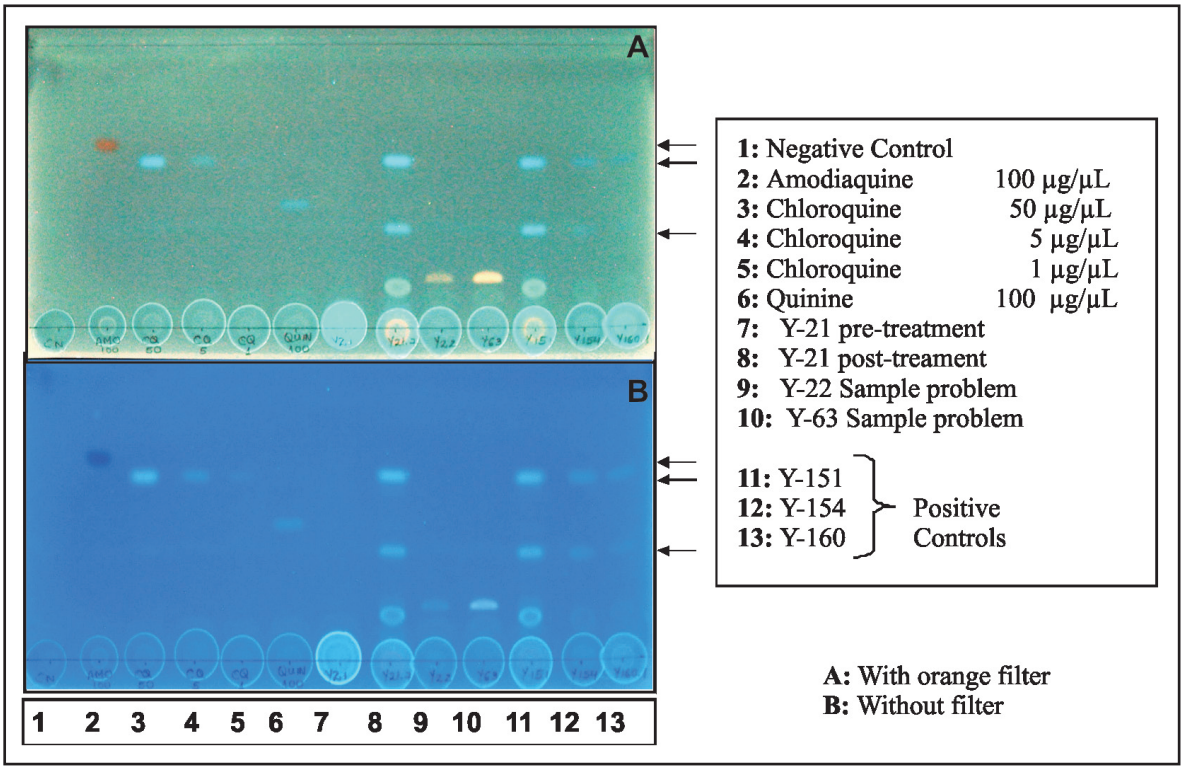
Malaric history and its relation with asymptomatic infections

Eleven of twelve people with asymptomatic infection had malaric history and another one had not malaric history, with a Mean (X) of 4.36 and a Median (X) of 4) (Table I). Two individuals with different number malaric episodes showed parasitemia levels that are over the microscopic threshold for detection remaining asymptomatic for 5 and 7 days.

DISCUSSION

Asymptomatic malaria is defined as *Plasmodium* infections that do not lead to clinical symptoms and therefore remain undetected by surveillance systems. These infections can still contribute to the transmission of the parasite in the population, which makes them particularly relevant in the context of malaria elimination. There are few

Fig. 5. Evaluation of antimalaric drugs in urine by thin layer chromatography.



studies about asymptomatic malaria probably due to absence of standard diagnostic criteria, since infected individuals may be in a pre-symptomatic period with parasitemia, and that could present symptoms later on (Laishram *et al.*, 2012; Galatas *et al.*, 2016).

In many studies the definition of asymptomatic malaria is based on the presence of parasites in peripheral TTBS, an axillary temperature <37.5ºC and the lack of malaria-related symptoms. Some studies include other criteria, such as longitudinal follow-up and parasite quantification. A longitudinal follow-up is important for differentiating between infections that seems to be asymptomatic at the moment of diagnosis, but that could become symptomatic after the initial detection (Laishram *et al.*, 2012; Galatas *et al.*, 2016).

There are few studies related to the pathophysiology of asymptomatic malaria infection, especially in the case of *P. vivax* infections. In spite of it has been proved that these individuals might influence transmission dynamics, since they might reach a prevalence 4-5 times higher than the symptomatic individuals and also, being infective for anophelines (Alves *et al.*, 2005; Laishram *et al.*, 2012). In the Cajigal Municipality the prevalence of asymptomatic cases was 8%. In Brazil, Alves *et al.* (2005) found an infection rate in *An. darlingi* of 1.2% for the asymptomatic carriers and 22% for the symptomatic carriers. Although the asymptomatic group infected mosquitoes at a much lower rate, these carriers usually remain infective longer than treated, symptomatic.

Also, the Cajigal Municipality is the most receptive malarious area in eastern Venezuela, reporting usually the highest prevalence in the Sucre State. Epidemiologically is considered an area of annual malaria transmission that might be responsible of exposure related immunity of the residents of this area. In fact, 11 of the 12 people with asymptomatic infection had 3-15 episodes of malaria, with a Mean (X) of 4.36 and a Median (X) of 4), which allows to infer that have developed some degree of immunity, thereby maintaining parasitemia without presenting a characteristic symptom, although not confirmed through immunological tests.

It has been well documented the presence of asymptomatic carriers in different geographic areas

worldwide, that is the case of Brazil, Ethiopia and Nigeria (Prata *et al.*; 1988; Aranha *et al.*, 1996; Alves *et al.*, 2002; Barbosa *et al.*, 2014; Tadesse *et al.*, 2015), as well as the Amazonian focus of southern Venezuela (Postigo *et al.*, 1998; Urdaneta *et al.*, 2003 (unpublished data); Marcano *et al.*, 2004), therefore it was also important to demonstrate its presence in the North-eastern focus of Venezuela (Sucre State), where it is only present *P. vivax* (Sojo-Milano, 2008).

In this research, it was found a prevalence of 8% of asymptomatic infections by *P. vivax* using PCR technique, confirming its higher sensitivity and specificity in relation to the TTBS and also, allowed to confirm that there were no *P. falciparum* infections. The present investigation is one of the few conducted in Venezuela focused on the detection of asymptomatic infections caused by *Plasmodium* species. Furthermore, this research was done in an area where there is only transmission for *P. vivax* and only few *P. falciparum* imported cases were from other endemic areas. This is one of the salient features of this study, since most of the other studies have been conducted in areas where *P. falciparum* was also present.

In Venezuela, there have been studies focused mainly on the Southern (Amazonas and Bolívar States) and Eastern focus (Anzoátegui, Monagas and Sucre States), where Postigo *et al.* (1998) reported asymptomatic malaria infections in 6% of the population that it did not detect by microscopy. In other areas of the Brazilian Amazon, asymptomatic malaria infection has been demonstrated in several cohorts of a longitudinal study, recording prevalence of 4.6, 4.2 and 0% respectively (Alves *et al.*, 2002).

In a more recent study in Brazilian Amazon, 56.6% of the *P. vivax* infections were asymptomatic (Barbosa *et al.*, 2014). In Nigeria evaluated 224 and 192 blood bank donors in periods of high and low transmission malaria and detected 41% and 19% of infection by *P. falciparum*, predominantly higher prevalence and lower parasitic loads during the peak of malaria transmission (Achidi, 1995). In Tierralta, Colombia 11.3% was found positive for *Plasmodium* spp. by TTBS of 212 individuals studied (Cucunubá *et al.*, 2008). In Colombia (Tierralta, Tumaco and Buenaventura), between October 2011 and January 2012 it was found 0.3% (n = 4) cases positives by TTBS and 9.7% (n = 113) by qPCR, 74% were positive for *P. vivax*, 22% for *P. falciparum* and 4% were mixed infections, which it is correlated to the overall parasite prevalence in this country (Vallejo *et al.*, 2015). In Rio Negro, Brazil in a cross sectional study found 0.92% of asymptomatic infection by *P. vivax* of 109 individuals (Suárez-Mutis *et al.*, 2007). Rodulfo *et al.* (2007) reported in the Amazonas and Sucre States (Venezuela) 93 microscopy positive from a group of 295 patients, of whom 66 had *P. vivax*, 26 *P. falciparum* and 1 mixed infection (*P. falciparum*/*P. vivax*). Rodríguez *et al.* (2010) conducted a prospective study in the Amazon region of Venezuela in April, September and December 2003 and reported 2%, 1% and 4% asymptomatic individuals respectively by TTBS.

Imwong *et al.* (2015) found in cross-sectional surveys in western Cambodia, the Thailand–Myanmar border and southwest Vietnam, where parasite prevalence was 224/5008 (4%) by mRDTs, 229/5111 (5%) by microscopy, and 988/4975 (20%) when assessed by HVUSqPCR; of these 164 (3%) were infected with *P. falciparum*, 357 (7%) with *P. vivax*, 56 (1%) with a mixed infection, and 411 (8%) had parasite densities that were too low for species identification. In Ngella, Central Islands Province, Solomon Islands (Southwest Pacific) was detected 468 *Plasmodium* spp. infections (prevalence=13.4%; 463 *P. vivax*, five mixed *P. falciparum*/*P. vivax*, no P. ovale or *P. malariae*) by qPCR, whereas by light microscopy 130 positive cases (prevalence = 3.7%; 126 *P. vivax*, three *P. falciparum* and one *P. falciparum*/*P. vivax*). The prevalence of *P. vivax* infection varied significantly among villages (range 3.0–38.5%, $p<0.001$) (Waltmann *et al.*, 2015). Although the TTBS is the common gold standard test for the diagnosis of malaria, however lacks sensitivity to detect asymptomatic infections by *Plasmodium* spp. (Barbosa *et al.*, 2014).

In this research the two infections identified by the conventional method were confirmed by PCR and OptiMAL® as *P. vivax*, one case weakly positive for *P. falciparum* for OptiMAL® rapid test was negative by PCR, so it was considered as a false positive. Since corresponded to a person who had left the Sucre State, and had never received blood transfusion and therefore unlikely *P. falciparum* infection. The sensitivity and specificity of OptiMAL® rapid test in this case based on the TTBS, was 100 and 99.32% respectively. Equivalent results were

reported in a study in Thailand, where they found flaws in the diagnosis of malaria by OptiMAL® rapid test, between 25.9% and 60.3% false positives for *P. vivax* and for *P. falciparum* respectively (Coleman *et al.*, 2002). These false positives could be explained by nonspecific binding of monoclonal antibodies conjugated to colloidal gold to capture. However, in a study carried out in Venezuela using this test it was not found inespecificity (Zerpa *et al.*, 2008). The sensitivity and specificity of the PCR respected to the TTBS Gold Standard, was 100 and 93.24% respectively, however, the rate of concordance between these two tests was very low ($\kappa = 0.265$), which is a consequence of the greater number of samples detected by PCR, while de TTBS did not detect 10 asymptomatic carriers.

The prevalence of asymptomatic carrier was lower than those obtained previously in southern Venezuela, in mining towns (7% prevalence by TTBS and 34% prevalence by PCR) (Urdaneta *et al.*, 2003, (unpublished data)) and 42%, 46% and 49% in indigenous populations in Amazonian State (Rodríguez *et al.*, 2010). The PCR is a technique able to detect 1-5 parasites/μL of blood of the five species of *Plasmodium* that affect humans and also the possibility to diagnose mixed infections (Singh *et al.*, 1999; Cox-Singh *et al.*, 2007), with a sensitivity 10 times higher than the TTBS and a specificity of 100% under good laboratory practices.

In spite of the fact that the molecular technologies are costly, more time consuming and requires equipment and trained personnel., they should be included in the central laboratories of Malaria Control Program, since they allow the detection of very low parasitemias, still below the threshold of diagnosis for microscopy or rapid tests. This would allow a periodic vigilance for the search of asymptomatic infections as it has been used in other countries (Harris *et al.*, 2010; Zoghi *et al.*, 2012).

The knowledge of the presence of asymptomatic carriers must induce the local sanitary authorities to revise the strategy of epidemiological surveillance and control in this region.

Conflicts of Interest

The Authors stated that there were no conflicts of interest in the performance of this work.

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