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Plasmodium spp. infection rates for some *Anopheles* spp. from Sifontes Municipality, Bolívar State, Venezuela

Tasas de infección de Plasmodium *spp. para algunos* Anopheles *spp. del municipio Sifontes, Estado Bolívar, Venezuela*

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SUMMARY

Bolivar state represents the most important focus of malaria transmission in Venezuela, most malaria cases are related to gold-mining, especially in the Sifontes municipality. Therefore, the aim of this study was to determine the rate of natural *Plasmodium* spp. infection in *Anopheles* spp. collected between September 2009 and January 2012 in Sifontes. Out of 1,633 *Anopheles* spp. collected, the most abundant species were *An. darlingi* Root (56.7%), *An. Albitarsis* Lynch Arribalzaga sensulato(30.6%) and *An. nuneztovari* Gabaldon s.l. (11.7%). All the mosquitoes were assayed individually by the polymerase chain reaction (PCR) and sixty-five were positive for *Plasmodium* spp. *An. albitarsis* s.l. had the highest infection rate, 5.4% (27/499; 95% CI: 3.63- 7.76), followed by *An. darlingi* with 4.0% (37/926; 95% CI: 2.86-5.45) and *An. nuneztovari* s.l. with 0.5% (1/191; 95% CI: 0.03-2.58). The overall infection rate was 3.9% (65/1,633; 95% CI:3.10-5.0). The rates of infection by *Plasmodium* species in mosquitoes were 3.7% (61/1633) for *P. vivax* and 0.2% (4/1633) for *P. falciparum*. The results show that the species *An. albitarsis* s.l.increased its rate of infection by *Plasmodium* more than 3 times in ten years. This natural infection of the vectors by *Plasmodium* may explain the current epidemiological situation of the Sifontes municipality as not only would the transmission remain, it would also be promoted.

Key words: Malaria, natural infection, *Plasmodium* spp., *Anopheles darlingi*, *An. albitarsis* s.l., Sifontes Municipality.

RESUMEN

El Estado Bolívar representa el foco más importante de transmisión de malaria en Venezuela; la mayoría de los casos están relacionados a la minería de oro, especialmente en el municipio Sifontes. Por lo tanto, el objetivo de este estudio fue determinar la tasa de infección natural de Plasmodium spp. en Anopheles spp. colectados entre septiembre de 2009 a enero de 2012 en Sifontes. De 1.633 Anopheles spp. colectados, las especies más abundantes fueron An. darlingi Root (56,7%), An. albitarsis Lynch Arribalzaga sensu lato(30,6%) y An. nunez tovari Gabaldon s.l. (11,7%). La reacción en cadena de la polimerasa (PCR) se realizó individualmente para todos los mosquitos y sesenta y cinco resultaron positivos para Plasmodium spp.An. albitarsis s.l. tuvo la tasa de infección mayor, 5,4% (27/499; 95% CI: 3,63- 7,76), seguido por An. darlingi con 4,0% (37/926; 95% CI: 2,86-5,45) yAn.nunez tovari s.l. con 0,5% (1/191; 95% CI: 0,03-2,58).Las tasas de infección para las especies de Plasmodium en mosquitos fueron 3,7% (61/1633) para P. vivax y 0,2% (4/1633) para P.falciparum. Los resultados muestran que la especie An. albitarsis s.l. aumentó su tasa de infección por Plasmodium más de 3 veces en diez años. Esta infección natural de los vectores por Plasmodium podría explicar la situación epidemiológica actual del Municipio Sifontes la cual no sólo permitiría que la transmisión permaneciera sino que se promoviera.

Palabras clave: *Malaria, infección natural,* Plasmodium *spp.,* Anopheles darlingi*,* An. albitarsis *s.l., Municipio Sifontes.*

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INTRODUCTION

Malaria is the most important parasitic disease in the world, the World Health Organization (WHO) has estimated that 214 million cases occurred in 2015 and 438,000 people died of malaria while more than 3.2 billion people are at risk of acquiring the disease worldwide (WHO, 2016). Latin America has made great efforts in the fight against malaria, though Venezuela is one of the few countries that fails to reduce the number of malaria cases, which could eventually endanger neighboring countries (WHO, 2016). Bolivar state represents the most important focus of malaria transmission in Venezuela and most malaria cases come from areas related to gold-mining, especially in the Sifontes municipality (Moreno *et al.*, 2014). Evidence indicates that *Anopheles darling* Root and *An. albitarsis* s.l. are the principal vectors in the zone (Moreno *et al.*, 2007, 2009; Rubio-Palis 2009).

Researchers worldwide have claimed that rise in malaria is linking to deforestation and associated environmental changes (Pattanayak *et al.*, 2006; Fornace *et al.*, 2016). Deforestation can produce new habitats for some anopheline species as it can favor specific anopheline larvae development (Moreno *et al.*, 2000, 2015a; Vittor *et al.*, 2009; Medina *et al.*, 2011).The increase in the number of *Anopheles* species increments the risk of human-mosquito contact and the risk of malaria infection. This is one of the main reasons why, in the Sifontes municipality where the deforestation is rising, the malaria transmission is quite significant.

The infection rates of *Plasmodium* species in *Anopheles* mosquito populations are necessary to provide estimates of the intensity of malaria transmission in a study area (Mahapatra *et al.*, 2006; Rubio-Palis, 2009). The method considered the "gold standard" is the observation of sporozoites in the salivary glands of individual mosquitoes, but it is very laborious, time-consuming, requires fresh samples and does not distinguish among all the *Plasmodium* species (WHO, 1975). Two other methods have been used for estimating the sporozoite rate: The enzyme-linked immunoabsorbent assay (ELISA) using monoclonal antibodies targeting the circumsporozoite protein (Beier, 2002) and the *Plasmodium*-specific polymerase chain reaction (PCR) (Snounou, 1993; Rubio *et al.*, 1999; Padley *et al.*, 2003). In general, the PCR technique is more sensitive than ELISA, particularly with low levels of the salivary gland sporozoites.

In the Sifontes municipality, exists a high risk of contracting malaria. During 2016 177,619 out of 240,613 (73.8%) malaria cases were reported in Bolivar state, of which 102,543 (57.7%) comes from this municipality (DGSA, 2016). Therefore, the aim of this study was to determine the rate of natural *Plasmodium* spp. infection in *Anopheles* spp. collected between September 2009 and January 2012 in San Isidro parish of Sifontes municipality, activities conducted within the framework for malaria entomological surveillance and control of the Environmental Health Direction of the Bolivar State (DSAEB, 2012).

MATERIALS AND METHODS

Study Area

The study was performed in two areas with several localities of the San Isidro parish in Sifontes municipality (lat 6º00' - 7º54' N, long 60º44' - 61º39' W), Bolivar state, Venezuela. Moreno *et al.* (2014) already described the area.The municipality, according to the morbidity of malaria and their geographical distribution, has been divided into three foci: Tumeremo to the North, El Dorado in the middle and San Isidro to the South.

In San Isidro parish, the first area "Las Claritas", is formed by residential urban conglomerates of the communities of Las Claritas, Ciudad Dorada and several small towns located along a twenty kilometer transecta side road 10 (San Miguel de Betania, Las Manacas, Tierra Blanca, El Granzón, San Marco). The second area, adjacent to the former, is one of the largest deposits of gold of the country (Las Cristinas mine). Informal, small-scale miners actively mine these deposits resulting in significant deforestation through forest clearing. Both areas, despite the closeness, constitute two separate epidemiological stratus with different demographic and landscape features, and represent the focus with the highest malaria incidence over the last decade in Sifontes municipality as well as in Bolivar state (Moreno *et al.*, 2014).

Mosquito collections

The female mosquitoes (1,633) were collected, both inside and outside houses, between September 2009-January 2012, monthly, 4-7 days in each month, using CDC light traps and up-draft ultra-violet traps (John W. Hock Co, Gainesville, FL), according to the protocol of Moreno *et al.* (2007). In the period August-December 2011 there was no mosquito collection, while in January 2012 it was only carried out in Las Cristinas. All *Anopheles* specimens were identified based on morphological features (Cova-García & Sutil, 1977; Faran & Linthicum, 1981; Rubio-Palis, 2000). After identification, groups of 5-10 *Anopheles* mosquitoes were stored in vials based on species, location and collection day, preserved in silica gel, transported to the Instituto de Investigaciones Biomédicas "Dr. Francisco Javier Triana Alonso" and stored at -80°C until their processing.

DNA extraction

The DNA was extracted from individual specimens using a phenol/chloroform method, resuspended in 50 µl of sterilized water and stored at -80ºC (Rivero *et al.*, 2004).

Nested PCR amplification

Detection and typing of *Plasmodium* spp. in *Anopheles* spp. was performed using a assay described by Snounou *et al.*, (1993). This nested PCR assay uses *Plasmodium* genus-specific primers rPLU6 (5`-TTAAAATTGTTGCAGTTAAAACG-3`) and rPLU5 (5`-CCTGTTGTTGCCTTAAACTTC-3`) for the initial PCR amplification followed by species-specific primers for the second amplification: *P. falciparum* rFAL1 (5`-TTAAACTGGTTT GGGAAAACCAAATATATT-3`) and rFAL2 (5`-ACACAATGAACTCAATCATGACT ACCCGTC-3´), *P. malariae* rMAL1 (5´- ATA ACATAGTTGTACGTTAAGAATAACCGC-3´) and rMAL2 (5´- AAATTCCCAT GCATAAAAAATTATACAAA-3´), *P. vivax*: rVIV1 (5`- CGCTTCTAGCTTAATCCA CATAACTGATAC-3´) and rVIV2 (5´- ACTTCCAAGCCGAAGCAAAGAAAGTCC TTA-3´), *P. ovaler* OVA1 (5´- ATCTCTTTTGCTATTTTTTAGTATTGGAGA-3´) and rOVA2 (5´-GGAAAAGGACACATTAATTG TATCCTAGTG-3´). The PCR amplifications were carried out in 25 µl reaction volumes using 50 ng of template DNA in a PTC-100 thermal cycler (MJ Research, Inc., Watertown, MA). Negative controls (all reagents except template) were run to detect possible contamination. The amplified products were visualized by electrophoresis in 2% agarose gels stained with ethidium bromide

Infection rate

The infection rate was estimated as the percentage of positive *Plasmodium* mosquitoes divided by the total number of mosquitoes assayed.

Data analysis

To estimate the infection rate, the mosquitoes collected were identified by species over all the villages and collection periods. To compare the observed differences in infection rates between anopheline species, the confidence intervals were calculated according to (Berry & Armitage, 1995). The program OpenEpi (Dean *et al.*, 2015) was used for data analysis.

RESULTS

Anopheles *spp. abundance*

The distribution of mosquito species collected by period and locality is shown in Table 1. Of a total of 1,633 *Anopheles* spp. collected, the most abundant species were *An. darlingi* (56.70%), *An. albitarsis* s.l.(30.56%) and *An. nuneztovari* Gabaldon s.l. (11.70%). In 2010, more mosquitoes of all species were collected because the collection was all year round instead of certain months of the other years.

With respect to locality, *An. albitarsis* s.l. population was eight times (58.57%/7.19%) more abundant in Las Cristinas than in San Isidro while *An. darlingi* and *An. nuneztovari* s.l. were 1.84 (72.23%/39.20%) and 12.79 (20.34%/1.59%) more abundant in San Isidro than in Las Cristinas during the period 2009-2011 (Table I). In January 2012, it was observed that the most abundant species in Las Cristinas was *An. darlingi* (47.25%) and *An. albitarsis* s.l. (39.01%). It was also noted that *An. nuneztovari* s.l. increased its population in that locality on the other years, almost 5 times (7.69%/1.59%). Moreover, *An.*

Table I. Anopheles species composition, locality and period distribution in the Sifontes municipality.

Locality	Year	Species n (%)					Total n
		An. darlingi	*An. albitarsis*	*An. triannulatus*	*An. strodei*	*An. nuneztovari*	
San Isidro	2009	53 (88.33)	6 (10)	-	1 (1.66)	-	60
	2010	442 (69.50)	49 (7.70)	-	-	145 (22.80)	636
	2011	98 (78.40)	4 (3.2)	1 (0.8)	-	22 (17.6)	125
	Sub-Total	593 (72.23)	59 (7.19)	1 (0.12)	1 (0.12)	167 (20.34)	821
Las Cristinas	2009	86 (42.57)	111 (54.95)	2 (0.99)	2 (0.99)	1 (0.50)	202
	2010	153 (37.68)	245 (60.34)	-	-	8 (1.97)	406
	2011	8 (36.36)	13 (59.09)	-	-	1 (4.54)	22
	Sub-Total	247(39.20)	369 (58.57)	2 (0.32)	2(0.32)	10 (1.59)	630
	2012	86 (47.25)	71 (39.01)	11 (6.04)	-	14 (7.69)	182
	Total	926 (56.70)	499 (30.56)	14 (0.85)	3 (0.18)	191 (11.70)	1633

n: Number of *Anopheles*. spp. analyzed for each period

triannulatus Neiva & Pinto s.l. went from having a very low abundance in both locations during the period 2009-2011 to 6% of abundance in 2012, representing an increase of 19 times in Las Cristinas (6.04%/0.32%).

Anopheles *spp. Infection by* Plasmodium *spp.*

The total of 1,633 mosquitoes were assayed individually by PCR (Table II). Sixty five were positive for *Plasmodium* spp. *Anopheles darlingi* and *An. albitarsis* s.l. were positive for *P. vivax* and *P. falciparum*, while *An. nuneztovari* s.l. was found to be only positive for *P. vivax*. *Anopheles albitarsis* s.l. had the highest infection rate, 5.41% (27/499; 95% CI: 3.63-7.76), followed by *An. darlingi* with 4.0% (37/926; 95% CI: 2.86-5.45) and *An. nuneztovari* s.l. with 0.52% (1/191; 95% CI:0.03-2.58).

The overall infection rate was 3.98% (65/1,633; 95% CI: 3.10-5.04) (Table 2). The rates of infection by *Plasmodium* species in mosquitoes were 3.7% (61/1633) for *P. vivax* and 0.2% (4/1633) for *P. falciparum* (Table II).

During the period September 2009 - July 2011, the highest rate of infection by *Plasmodium* spp. was in Las Cristinas with 6.4% (40/626; 95% CI: 4.56-8.52), distributed in *An. darlingi* 8.09% (20/247; 95% CI: 5.08-12.28), *An. albitarsis* s.l. 5.15% (19/369; 95% CI:3.19-7.89) and *An. nuneztovari* s.l. 10% (1/10; 95% CI: 0.5-49.32). In addition, a total of 3 *An. triannulatus* and 3 *An. strodei* were analyzed but none was infected. In San Isidro the rate of infection by *Plasmodium* spp. was 2.19% (18/821; 95% CI: 1.34-3.40) distributed in *An. darlingi* 2.36% (14/593; 95% CI: 1.34-3.87) and *An. albitarsis* s.l. 6.78% (4/59; 95% CI: 2.15-16.35). Here a total of 167 *An. nuneztovari* s.l., 1 *An. triannulatus* and 1 *An. strodei*were analyzed but none was infected (Table II). In January 2012, the collections were only in Las Cristinas and *An. albitarsis* s.l. had the highest infection rate by *Plasmodium* spp. with 5.63 % (4/71; 95% CI: 1.79-13.59) followed by *An. darlingi* with 3.5% (3/86; 95% CI: 0.88-9.49). Likewise, 14 *An. nuneztovari* s.l. and 11 *An. triannulatus* were analyzed but none was infected (Table II).

DISCUSSION

Anopheles albitarsis s.l. had a higher infection rate by *Plasmodium* spp. (5.4%) than *An. darlingi* (4.0%) for all months of the years studied, except in 2011 when it was collected no infected specimen, probably because *An. albitarsis* s.l. represented only 11.56% of the total mosquitoes collected, compared with 72.11% of *An. darlingi*. This result differs from Moreno *et al.*, (2009) who worked in the same Sifontes municipality during 1999-2000 and reported a sporozoite rate ~ 3 times less for *An. albitarsis* s.l. (0.27%) than for *An. darlingi* (0.82%). It could mean that the species *An. albitarsis* s.l. had to increase its rate of infection by *Plasmodium* more than 3 times in ten years.

Table II. Natural *Plasmodium* infection in *Anopheles* spp. collected in Las Cristinas and San Isidro during Sept. 2009- Jan. 2012.

Species	Year	Las Cristinas				San Isidro				Total *Plasmodium* spp. IR[c] (95% CI)
		N[a]	*P. vivax* % (n[b])	*P. falciparum* % (n[b])	*Plasmodium* spp. IR[b] (95% CI)	N[a]	*P. vivax* % (n[b])	*P. falciparum* % (n[b])	*Plasmodium* spp. IR[c] (95% CI)	
An. darlingi	2009	86	3.50 (3)	-	3.50 (3) [0.88 – 9.49]	53	-	-	-	
	2010	153	11.11 (17)	-	11.11 (17) [6.68 – 17.43]	442	0.90 (4)	-	0.90 (4) [0.28–2.18]	
	2011	8	-	-	-	98	9.18 (9)	1.02 (1)	10.20 (10) [5.18–18.19]	
	2009-2011	247	8.09 (20)	-	8.09 (20) [5.08-12.28]	593	2.19 (13)	0.17 (1)	2.36 (14) [1.34–3.87]	
	2012	86	1.16 (1)	2.32 (2)	3.5 (3) [0.88 – 9.49]	-	-	-	-	
Sub-Total	All	333	6.30 (21)	0.60 (2)	6.90 (23) [4.48 – 10.20]	593	2.19 (13)	0.17 (1)	2.36 (14) [1.34–3.87]	4.00 (37/926) [2.86–5.45]
An. albitarsis s.l.	2009	111	10.81 (12)	-	10.81 (12) [5.85–18.38]	6	-	-	-	
	2010	245	2.85 (7)	-	2.85 (7) [1.25–5.65]	49	8.16 (4)	-	8.16 (4) [2.59–19.69]	
	2011	13	-	-	-	4	-	-	-	
	2009-2011	369	19	-	5.15 (19) [3.19–7.89]	59	6.78 (4)	-	6.78 (4) [2.15–16.35]	
	2012	71	4.22 (3)	1.41 (1)	5.63 (4) [1.79–13.59]	-	-	-	-	
Sub-total	All	440	5.00 (22)	0.23 (1)	5.23 (23) [3.39–7.72]	59	6.78 (4)	-	6.78 (4) [2.15–16.35]	5.41 (27/499) [3.63–7.76]
An. nuneztovari	2009	1	1	-	100 (1) [5.00–49.32]	-	-	-	-	
	2010	8	-	-	-	145	-	-	-	
	2011	1	-	-	-	22	-	-	-	
	2009-2011	10	1	-	10 (1) [0.50–49.32]	167	-	-	-	
	2012	14	-	-	-	-	-	-	-	
Sub-total	All	24	1	-	4.17 (1) [0.21-20.55]	167	-	-	-	0.52 (1/191) [0.03-2.58]
Others	2009-2011	6	-	-	-	2	-	-	-	-
	2012	11								
Total		812	44	3	5.79 (47) [4.30-7.63]	821	17	1	2.19 (18) [1.34–3.40]	3.98 (65/1633) [3.10-5.04]

N[a] Number of *Anopheles* spp. analyzed; n[b] Number of *Anopheles* spp. infected with *Plasmodium* spp; [c]Infection Rate: Number of positive *An. spp.* (n[b]) per number of total analyzed (N[a]) per 100, determined for each locality, IR = [(n[b] / N[a]) × 100]

In spite, however, of the advantages of molecular diagnostic tests (most accuracy and sensitivity) to detect and identify the malaria parasite species, employment of these techniques for recognizing precisely infective stage mosquitoes and assessing sporozoite rates, needs to get rid of the abdomen of the mosquito. That is because if mosquitoes recently fed on human blood infected with malaria (erythrocytic form), false-positive results could be obtained. Here, in the present study, all positive *Anopheles* mosquitoes were considered to have infective sporozoites, and therefore the results could overestimate the true infection rate. However, it is important to be aware that this study gave emphasis to the relative difference between the infection rate of *An. albitarsis* s.l. and *An. darlingi* which could compensate for the overestimation of the absolute values and give a direct insight into the true scale of difference between both *Anopheles*.

It was shown that *P. vivax* infected more than *P. falciparum* the main *Anopheles* species present, 11.5 (3.67% *P. vivax*/0.32% *P. falciparum*) and 26.0 (5.21% *P. vivax*/0.20% *P. falciparum*) times more for *An. darlingi* and *An. albitarsis* s.l. respectively. This result was also greater than the one found in the Sifontes municipality a decade ago when the rate of infection of *P. vivax* was only 3.7 times more than *P. falciparum* (Moreno *et al.*, 2009). Although both epidemiological and sampling situations are not comparable, this high natural infection of the vectors by *Plasmodium* may explain the current epidemiological situation of the Sifontes municipality.

Anopheles albitarsis s.l. was considered an opportunistic vector (Moreno *et al.*, 2000, 2004, 2007) and had also been proposed as an emerging vector

(Moreno *et al.*, 2009) in Bolivar state. Although this study confirms that *An. albitarsis* s.l, and *An. darlingi* are primarily responsible for the malaria endemicity in the Sifontes municipality (Moreno *et al.*, 2009), it demonstrates for the first time that *An. albitarsis* s.l. has a higher rate of *Plasmodium* infection than *An. darlingi*. These results suggest that *An. albitarsis* s.l. is gradually becoming the main species in Sifontes by displacing *An. darlingi*. However, further studies on the bionomics of these vectors are required to confirm this hypothesis.

Anopheles albitarsis s.l. has already been incriminated as a primary malaria vector in Amapá state of Brazil in a region of northeastern Amazonia (Conn *et al.*, 2002). It was also reported that this species was more abundant than *An. darlingi* in Roraima, Brazil during the period 2001-2002 (Póvoa *et al.*, 2006). The emergence of *An. albitarsis* s.l. was also informed in Puerto Carreño, Colombia and the report concluded that *An. albitarsis* s.l. with *An. darlingi* contribute to maintaining endemic malaria all year round in this region (Jiménez *et al.*, 2012). At this point, it is important to note that, as with other species of the subgenus *Nyssorhynchus*, the taxon *An. albitarsis* s.l. is a complex of at least nine species morphologically indistinguishable from each other, of which only six have been formally described (Ruíz-López *et al.*, 2012). At present, the bionomics and ecology of the species within this complex are not known with certainty (Ruíz-López *et al.*, 2012). Of these, only *An. albitarsis* F has been confirmed in Bolivar state (Rubio-Palis *et al.*, 2013).

The fact that *An. albitarsis* s.l. is a primary malaria vector in the Sifontes municipality may be due to deforestation from mining that impact the rainforest environment causing major ecological changes and favoring habitat for *An. albitarsis* s.l. Larvae of this species are common and abundant in man-made shallow water bodies exposed to sunlight, characteristic habitats generated by mining activities (Moreno *et al.*, 2015a, 2015b). By contrast, *An. darlingi* is usually the predominant species in environments partially shaded, near the borders of forests with high humidity and rainfall (Berti*et al.*, 2008, Moreno *et al.*, 2015a, 2015b). It has been reported that in Ramal do Granada, Brazil, populations of *An. darlingi* decreased depending on deforestation that is caused by human activity (Moutinho *et al.*, 2011). On the other hand, the spatial distribution of *An. albitarsis* s.l. in the Atlantic forest in southeastern Brazil, correlated with open areas or deforested lands which are associated with changes in the natural ecosystem (Laporta *et al.*, 2011). Also, in this case, a significant association between the presence of *An. albitarsis* s.l. with malaria cases was found.

During the study period (September 2009-January 2012), the Sifontes municipality reported 60,073 accumulative malaria cases which represented 54.5% of the nationwide malaria cases and 42,748 (71.1%) were registered in San Isidro parish (DSAEB, 2009-2012). In this parish, the area of Las Claritas, very deforested for being a mining town (Las Cristinas mine) located at km 88, was the one with the highest prevalence of *An. albitarsis* s.l. (54%) compared to *An. darlingi* (41%). These ecological changes that favor *An. albitarsis* s.l. habitats together with the massive convergence of people from different parts of Venezuela and from other countries who can carry the parasite, suggest that malaria transmission will continue to increase, especially if the natural vegetation continues reducing in the municipality. To clarify this point, it would be necessary to study the dynamics of malaria incidence and of the vector populations between partially and totally deforested areas to determine the relationships among variables under the two conditions.

Other species present in the Sifontes municipality were *An. nuneztovari* s.l., predominant vector in western Venezuela (Rubio-Palis, 2009), and also, albeit to a lesser extent, *An. triannulatus* s.l. which is considered a secondary vector (Sinka *et al.*, 2010), although recently there was reported that it was one of the most abundant in Colombia (Rosero *et al.*, 2013).

A key element of the malaria prevention and control is the surveillance of mosquito populations. This includes various factors like the mosquito population abundance, *Anopheles* and *Plasmodium* species circulating in a certain area, and the *Anopheles* infection rates, especially in areas in which there are several *Anopheles* species that can transmit malaria, like the Sifontes municipality (Galardo *et al.*, 2007).The precise determination of this last factor, according to the results of this study, should be considered to strengthen public health laboratories and reorient strategies for controlling malaria vectors in the Sifontes municipality.

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Conflict of interest

The authors declare that they have no conflict of interest.

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