Anopheles species composition explains differences in *Plasmodium* transmission in La Guajira, northern Colombia

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Malaria in La Guajira, the most northern state of Colombia, shows two different epidemiological patterns. Malaria is endemic in the municipality of Dibulla whereas in Riohacha it is characterised by sporadic outbreaks. This study aimed to establish whether differences in transmission patterns could be attributed to different vector species. The most abundant adult female species were Anopheles aquasalis, *exclusive to Riohacha, and* Anopheles darlingi, *restricted to Dibulla.* Anopheles *mosquitoes were identified using morphology and the molecular markers internal transcribed spacer 2 and cytochrome c oxidase I. All specimens (n = 1,393) were tested by ELISA to determine natural infection rates with* Plasmodium falciparum *and* Plasmodium vivax. An. darlingi *was positive for* P. vivax *210, with an infection rate of 0.355% and an entomological inoculation rate of 15.87 infective bites/person/year.* Anopheles albimanus *larvae were the most common species in Riohacha, found in temporary swamps; in contrast, in Dibulla* An. darlingi *were detected mainly in permanent streams. Distinctive species composition and larval habitats in each municipality may explain the differences in* Plasmodium *transmission and suggest different local strategies should be used for vector control.*

Key words: *Anopheles* - *Plasmodium* - malaria

In Colombia, malaria is the most important vector-borne disease, with an average of 123,000 cases per year during the last decade (WHO 2012). *Plasmodium vivax* is responsible for 62.9% of these cases; *Plasmodium falciparum* for 37% and *Plasmodium malariae* for 0.1% (WHO 2012). In the northern state of La Guajira, in the Caribbean region, malaria has not been a major public health problem except in the foothills of the Sierra Nevada de Santa Marta, particularly in the municipality of Dibulla where *P. falciparum* malaria cases predominated every year in the 90's (Cáceres et al. 2000). Nevertheless, since the mid-1990s a rise in the overall number of cases has been consistently reported. Between 1990-1995, the average annual number of cases ranged from 40-150. This increased to 500 between 1997-1998. An exponential increment of cases was reported in the period December 1999-November 2000. A total number of 5,853 cases and 12 deaths were reported, by far the largest outbreak reported in this region. The municipalities of Riohacha and Dibulla were the most affected (González 2000). After this period, cases decreased to an average of 150 between 2000-2007. In 2008-2009, another outbreak occurred in which Dibulla was the most affected, contributing 1,617 cases out of 3,041, mainly caused by *P. vivax* (SIVIGILA 2013). Subsequently, a Global Fund project (PAMAFRO 2011) intensified the distribution of insecticide treated nets in Dibulla and malaria cases declined to 148 cases (10% of the state) (SIVIGILA 2013).

Even though Riohacha and Dibulla have been the localities most affected during malaria outbreaks, ecological conditions and epidemiological patterns of the disease are distinctive in each municipality. Riohacha is located in a desert valley and cases of malaria, mainly *P. vivax*, only appear in high numbers at the end of the rainy season during the last few months of the year. On the other hand, Dibulla is located in the foothills of the Sierra Nevada de Santa Marta, where malaria is endemic, cases of *P. falciparum* were more common in the 90's (Cáceres et al. 2000) and *P. vivax* predominated from the 2008-2009 outbreak (SIVIGILA 2013).

Entomological studies conducted in this region are scarce. In 1957 the former Malaria Eradication Service (SEM) reported the presence of nine species of *Anopheles* (*currently known as species complex): *Anopheles albimanus* Wiedemann, *Anopheles pseudopunctipennis** Theobald, *Anopheles darlingi* Root, *Anopheles triannulatus** (Neiva & Pinto), *Anopheles punctimacula* Dyar & Knab, *Anopheles albitarsis** Galvão & Damasceno, *Anopheles aquasalis* Curry, *Anopheles argyritarsis* Robineau-Desvoidy and *Anopheles neomaculipalpus* Curry (SEM 1957). Studies carried out during the 1999-2000 malaria outbreak recorded eight species, seven of which had already been registered by SEM and one species, *Anopheles trinkae* Faran, 1979, reported for the first time in this area (Cáceres et al. 2000). Among these

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species *An. albimanus* was proposed as the main malaria vector based on its abundance and presence at all sampling sites, but natural infectivity rates were not measured (Cáceres et al. 2000, Porras et al. 2001). However, species well-known as malaria vectors in other Neotropical countries, such as *An. darlingi*, *An. pseudopunctipennis s.l.* and *An. aquasalis* (Grillet 2000, Sinka et al. 2010, Hiwat & Bretas 2011) are also present in La Guajira and may be involved in local transmission.

Given the differences in *Plasmodium* transmission in Dibulla and Riohacha and the diversity of species reported in La Guajira, this study aimed to identify the species of *Anopheles* present in these two municipalities and to determine their natural infectivity with *Plasmodium* as a first step to incriminate the malaria vectors in the region.

La Guajira is the northern most part of South America, bounded by the Caribbean and Bolivarian Republic of Venezuela. Riohacha is the state capital and has approximately 170,000 inhabitants in an area of 20,848 km^2. It is located in a desertic area with annual rainfall of 1,000 mm. Dibulla has a population of 21,078, an area of 6,633 km^2 and is characterised by humid tropical forest weather with 3,000-5,000 mm rainfall/year (Gobernación de La Guajira 2008).

Within each municipality, a household was selected taking into account local malaria endemicity, the presence of *Anopheles* mosquitoes and the feasibility of access throughout the sampling period: Radio Delfin in Riohacha 11º32'645"N 72º53'944"W and Las Flores in Dibulla 11º14'813"N 73º09'951"W (Fig. 1). Five mosquito surveys of four days each were conducted between 2007-2008 in both localities, including three surveys in the dry season (May 2008-July 2007, 2008) and two during the rainy season (August-September 2008).

Human landing catches (HLC) were undertaken from 06:00 pm-06:00 am during the first night in each sampling area to determine the peak of activity of anthropophilic mosquitoes. Consecutive HLC were carried out only during the biting peak between 08:00 pm-12:00 am. Each catch was conducted for intervals of 50 min followed by 10 min of observation on the surfaces of houses to search for resting mosquitoes. One collector was located inside and another was outside each house simultaneously and they switched positions every hour to avoid bias due to differences in attractiveness or catching ability. Mosquitoes collected were killed the next day in lethal chambers with ethyl acetate. Then they were packed individually in 0.5 mL Eppendorf tubes and stored in bags containing silica gel. Data were analysed using geometric means to determine the biting rate, interpreted as the average number of bites/human/h indoors and outdoors. A student *t* test was used to determine if there was a difference between the average of mosquitoes collected during the dry and rainy seasons. Additionally, nightly collections were made in animal shelters, most often in cattle and goats stables, by actively searching for resting mosquitoes in a radius of 100 m around each house.

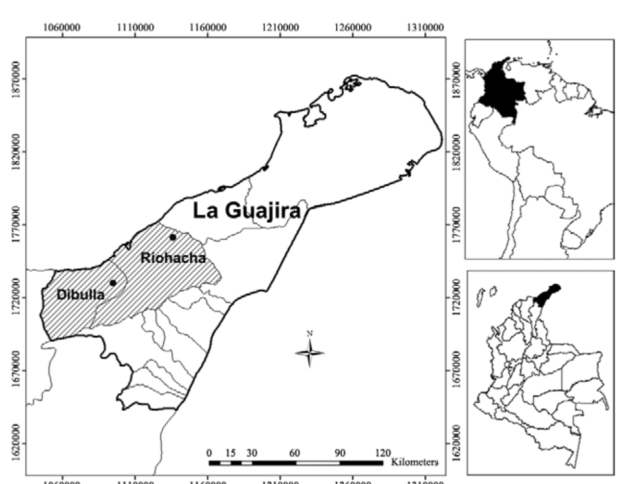

Fig. 1: map of the study area, department of La Guajira, Colombia, showing the municipalities of Riohacha (11º32'645"N 72º53944"W) and Dibulla (11º14'813"N 73º09'20"W). Dots indicate the exact location of sampling households.

In each locality searching for breeding sites was conducted within 100 m of each of the houses. Sampling was done by dipping 10 times per m^2 with a standard dipper (350 mL). Collected larvae were maintained for linked rearing and the larval and pupal skins kept for taxonomic determination.

All collected specimens were identified based on morphological traits (González & Carrejo 2009). Identifications were confirmed by internal transcribed spacer (ITS)2[ITS2F(5'-TGTGAACTGCAGGACACATGAA-3') and ITS2R (5'-ATGCTTAAATTTAGGGGGTAGTC-3')] and cytochrome c oxidase I (COI) [TY-J-1400 (5'-TACAATTTATCGCCTAAACTTCAGCC-3') and UEA-6 (5'-TTAATTCCTGTAGGNACAGCAATAATTAT-3')].

The determination of mosquitoes naturally infected with *Plasmodium* was performed following the standard methodology of the ELISA kit distributed by the Centers for Disease Control (USA) using *P. vivax* 210, 247 and *P. falciparum* monoclonal antibodies.

This study was approved by the Ethical Committee of the Faculty of Medicine of the National University of Colombia, protocol 1608.

Considering morphological characteristics and ITS2 and *COI* sequences, seven species of *Anopheles* were identified for the region: *An. albimanus* (KC354815-7, KC3548224-5), *An. aquasalis* (KC354812-3, KC354821), *An. darlingi* (KC894593-5), *An. punctimacula* (KC354809, KC354818), *An. pseudopunctipennis s.l.* (KC354810-1, KC354819-20), *An. argyritarsis* and *An. triannulatus s.l.*

A total of 1,343 specimens were collected by HLC and 47 larval series were successfully reared. A similar proportion of specimens were collected in each municipality, however, the species composition was different. In Riohacha, *An. albimanus* larvae was found in a swamp and in a well, as well as in sympatry with *An. pseudopunctipennis s.l.* in a stream margin (total = 17). In Dibulla *An. albimanus*, *An. darlingi*, *An. argyritarsis*, *An. punctimacula* and *An. pseudopunctipennis s.l.* were collected in a stream margin (total = 30 larvae).

TABLE

Anopheles species caught by human landing catches (HLC) and resting outdoors in the municipalities of Riohacha and Dibulla, Colombia, 2008-2009

		HLC								
		Dry season			Rainy season					
Municipality	Species	GM	95% CI	n	GM	95% CI	n	Student *t* test (p)	Resting	Total
Riohacha	*An. aquasalis*	0.24	0-0.9	30	82.7	55.1-124	639	12.03 (< 0.001)	-	669
	An. albimanus	-	-	0	2.81	0.1-11.8	58	-	-	58
Dibulla	*An. darlingi*	17.64	9.7-31.2	458	5.26	2.6-9.6	45	2.57 (0.018)	17	520
	An. albimanus	3.93	2.2-6.4	85	1.1	0.3-2.4	10	2.57 (0.017)	6	101
	An. punctimacula	-	-	1	-	-	0	-	1	2
	An. triannulatus s.l.	-	-	0	0.64	0-1.5	6	-	25	31
	An. pseudopunctipennis s.l.	0.46	0.14-0.87	9	0.22	0-0.6	2	0.92 (0.36)	1	12
Total		-	-	583	-	-	760	-	50	1,393

CI: confidence intervals; GM: geometric means.

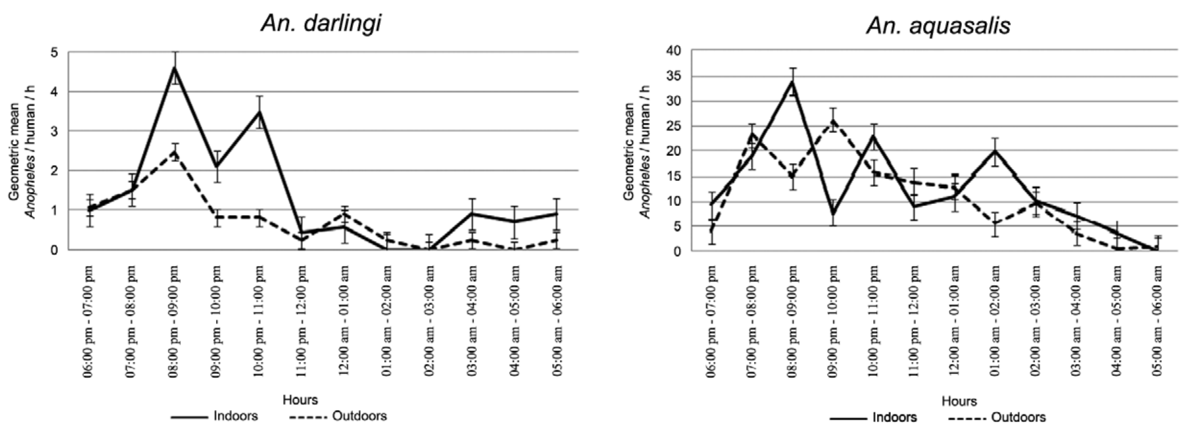

Fig. 2: human landing activity of *Anopheles aquasalis* in the municipality of Riohacha and *Anopheles darlingi* in the municipality of Dibulla, La Guajira, Colombia.

From HLC the most abundant species was *An. aquasalis* (48%), collected only in Riohacha, followed by *An. darlingi* (37.3%), found only in Dibulla. *An. albimanus* was collected in both municipalities at low densities (11.4%). Other species such as *An. punctimacula*, *An. triannulatus s.l.* and *An. pseudopunctipennis s.l.* were found in lower abundance representing 3.2% of the total catches (Table). No evidence was found of the presence of *An. trinkae*, as previously reported in this region (Cáceres et al. 2000).

The abundance of *An. aquasalis* and *An. albimanus* in Riohacha increased as the rainy season progressed, whereas in Dibulla a higher abundance of *An. darlingi* was found during the dry season. The differences between the two seasons in the geometric mean number of the mosquitoes collected for each of those species were statistically significant (Table). Resting mosquitoes (n = 50) were only collected outdoors in Dibulla near goats (*Capra hircus*) stables. The most abundant species associated with goats, the most common regional domestic animal, were *An. triannulatus s.l.* (50%), followed by *An. darlingi* (34%) and *An. albimanus* (12%) (Table).

Human landing activity was measured by hour for the most abundant species in each area, *An. aquasalis* in Riohacha and *An. darlingi* in Dibulla. *An. aquasalis* was present throughout the night, both indoors and outdoors, with a slight decrease in its activity after 02:00 am. For this species the biting rate was 153 bites/human/night. *An. darlingi* showed a higher activity during the first six hours of the night, both indoors and outdoors. After midnight, its density dropped, but the species was still present. For *An. darlingi* the biting rate was 12.24 bites/human/night (Fig. 2).

All field-collected adult specimens were tested by ELISA. Only *An. darlingi* (n = 2) was positive for the circumsporozoite protein of *P. vivax* 210. The positive samples were collected in Dibulla at 01:00 am in July 2008 at the end of the dry season. Infection rate was 0.355 and the estimated entomological inoculation rate (EIR) for this species was 15.8 infective bites/person/year.

Plasmodium transmission in La Guajira is characterised by outbreaks. This explains the intermittent nature of vector control programmes, based primarily on the treatment of mosquito larval habitats with biological larvicides such as *Bacillus sphaericus* and indoor residual spraying, usually with deltamethrin (0.02 g/m^2). From 2008, when the Global Fund project (PAMAFRO 2011) began, the use of insecticide treated nets has been promoted and distributed by the malaria program, particularly for Dibulla. However, efficiency and effectiveness of these control measures remain unknown, in part because little information regarding the role of different species of *Anopheles* in *Plasmodium* transmission was obtained prior to insecticide treated net usage to enable an objective evaluation.

In our study we found that different species of *Anopheles* are correlated with different geographic areas and type of *Plasmodium* transmission. In Riohacha, *An. aquasalis* was the most abundant species, followed by *An. albimanus* and *An. pseudopunctipennis s.l.* The latter was collected only in the larval stage. In Dibulla a higher diversity of species was found, with *An. darlingi* predominating. Although the two collection sites are only separated by 53 km, the difference in species composition might be due to the type of larval habitats in each municipality. Riohacha had a higher proportion of temporary larval habitats (61.5%, n = 13), characterised by large flooded areas present mainly during the rainy months. The remaining proportion (38.5%) consisted of permanent larval habitats, specifically man-made wells, in which only *An. albimanus* larvae were found. In our collection sites we did not find breeding sites for *An. aquasalis*, despite the adult density and the presence of saline mangroves referred to as preferred larval habitats for this species (Berti et al. 1993, Grillet 2000). In Dibulla, temporary larval habitats accounted for only 9.1% (n = 11) and mainly consisted of animal tracks. In this municipality the most important larval habitats were permanent streams with slow currents, partial shade and emergent vegetation, which are known to be suitable habitats for several *Anopheles* species including *An. darlingi* (Faran 1980, Sinka et al. 2010).

In Dibulla, *An. darlingi* was positive for *P. vivax* 210, with an infection rate of 0.35%. The present finding, in combination with high anthropophily, confirms the role of *An. darlingi* as the primary vector in this region. This result widens the geographic range within which this species has been established as the main malaria vector, adding La Guajira to the eastern region of Colombia, including the Amazon and other regions like Urabá, Bajo Cauca and Magdalena in the northwest of the country (Olano et al. 2001, Gutierrez et al. 2010, Ahumada et al. 2012).

The EIR for *An. darlingi* was 15 infective bites/person/year, about one/month, and may explain the endemicity of malaria in this area. The EIR reported in this study is similar to that reported for this species in French Guiana where the values for the three localities were 22.8, 27.4 and 14.4 (Girod et al. 2008), but much lower than the rate of 129 reported in the region of Ocamo, in the Venezuelan Orinoco (Magris et al. 2007). These differences in the EIR for the same species might be due to the biting rates reported in different geographic areas rather than the sporozoite rate, considering the similarities in the infectivity results in the different areas and the wide range of densities. This may be relevant as it shows the capacity of this species to maintain transmission even at a low density.

In the Riohacha area, neither *An. aquasalis* nor *An. albimanus* were positive for the circumsporozoite protein. Studies in Brazil and Venezuela (Berti et al. 1993, Povoa et al. 2003), where *An. aquasalis* is a recognised vector, have found infection rates between 0.7-1.18, suggesting that the number of mosquitoes tested in the present study (n = 690) was probably not sufficient to determine the natural infection in this species. Additionally, *An. aquasalis* and *An. albimanus* have mainly zoophilic behaviour (Zimmerman 1992) which implies that high densities of either species is needed to have a major impact in *Plasmodium* transmission (Deane 1986, Berti et al. 1993, Montoya-Lerma et al. 2011).

In conclusion, the type of *Plasmodium* transmission in La Guajira seems to be related to the *Anopheles* species present. In Dibulla the vector is *An. darlingi* and therefore, due to anthropophilic behaviour and indoor biting throughout the night, the use of insecticide treated nets is recommended as a method of local control. However, any complementary control activity to reduce the human-vector contact might be beneficial, because of *An. darlingi* activity in the earlier part of the night when people might be outdoors. In Riohacha, where the potential vectors appear to be *An. albimanus* and *An. aquasalis,* personal protection and treatment of breeding sites should be the main control strategy, alongside any complementary indoor control activities such as residual spraying or the use of insecticide treated nets.

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