Species composition and natural infectivity of anthropophilic *Anopheles* (Diptera: Culicidae) in the states of Córdoba and Antioquia, Northwestern Colombia

Lina A Gutiérrez[1]/+, John J González[2], Giovan F Gómez[1], Martha I Castro[1,2], Doris A Rosero[1], Shirley Luckhart[3], Jan E Conn[4], Margarita M Correa[1]

[1]Grupo de Microbiología Molecular, Escuela de Microbiología, Universidad de Antioquia, Calle 67 53-108, 5-430 Medellín, Colombia [2]Unidad de Entomología, Laboratorio de Salud Pública, Secretaría de Salud Departamental de Córdoba, Montería, Colombia [3]Department of Medical Microbiology and Immunology, University of California at Davis, California, USA [4]Griffin Laboratory, Wadsworth Center, New York State Department of Health, New York, USA

Malaria is a serious health problem in the states of Córdoba and Antioquia, Northwestern Colombia, where 64.4% of total Colombian cases were reported in 2007. Because little entomological information is available in this region, the aim of this work was to identify the Anopheles *species composition and natural infectivity of mosquitoes distributed in seven localities with highest malaria transmission. A total of 1,768* Anopheles *mosquitoes were collected using human landing catches from March 2007-July 2008. Ten species were identified; overall,* Anopheles nuneztovari *s.l. was the most widespread (62%) and showed the highest average human biting rates. There were six other species of the* Nyssorhynchus *subgenus:* Anopheles albimanus *(11.6%),* Anopheles darlingi *(9.8%),* Anopheles braziliensis *(6.6%),* Anopheles triannulatus *s.l. (3.5%),* Anopheles albitarsis *s.l. and* Anopheles oswaldoi *s.l. at < 1%; and three of the* Anopheles *subgenus:* Anopheles punctimacula, Anopheles pseudopunctipennis *s.l. and* Anopheles neomaculipalpus *at < 1% each. Two species from Córdoba,* An. nuneztovari *and* An. darlingi, *were found to be naturally infected by* Plasmodium vivax *VK247, as determined by ELISA and confirmed by nested PCR. All species were active indoors and outdoors. These results provide basic information for targeted vector control strategies in these localities.*

Key words: malaria - *Anopheles* - biting activity - natural infectivity - Northwestern Colombia

Malaria remains an important public health problem in Colombia. Of the 110,480 malaria cases reported in 2007 by the Colombian National Institute of Health, 31.9% were from Córdoba and 32.5% from Antioquia. These regions report significant numbers of autochthonous malaria cases, particularly those caused by *Plasmodium vivax* Grassi & Felleti (INS 2007).

It is a challenge to determine all of the factors involved in the dynamics of malaria transmission in a given geographic area. However, knowledge about the presence, distribution and abundance of anopheline vector species is critical to facilitate development of efficient vector control policies (Loaiza et al. 2008). Initial efforts to describe *Anopheles* species distribution in Colombia were conducted by the Servicio de Erradicacion de la Malaria (SEM) more than 50 years ago. These studies reported 16 *Anopheles* species from Antioquia and 11 species from Córdoba (SEM 1957). A recent review of *Anopheles* species distribution in Colombia (González & Carrejo 2007), which included SEM reports and data gathered from scientific papers, showed a high diversity of *Anopheles* species in both states: *Anopheles albimanus* Wiedemann, *Anopheles apicimacula* Dyar & Knab, *Anopheles argyritarsis* Robineau-Desvoidy, *Anopheles braziliensis* (Chagas), *Anopheles costai* Fonseca & Ramos, *Anopheles darlingi* Root, *Anopheles eiseni* Coquillett, *Anopheles marajoara* Galvão & Damasceno, *Anopheles neomaculipalpus* Curry, *Anopheles nuneztovari* Gabaldon, *Anopheles oswaldoi* (Peryassu), *Anopheles pseudopunctipennis* Theobald, *Anopheles punctimacula* Dyar & Knab, *Anopheles rangeli* Gabaldon, Cova Garcia & Lopez, *Anopheles strodei* Root and *Anopheles triannulatus* Neiva & Pinto. *Anopheles malefactor* Dyar & Knab and *Anopheles neivai* Howard Dyar & Knab were reported only in Antioquia and *Anopheles aquasalis* Curry only in Córdoba. Several of these species, including *An. albimanus*, *An. darlingi* and *An. nuneztovari* s.l., are primary malaria vectors in other regions of Colombia and *An. rangeli*, *An. oswaldoi* s.l., *An. neivai* and *An. marajoara* are of regional/local importance (Herrera et al. 1987, Olano et al. 2001, Quiñones et al. 2006, Gutiérrez et al. 2008).

Morphological and molecular studies have demonstrated high levels of intra-individual variation and inter-specific similarities in *Anopheles* (Harbach 2004, Marrelli et al. 2005, Li & Wilkerson 2007, Calle et al. 2008). In Colombia, the geographical distribution of members of species complexes and their potential role(s) in malaria transmission are poorly understood.

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+ Corresponding author: liangutibui@gmail.com
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Examples include the Albitarsis Complex (Wilkerson et al. 1995, Lehr et al. 2005), *An. triannulatus* s.l. (Silva-Do-Nascimento et al. 2006), *An. nuneztovari* s.l. (Kitzmiller et al. 1973, Conn et al. 1998, Mirabello & Conn 2008) and *An. pseudopunctipennis* s.l., a recognised malaria vector in Mexico (Rodriguez et al. 2000, Joy et al. 2008), Bolivia (Lardeux et al. 2007) and Argentina (Dantur Juri et al. 2009). It is important to clarify the role that *Anopheles* species from Córdoba and Antioquia have in malaria transmission because primary malaria vectors may coexist with other species that are potential vectors or vectors of regional/local importance (Moreno et al. 2005, Póvoa et al. 2006, Quiñones et al. 2006, Gutiérrez et al. 2008). It has been observed that species distribution and behaviour can vary temporally in relation to climate variations (Tadei et al. 1998, Gevrey & Worner 2006) and furthermore, recent studies suggest that geographic and climatic variation, in addition to the cultural diversity observed in endemic regions of Colombia, may affect malaria transmission (Poveda et al. 2001, Mantilla et al. 2009). The aim of this study was to provide current information on anopheline species geographical distribution and detection of mosquitoes naturally infected with *Plasmodium falciparum* Welch and *P. vivax*, VK210 and VK247 collected in close proximity to humans in various localities within Antioquia and Córdoba. This paper provides a revision of anthropophilic *Anopheles* species composition, feeding behaviour and vector incrimination through the evaluation of indoor and peri-domestic areas in these localities.

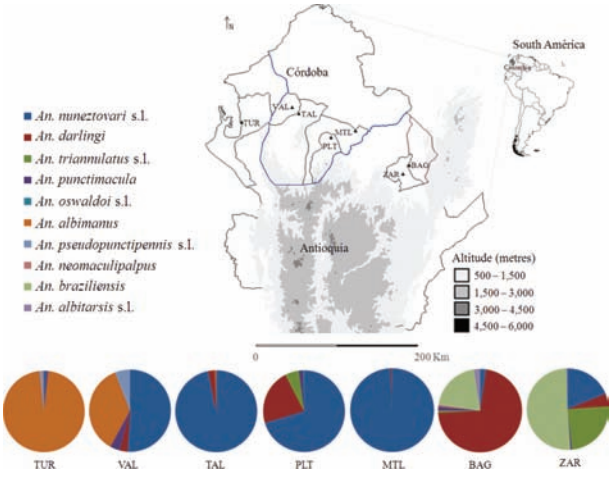
MATERIALS AND METHODS

Study sites - *Anopheles* mosquitoes were collected in localities from the states of Córdoba and Antioquia (departments), Northwestern Colombia. Both states include areas in the Caribbean and the Andean Regions, which correspond to Coastal and Piedmont ecoregions, respectively, according to the classification of Rubio-Palis and Zimmerman (1997). These areas are characterised by diverse flora and wildlife, which are influenced by the contrasting topographies and temperature variations. The sites had temperatures up to 26ºC and relative humidity > 80% (IGAC 2002). Localities with the highest official malaria reports from each state were selected for this study and included in Antioquia: Turbo (TUR; 8°05'N, 76°44'W), Zaragoza (ZAR; 7°29'N, 74°51'W) and El Bagre (BAG; 7°35'N, 74°49'W); these localities represented 36% of the total cases reported for Antioquia (DSSA 2008). In Córdoba, the localities sampled were: Valencia (VAL; 8°15'N, 76°08'W), Tierralta (TAL; 8°10'N, 76°03'W), Puerto Libertador (PLT; 7°54'N, 75°40'W) and Montelibano (MTL; 7°59'N, 75°25'W) (Figure); these localities represented 97.85% of the total cases for Córdoba (Gobernación de Córdoba 2008).

The main economic activities in VAL, TAL, MTL and PLT (Córdoba) and TUR (Antioquia) are crop and livestock production, while in BAG and ZAR (Antioquia), alluvial mining, followed by livestock, sorghum and rice production are the most important economic activities. In this study, most field visits could not be planned according to seasonal or temporal climatic changes. Rather, collections were based on accessibility to the localities and on confirmation of local security.

Mosquito collection and species identification - The collection of adult mosquitoes by human-landing catches (HLC) were conducted under an informed consent agreement using a protocol and collection procedures that were reviewed and approved by a University of Antioquia Institutional Review Board. Indoor and peri-domestic collections (within ~5 m of each house) were performed from 18-22 h ± 2 h, between March 2007-July 2008. At least one collection per field trip was conducted overnight (18-6 h) in TAL, PLT, MTL and TUR. Collections were conducted in most cases by at least three human baits per shift. In addition, larval habitats were visited for collection at one location in each state, ZAR and MTL. For some species it was possible to obtain and rear field-collected larvae to support species identification. Adult mosquitoes and immature stages available (larvae, pupae or their exuviae) were identified based on morphological features according to the taxonomic key by Gonzalez and Carrejo (2007). Because mis-assignments occur often among species in the Oswaldoi Group of *Nyssorhynchus* based on morphological characteristics (Ruiz et al. 2005), we verified species assignation for all the Oswaldoi Group specimens using a PCR-RFLP assay based on ITS2 sequences (Zapata et al. 2007, Cienfuegos et al. 2008). The DNA was obtained from a single leg added to a PCR mixture or genomic DNA obtained from individual abdomens. In addition, some of the mosquitoes identified as *An. albimanus* or *An. darlingi* were selected at random and their species assignment was molecularly confirmed using the PCR-RFLP-ITS2 assay (Zapata et al. 2007).

Natural infectivity status - Natural infectivity by *P. falciparum* and *P. vivax* (VK210 and VK247) was evaluated using a standard ELISA protocol (Wirtz et al. 1987a, b, 1991, 1992) on pooled heads and thoraces



Distribution of specimens and collection localities in states of Antioquia and Córdoba, Colombia.

of five mosquitoes of the same species. To perform the ELISA assays, pools were macerated in 50 µL BB-IGEPAL buffer and the volume adjusted to 250 µL with BB buffer. Positive ELISA pools were analysed by nested genus-specific PCR (Singh et al. 1999) on individual mosquito abdomens to determine the specific infected mosquito within each pool.

Data analysis - Descriptive statistics were performed using GraphPad Prism version 4.00 (GraphPad 1992-2003) and $p < 0.05$ as the cut-off for statistical significance. Infectivity rate (IR) for each species was expressed as the number of positive individuals (np) per number of total analysed (nt) per 100 determined per site and per state [IR = (np/nt) x 100]. In addition, the confidence interval (CI 95%) was calculated to indicate the reliability of the estimated value, under the assumption of a binomial distribution, using the EPIDAT program, version 3.1 (OPS/OMS 2006). Human-biting activity was registered directly from HLCs. Hourly data from all collections were grouped and the total number of bites per hour was obtained for each species by site. Overall averages of the human-biting rate (HBR) were estimated from HLCs for each species. These data were expressed as the number of bites per person per night (b/p/n) during nights with 6 h collections and nights with 12 h collections. Values of HBR per species, per site (log transformed) were tested for differences among species using one-way ANOVA.

RESULTS

Species composition and identification of anthropophilic anophelines - A total of 1,768 *Anopheles* mosquitoes were collected by HLC at seven localities from Córdoba and Antioquia, during 24 field trips and approximately 480 h of sampling, with each site visited at least twice. Ten *Anopheles* species of two subgenera: *Nyssorhynchus* (7 species) and *Anopheles* (3 species) were identified. Collection dates and sample sizes are shown in Table I. As expected, most morphological misidentifications occurred among the Oswaldoi Group. In particular, *An. nuneztovari* s.l., *An. oswaldoi* s.l. and *An. rangeli* were confirmed by PCR-RFLP-ITS2 assays performed on individual adults (Zapata et al. 2007, Cienfuegos et al. 2008). Immature stages and male genitalia (when available) were also used to confirm adult identification. Immature stages were identified for *An. nuneztovari* s.l. (n = 3), *An. triannulatus* s.l. (n = 1) and *An. albitarsis* s.l. (n = 1) from MTL in Córdoba, collected from sunlit flooded pasture fields and also for *An. nuneztovari* s.l. (n = 2), *An. triannulatus* s.l. (n = 5) and *An. braziliensis* (n = 1), collected from mining excavations, with partial light exposure, from BAG and ZAR in Antioquia. Anthropophilic species composition by locality, as determined by morphological and molecular tests, is illustrated in Figure and Table I.

A total of 1,201 specimens were identified from Córdoba. *An. nuneztovari* s.l. and *An. darlingi* were found in all localities, but *An. nuneztovari* s.l. was the predominant species, corresponding to 88% of the total specimens identified. *An. darlingi* accounted for 6%, with the highest density in PLT. Five other species represented 6% of the identified specimens and were distributed as follows: *An. punctimacula* was found in VAL, PLT and MTL, *An. pseudopunctipennis* s.l. in VAL, *An. albimanus* was the second most abundant species in VAL and it was also found in TAL, *An. triannulatus* s.l. in PLT and *An. oswaldoi* s.l. in PLT and MTL. In Antioquia, 482 specimens were identified and the predominant species was different in each locality. In TUR, *An. albimanus* was the most abundant species (96.6%); four other species were present at ≤ 1.1%: *An. nuneztovari* s.l., *An. pseudopunctipennis* s.l., *An. punctimacula* and *An. neomaculipalpus*. In ZAR, the most predominant species, in order of abundance, were *An. braziliensis*, *An. triannulatus* s.l. and *An. nuneztovari* s.l., with *An. darlingi* (5%), *An. punctimacula* and *An. albitarsis* s.l. each present at < 1%. In BAG, the most common species were *An. darlingi* and *An. braziliensis*, with *An. nuneztovari* s.l., *An. triannulatus* s.l., *An. punctimacula*, *An. neomaculipalpus* and *An. albitarsis* s.l. each present at less than 2.5%.

Anopheles species naturally infected with Plasmodium - To determine the natural infectivity of *Anopheles* species collected in Córdoba and Antioquia, 1,616 specimens corresponding to 463 pools with up to five individuals per pool were analysed by ELISA (Table II). No species collected from Antioquia was infected. In contrast, two species collected in Córdoba, *An. nuneztovari* s.l. and *An. darlingi*, were found naturally infected. Of 1,047 *An. nuneztovari* s.l. tested, four individuals were found infected with *P. vivax* VK247 which represented a total IR for this species in Córdoba of 0.382% (CI: 0.104-0.975); three individuals were from MTL, representing an IR of 0.489% (3/613; CI: 0.101-1.424) for this locality and the other *An. nuneztovari* s.l. was from TAL, corresponding to an IR of 0.649% (1/154; CI: 0.016-3.565). From a total of 166 *An. darlingi* analysed, one individual from PLT was found infected with *P. vivax* VK247, resulting in an IR of 1.587% (1/63; CI: 0.040-8.530) for this locality and 1.449% (1/69; CI: 0.037-7.812) for Córdoba. No specimens were ELISA-positive for *P. falciparum*. The genus-specific nested PCR confirmed the infectivity of five individual *Plasmodium*-infected mosquitoes that were ELISA-positive for *P. vivax* VK247.

All species collected registered biting activity both indoors and outdoors. Four of the five infected specimens were caught indoors, one *An. darlingi* from PLT, one *An. nuneztovari* s.l. from TAL and two from MTL; only one infected *An. nuneztovari* s.l. was caught outdoors, at midnight, from MTL. *An. nuneztovari* s.l. and *An. darlingi* showed biting activity throughout the night, mainly between 19-3 h. No *An. nuneztovari* s.l. specimens from PLT were found to be infected; however, this species was the most prevalent and showed the highest HBR in all field trips (data not shown). Overall HBR means were not significantly different among mosquito species ($F = 0.3684$; $p = 0.8963$). The greatest HBR were obtained for *An. nuneztovari* s.l. and for *An. albimanus*. The highest HBR for *An. nuneztovari* s.l. was recorded from MTL in Córdoba (2.3 b/p/n) and for *An. albimanus* from TUR in Antioquia (1.7 b/p/n).

TABLE I

Anopheles species composition in seven localities of states of Córdoba and Antioquia, Colombia, March 2007-July 2008

Locality	Year	Month (number of days)	*Anopheles* collected n	Predominant species (%)	Other species (%)
VAL	2007	March (1)	105	*Anopheles nuneztovari* s.l. (51)	*Anopheles pseudopunctipennis* s.l. (6)
		April (3)		*Anopheles albimanus* (36)	*Anopheles punctimacula* (4)
					Anopheles darlingi (3)
TAL[a]	2007	July (4)	193	*An. nuneztovari* s.l. (96.5)	*An. darlingi* (2.5)
		August (3)			*An. albimanus* (0.6)
		September (2)			
		October (1)			
PLT[a]	2007	July (3)	340	*An. nuneztovari* s.l. (70.4)	*Anopheles triannulatus* s.l. (5.5)
		August (3)		*An. darlingi* (22)	*An. punctimacula* (1.7)
		September (3)			*Anopheles oswaldoi* s.l. (0.3)
		October (2)			
		November (2)			
		December (2)			
MTL[a]	2007	July (5)	648	*An. nuneztovari* s.l. (99.4)	*An. darlingi* (0.3)
		August (2)			*An. punctimacula* (0.2)
		October (3)			*An. oswaldoi* s.l. (0.2)
		November (2)			
TUR[a]	2007	November (3)	174	*An. albimanus* (96.6)	*An. nuneztovari* s.l. (1.1)
	2008	May (1)			*An. pseudopunctipennis* s.l. (1.1)
					An. punctimacula (0.9)
					Anopheles neomaculipalpus (0.6)
ZAR	2008	January (2)	180	*Anopheles braziliensis* (50.6)	*An. darlingi* (5)
		March (4)		*An. triannulatus* s.l. (24.4)	*An. punctimacula* (0.6)
		June (1)		*An. nuneztovari* s.l. (18.9)	*Anopheles albitarsis* s.l. (0.6)
		July (2)			
BAG	2008	March (3)	128	*An. darlingi* (71.9)	*An. nuneztovari* s.l. (2.3)
		May (3)		*An. braziliensis* (20.3)	*An. albitarsis* s.l. (2.3)
					An. punctimacula (1.6)
					An. triannulatus s.l. (0.8)
					An. neomaculipalpus (0.8)

Antioquia: BAG: El Bagre (El Sabalito Sinaí, Guachí, La Bonga and La Sardina); TUR: Turbo (Camerum and Yarumal); ZAR: Zaragoza (El Retiro, San Antonio and San Juan de Pelusa). Córdoba: MTL: Montelibano (Puerto Anchica); PLT: Puerto Libertador (La Bonga); TAL: Tierra Alta (Alto Guarumal); VAL: Valencia (Mieles abajo). *a*: in average 1-2 collections of adult mosquitoes was made overnight per field trip (18-6 h).

DISCUSSION

An. nuneztovari s.l. was found in all sites sampled from Córdoba and Antioquia and was the most prevalent species in all sites from Córdoba. In this study, the species composition of anthropophilic anophelines was lower than reported previously in Córdoba and Antioquia (SEM 1957, González & Carrejo 2007). Four species reported in these municipalities ~50 years ago, *An. apicimacula* (in TUR), An. *costai* (in ZAR and MTL), *An. rangeli* and *An. strodei* (in TUR, ZAR, VAL, PLT and MTL), were not found in the present study. *Anopheles nuneztovari* s.l. is still present in the same sites where it was previously reported, *An. albimanus* continues to be detected in TUR, TAL and VAL, *An. punctimacula* in TUR and ZAR, *An. triannulatus* s.l. and *An. braziliensis* in ZAR, *An. darlingi* in ZAR and in all sites in Córdoba, while An. *pseudopunctipennis* s.l. was collected in TUR and VAL and *An. neomaculipalpus* in TUR (Figure, Table I). *An. marajoara* was previously reported in ZAR (González & Carrejo 2007) and *An. albitarsis* s.l. was distributed in BAG and ZAR, albeit at a low density. Additional molecular procedures must be conducted to determine which species of the albitarsis complex is/are present in these localities because of their potential importance in malaria transmission in this zone.

The reduced diversity of *Anopheles* species in these sites compared to previous studies may have resulted from: (i) changes in ecological conditions and human activities (Wolda & Galindo 1981, Conn et al. 2002, Yasuoka & Levins 2007, Dantur Juri et al. 2009), (ii) failure

TABLE II

Detection of natural infection on *Anopheles* spp females collected in seven localities of states of Córdoba and Antioquia, Colombia

Anopheles species by subgenera	Number collected (percent identified of total)	Natural infection analysed (individuals infected by *Plasmodium vivax* VK247)[a]	HBR[b] (mean ± SE) overall	HBR[c] (mean ± SE) overall
Nyssorhynchus				
Anopheles nuneztovari s.l.	1,096 (62)	1,047 (4)	0.816 ± 0.451	0.527 ± 0.483
Anopheles albimanus	205 (11.6)	204	0.457 ± 0.380	0.153 ± 0.305
Anopheles darlingi	174 (9.8)	166 (1)	0.163 ± 0.116	0.040 ±0.073
Anopheles braziliensis	117 (6.6)	115	0.114 ± 0.082	[e]
Anopheles triannulatus s.l.	61 (3.5)	55	0.047 ± 0.038	0.007 ± 0.015
Anopheles albitarsis s.l.	4 (0.2)	4	0.006 ± 0.004	[e]
Anopheles oswaldoi s.l.	2 (0.1)	2	0.001 ± 0.001	[e]
Anopheles				
Anopheles punctimacula	14 (0.8)	13	0.019 ± 0.008	0.005 ± 0.006
Anopheles pseudopunctipennis s.l.	8 (0.5)	8	0.014 ± 0.011	0.007 ± 0.015
Anopheles neomaculipalpus	2 (0.1)	2	0.004 ± 0.003	[e]
Anopheles spp[d]	85 (4.8)			
Total (individuals)	1,768	1,616	-	-

a: species with infected specimens as determined by nested PCR of individual abdomens of positive pools; b: average bites per person per night (b/p/n) obtained from a mean of three collectors between 4-9 d/6h per day replicates for all sites; *c*: average b/p/n obtained from a mean of three collectors between 10-15 d/12h per day replicates for all sites; *d*: specimens too damaged to identify; *e*: specimens not collected; HBR: human-biting rate.

to detect higher species diversity because of differences in the sites sampled/localities, (iii) low and sporadic number of observations and (iv) our use of molecular tools to support species assignments, eliminating previous inaccuracies in species identification. Initially, most misidentifications were detected for members of the Oswaldoi Group - *An. strodei*, *Anopheles benarrochi*, *An. rangeli* and *An. oswaldoi* s.l. - species characterised by high morphological similarities in adult females. The molecular patterns for those specimens corresponded to *An. nuneztovari* s.l., a species that is also characterised by high intra-specific morphological variability (Ruiz et al. 2005, Calle et al. 2008, Fajardo et al. 2008).

Our collections of *An. nuneztovari* s.l. and *An. pseudopunctipennis* s.l. in VAL are consistent with recent reports by Parra-Henao and Alarcon (2008) from four sites in this locality that confirm that *An. nuneztovari* s.l. is the most prevalent species (91.1%) with the highest biting activity outdoors, at 21 h (data not shown). These authors also collected *An. pseudopunctipennis* s.l. (4%) and two additional species not detected in our study: *An. neomaculipalpus* (4.4%) and *Anopheles evansae* (0.5%). In contrast, our data showed that *An. albimanus* was the second most abundant species and in addition, we found *An. punctimacula* and *An. darlingi* in Mieles Abajo, VAL. The differences in the species composition between these studies could derive from differences in sampling sites, times of the year and collection hours.

Anopheles nuneztovari s.l. biting behaviour varied at each site. For example, in TAL this species showed the highest biting frequency indoors at 23 h , while in MTL occurred outdoors at 20 h and in PLT it was indoors at 22 h (data not shown). In VAL, the three primary malaria vectors of Colombia co-occurred: *An. albimanus*, *An. darlingi* and *An. nuneztovari* s.l. However, no naturally infected specimens were detected at VAL, probably because of the small sample size (105 specimens) tested. These species are able to transmit the parasites to humans (SEM 1957, Herrera et al. 1987, Gutiérrez et al. 2008) and in this study they showed human-biting activity, both indoors and outdoors, suggesting that further studies should be conducted to determine their role in malaria transmission in VAL.

All species collected in this study were anthropophilic and endo-exophagic. *Plasmodium*-infected *An. darlingi* and *An. nuneztovari* s.l. from PLT and MTL-TAL, respectively, were collected throughout the night (18-6 h), but in general these mosquitoes species were more active between 19-3 h. These data agree with results from longitudinal entomological and epidemiological studies carried out by Moreno et al. (2007) in the Southern Venezuela, where *An. darlingi* biting activity was evident through the night. In TUR, a Caribbean coast locality, *An. albimanus* was the predominant species (96.6%) and exhibited biting activity both indoors and in peri-domestic areas, especially between 18-22 h, with a peak activity indoors at 20 h (data not shown). Previous work also showed that this species predominated in the Colombian Caribbean region (Gutiérrez et al. 2008).

An. triannulatus s.l. has been traditionally considered zoophilic (Faran & Linthicum 1981) and it has not been reported to be naturally infected with *Plasmodium*

spp in endemic areas of Colombia. In this work, *An. triannulatus* s.l. showed anthropophilic activity and it was active indoors and outdoors, mainly between 18-1 h in PLT, ZAR and BAG. Similarly, Brochero et al. (2006) found *An. triannulatus* s.l. indoors and outdoors in Northeastern Colombia, exhibiting indoor biting activity similar to *An. nuneztovari* s.l. the main malaria vector. *An. triannulatus* s.l. constitutes a species complex with differences in malaria transmission ability (Silva-Do-Nascimento et al. 2006, Galardo et al. 2007); therefore, it will be important to investigate which species of the complex is/are present in these endemic areas and their potential as local vectors.

The overall average HBRs estimated for these species were in the range of values reported for the same species in other areas (Olano et al. 1997, Loaiza et al. 2008). Previous HBRs detected for *An. albimanus* in Buenaventura (Colombian Pacific region) were 0-7.1 (Olano et al. 1997) and 0.1-3.5 in Isla Pino (Panamá) (Loaiza et al. 2008). Also, HBR values for *An. darlingi*, *An. nuneztovari* s.l., *An. albitarsis* s.l. and *An. braziliensis* agree with those found in Boa Vista, Roraima, Brazil for the same species (da Silva-Vasconcelos et al. 2002, Póvoa et al. 2006). Specimens of *An. darlingi*, *An. triannulatus* s.l., *An. oswaldoi* s.l., *An. braziliensis*, *An. albitarsis* s.l., *An. punctimacula* and *An. pseudopunctipennis* s.l. were less abundant and thus HBRs by site for these species were lower than 1 b/p/n. Nonetheless, the presence of these species could have important epidemiological implications with respect to malaria transmission because of their high anthropophilic behaviour.

Two species, *An. nuneztovari* s.l. and *An. darlingi*, were found to be infected with *P. vivax* VK247 in Córdoba. These two species have been previously identified to be involved in transmission in others areas in Colombia. Reports by SEM (1957) showed that *An. darlingi* from Northern and Southern Colombia (Barrancabermeja, Santander; Villavicencio, Meta and Rioacha in Guajira) and *An. nuneztovari* s.l. from Cucuta in state of Norte de Santander were positive for *Plasmodium* sporozoites in the midgut and salivary glands. Also, Herrera et al. (1987) found *An. darlingi* infected with *P. falciparum* in Southwestern Colombia (Puerto Lleras, Meta) at IRs of 0.1% (indoors) and 0.06% (outdoors). A recent study that estimated natural IRs in the malaria vectors *An. albimanus* and *An. neivai* (Gutiérrez et al. 2008) and the present study implicates *An. darlingi* and *An. nuneztovari* s.l. In general, these two studies show that IRs for Colombian *Anopheles* species are low, but still enough to maintain malaria transmission in these endemic areas (INS 2007, WHO 2008).

Vector control is an important component of malaria control programs because it is one of the most efficient strategies to prevent transmission (WHO 2006). Our study identifies two vector species in Córdoba, Colombia, a highly endemic region. This information contributes to a better knowledge of the species with anthropophilic preferences in these areas. This information could help direct vector management activities in this region.

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