Anthropophilic Anopheles species composition and malaria in Tierradentro, Córdoba, Colombia

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Malaria is still a primary health problem in Colombia. The locality of Tierradentro is situated in the municipality of Montelíbano, Córdoba, in the northwest of Colombia, and has one of the highest annual parasite index of malaria nationwide. However, the vectors involved in malaria transmission in this locality have not yet been identified. In this study, the local anthropophilic Anopheles composition and natural infectivity with Plasmodium were investigated. In August 2009, 927 female Anopheles mosquitoes were collected in eight localities using the human landing catch method and identified based on their morphology. Cryptic species were determined by restriction fragment length polymorphism-internal transcribed spacer (ITS)2 molecular analysis. Eight species (Anopheles nuneztovari s.l. (92.8%), Anopheles darlingi (5.1%), Anopheles triannulatus s.l. (1.8%), Anopheles pseudopunctipennis s.l. (0.2%), Anopheles punctimacula s.l. (0.2%), Anopheles apicimacula (0.1%), Anopheles albimanus (0.1%) and Anopheles rangeli (0.1%) were identified and species identity was confirmed by ITS2 sequencing. This is the first report of An. albimanus, An. rangeli and An. apicimacula in Tierradentro. Natural infectivity with Plasmodium was determined by ELISA. None of the mosquitoes was infectious for Plasmodium. An. nuneztovari s.l. was the predominant species and is considered the primary malaria vector; An. darlingi and An. triannulatus s.l. could serve as secondary vectors.

Key words: malaria - Anopheles - Plasmodium - infectivity - Colombia

Colombia is among the 31 high-malaria-burden countries (Aregawi et al. 2009); although the total number of cases decreased by 45% from 2003 (125,064) to 2012 (56,175), malaria remains a major health problem. In 2012, the departments of Antioquia and Córdoba, situated in the northwest of the country, reported 53.1% of the total cases [National System for Public Health Surveillance (SIVIGILA)]. Recent studies of the anthropophilic Anopheles composition and natural mosquito infectivity with Plasmodium in the municipality of Montelíbano have led to the identification of four Anopheles species: Anopheles nuneztovari s.l., Anopheles darlingi, Anopheles oswaldoi s.l. and Anopheles punctimacula s.l.

Of those, An. nuneztovari s.l. (99.4%) was the most abundant species in the municipality. An. nuneztovari s.l. was also found to be infected with Plasmodium vivax [infectivity rate (IR) = 0.489%] and is therefore considered the main malaria vector in the municipality (Gutiérrez et al. 2009). Naranjo-Díaz et al. (2013) analysed species diversity in the neighbouring municipalities of Tierralta and Puerto Libertador and identified six species: An. nuneztovari s.l., An. darlingi, Anopheles triannulatus s.l., Anopheles pseudopunctipennis s.l., An. punctimacula s.l. and Anopheles argyritarsis, of which An. nuneztovari s.l. (IR = 0.05-0.10) and An. triannulatus s.l. (IR = 1.52) were found to be infective for P. vivax VK247 and An. darlingi (IR = 0.09) for P. vivax VK210. Tierradentro is one of the localities of Montelíbano that has a high malaria incidence. However, the previous entomological studies were performed in the urban sector only due to serious disturbances affecting law, order and security.

This study aimed to investigate urban and rural mosquito populations and their natural Plasmodium infectivity to determine malaria vectors in the locality of Tierradentro, Montelíbano. Eight species (complexes) of the subgenera Nyssorhynchus and Anopheles were identified: An. nuneztovari s.l., An. darlingi, An. triannulatus s.l., An. pseudopunctipennis s.l., An. punctimacula s.l., Anopheles apicimacula, Anopheles albimanus and Anopheles rangeli. Species identity was confirmed by restriction fragment length polymorphism-internal transcribed spacer (RFLP-ITS2) analysis, ITS2 sequencing and National Center for Biotechnology Information (NCBI) BLAST search. None of the mosquitoes was found to be infectious for Plasmodium (IR < 0.1%).

The locality of Tierradentro is situated in the northwest of Colombia in the south of Córdoba in the municipality of Montelíbano (Supplementary data). The urban centre is located along the San Jorge River at 55 m above mean sea level (07º48’50”N 75º52’40”W). The tropical climate is characterised by a monthly mean temperature of 25.6-27.1°C (SIPLAN 2008). The average annual precipitation is 2,386 mm and ranges from 350 mm in August to 20 mm in January (Hydrometrical Station Cuba Hda). The region is mountainous and the vegetation consists of natural forests, gallery forests, stubble, pastures and cropland.
The total population of Tierradentro is 6,447, with 77.75% inhabiting the urban sector and 22.25% the rural regions (SIPLAN 2008). From 2003-2009, Montelíbano reported 6.29% of the nationwide malaria cases (664,489); 42,039 (0.2%, KF436937), and 6,290 (0.1%, KF436939), An. apicimacula (0.1%, KF436935) and An. rangeli (0.1%). NCBI BLAST identification resulted in 99% sequence identity for An. nuneztovari B/C (Fritz et al. 1994, Sierra et al. 2004, Marrelli et al. 2005), An. darlingi (Marrelli et al. 2005), An. pseudopunctipennis s.l. (Miller et al. 1997, M Herrera et al., unpublished observations), An. triannulatus of the lineage NW (Rosero et al. 2012, Moreno et al. 2013), An. punctimacula s.s. (Cienfuegos & Correa (GU477275) (Loaiza et al. 2013)), An. albimanus (L78065) (Cienfuegos et al. 2011) and An. apicimacula (Loaiza et al. 2013). The species composition differed in each locality. An. nuneztovari s.l. was the most abundant species in all localities [CLA (198/201, 98.5%), BEL (64/67, 95.5%), BSC (131/164, 79.9%), BSM (171/183, 93.4%), SAN (36/40, 90%), VAL (144/160, 90%), VIN (19/21, 90.5%) and ISA (10/11, 90.9%)], due to its adaptability to variable larval habitats, particularly artificial ones (Tadei et al. 1998, Tadé & Thatcher 2000). In accordance with former studies, this species is considered the primary malaria vector (Gutiérrez et al. 2009). An. darlingi was largely collected in BSC (20/163, 12.3%) and VAL (14/160, 8.6%), with a few specimens from CLA (2/210, 1%), BEL (1/67, 1.5%), SAN (1/40, 2.5%) and BSM (3/183, 1.6%). BSC and VAL are located in partly deforested flat areas that constitute perfect breeding sites for this species (Hiwat & Bretas 2011). An. triannulatus s.l. showed no restriction to specific localities and was encountered in BSM (8/183, 4.4%), SAN (2/40, 5%), BSC (2/163, 1.2%), CLA (1/201, 0.5%), BEL (1/67, 1.5%) and VAL (1/160, 0.6%). As a habitat generalist, this species shows a wide distribution across Latin America, with no environmental constraints and is considered a regionally important malaria vector (McKeon et al. 2013). Although the An. darlingi and An. triannulatus s.l. density was low and locally restricted, these species could constitute regional secondary malaria vectors due to their high IR (> 1.5) (Gutiérrez et al. 2009). An. apicimacula (VEN: 1/21, 4.8%), An. pseudopunctipennis s.l. (VEN: 1/21, 4.8%; ISA: 1/11, 9.1%) and An. punctimacula s.l. (SAN: 2/40, 5%) were found in low numbers in dispersed villages and solitary farms in forested mountainous areas with little human activity and natural small water bodies. The latter two species have historically been collected in small numbers and have never been found to be infected with Plasmodium. Nevertheless, they are widely distributed and are considered secondary vectors of local importance in some regions. However, these species are not of epidemiological relevance in Colombia (Olano et al. 2001, Gutiérrez et al. 2008).

We for the first time registered specimens of An. albimanus, An. rangeli and An. apicimacula in Montelíbano. The increase in species diversity for the region may have resulted from the inclusion of urban and rural collection sites with different ecological conditions. An. albimanus is commonly associated with the low coastal regions of the Caribbean and Pacific in Colombia, where it is a primary malaria vector. An. albimanus may also be found at higher elevations (Montoya-Lerma et al. 2011) and has previously been reported for the neighbouring municipality of Tierralta (Gutiérrez et al. 2009). An.
rangeli has been considered to be a main local malaria vector in the department of Putumayo, in the south of Colombia (Quiñones et al. 2006), and recent studies provide evidence for Anopheles benarrochi B being mainly responsible for Plasmodium transmission in that region (Orjuela et al. 2013). Little is known about the distribution and taxonomic status of An. apicimaculata and its epidemiologic importance for malaria transmission.

Constant epidemiological surveillance is essential because human activities, climate change and evolutionary processes might have an impact on the geographical and temporal distribution, susceptibility and efficiency of the vectors and the lifecycles of the pathogens (Gould & Higgs 2009). The high Anopheles density in the urban centre and the villages along roads is most likely the result of artificial water bodies (road ditches, fish breeding pools, drinking troughs), which provide adequate larval habitats for Anopheles, particularly An. nuneztovari s.l. (Rodríguez et al. 2010). Structural interventions for avoiding stagnant water near settlements will likely significantly reduce malaria transmission and cases.

Further studies should focus on breeding and crossing experiments to clarify the taxonomic status of the Anopheles species complexes. Contemporary studies have focused on applying molecular markers for species identification; however, in spite of their utility for analysing population structures, evolutionary events and speciation tendencies, such studies do not provide conclusive results regarding species differentiation. Furthermore, the presence of other arthropod-borne pathogens, e.g., arboviruses and possible synergies between different pathogen life cycles should be extensively studied to acquire comprehensive, integrated knowledge on the transmission cycles of infectious diseases.

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REFERENCES

Aregawi M, Cibulskis RE, Otten M, Williams R 2009. World malaria report 2009. Available from: who.int/malaria/world_malaria_report_2009/en/.

Carrejo NS, González R 2007. Introducción al estudio taxonómico de Anopheles de Colombia, Universidad del Valle, Cali, 237 pp.

Cienfuegos AV, Gómez GF, Córdoba LA, Luckhart S, Conn JE, Correa MM 2008. Diseño y evaluación de metodologías basadas en PCR-RFLP de ITS2 para la identificación molecular de mosquitos Anopheles spp (Diptera: Culicidae) de la Costa Pacífica de Colombia. Rev Biomed 19: 35-44.

Cienfuegos AV, Rosero DA, Naranjo N, Luckhart S, Conn JE, Correa MM 2011. Evaluation of a PCR-RFLP-ITS2 assay for discrimination of Anopheles species in northern and western Colombia. Acta Trop 118: 128-135.

Fritz GN, Conn J, Cockburn A, Seawright J 1994. Sequence analysis of the ribosomal DNA internal transcribed spacer 2 from populations of Anopheles nuneztovari (Diptera: Culicidae). Mol Biol Evol 11: 406-416.

Gould EA, Higgs S 2009. Impact of climate change and other factors on emerging arbovirus diseases. Trans R Soc Trop Med Hyg 103: 109-121.

Gutiérrez LA, González JJ, Gómez GF, Castro MI, Rosero DA, Luckhart S 2009. Species composition and natural infectivity of anthropophilic Anopheles (Diptera: Culicidae) in the states of Córdoba and Antioquia, northwestern Colombia. Mem Inst Oswaldo Cruz 104: 1117-1124.

Gutiérrez LA, Naranjo N, Jaramillo LM, Muskus C, Luckhart S, Conn JE, Correa MM 2008. Natural infectivity of Anopheles species from the Pacific and Atlantic regions of Colombia. Acta Trop 107: 99-105.

Hiwat H, Bretas G 2011. Ecology of Anopheles darlingi Root with respect to vector importance: a review. Parasit Vectors 4: 177.

Loaiza JR, Scott ME, Berjingham E, Sanjur OI, Rovira JR, Butari LC, Linton Y, Bickersmith S, Conn JE 2013. Novel genetic diversity within Anopheles punctimaculata s.l.: phylogenetic discrepancy between the barcode cytochrome c oxidase I (COI) gene and the rDNA second internal transcribed spacer (ITS2). Acta Trop 128: 61-69.

Marrelli MT, Floeter-Winter LM, Malafronte RS, Tadei WP, Lourenço-de-Oliveira R, Flores-Mendoza C, Marinotti O 2005. Amazonian malaria vector anopheline relationships interpreted from ITS2 rDNA sequences. Med Vet Entomol 19: 208-218.

McKeon SN, Schlichting CD, Povoa MM, Conn JE 2013. Ecological suitability and spatial distribution of five Anopheles species in Amazonian Brazil. Am J Trop Med Hyg 88: 1079-1086.

Miller BR, Crabtree MB, Savage HM 1997. Phylogenetic relationships of the Culicomorpha inferred from 18S and 5.8S ribosomal DNA sequences (Diptera: Nematocera). Insect Mol Biol 6: 105-114.

Montoya-Lerma J, Solarte YA, Giraldo-Calderón GI, Quiñones ML, Ruiz-López F, Wilkerson RC, González R 2011. Malaria vector species in Colombia - A Review. Mem Inst Oswaldo Cruz 106 (Suppl. 1): 223-238.

Moreno M, Bickersmith S, Harlow W, Hildebrandt J, McKeon SN, Silva-do-Nascimento TF 2013. Phylogeography of the neotropical Anopheles triannulatus complex (Diptera: Culicidae) supports deep structure and complex patterns. Parasite Vectors 6: 47.

Naranjo-Díaz N, Rosero DA, Rua-Uríbe G, Luckhart S, Correa MM 2013. Abundance, behavior and entomological inoculation rates of anthropophilic anophelines from a primary Colombian malaria endemic area. Parasit Vectors 6: 61.

Olano VA, Brochero L, Sáenz R, Quiñones ML, Molina JA 2001. Mapas preliminares de la distribución de especies de Anopheles vectores de malaria en Colombia. Biomedica 21: 402-408.

Orjuela LI, Herrera M, Erazo H, Quiñones ML 2013. Especies de Anopheles presentes en el departamento del Putumayo y su infección natural con Plasmodium. Biomedica 33: 42-52.

Quiñones ML, Ruiz F, Calle DA, Harbach RE, Erazo HF, Linton Y-M 2006. Incrimination of Anopheles (Nyssorhynchus) rangeli and An. (Nys.) oswaldoi as natural vectors of Plasmodium vivax in Southern Colombia. Mem Inst Oswaldo Cruz 101: 617-623.
Rodriguez VC, Carrascal DR, Jimenez MM, Gutierrez P, Ahumada M, Quiiones ML 2010. Proyecto piloto nacional de adaptación al cambio climático (INAP) - Comonente D salud humana malaria - Informe. Available from: conservation.org.co/wp-content/themes/CI-Colombia/images/ci/2012/07/Anexo-25.-Sistema-de-Vigilancia-Malaria.pdf.

Rosero DA, Jaramillo LM, Gutierrez LA, Conn JE, Correa MM 2012. Genetic diversity of Anopheles triannulatus s.l. (Diptera: Culicidae) from northwestern and southeastern Colombia. Am J Trop Med Hyg 87: 910-920.

Sierra DM, Velez ID, Linton Y 2004. Malaria vector Anopheles (Nyssorhynchus) nuneztovari comprises one genetic species in Colombia based on homogeneity of nuclear ITS2 rDNA. J Med Entomol 41: 302-307.

SIPLAN 2008. Sistema de Planeación Zonal Comunitario, Subregión Alto San Jorge, Departamento de Córdoba, Municipio de La Apartada, Montelíbano y Puerto Libertador. Fundación San Isidro. Available from: fundacionsanisidro.org/, in construction.

Tadei WP, Thatcher BD 2000. Malaria vectors in the Brazilian Amazon: Anopheles of the subgenus Nyssorhynchus. Rev Inst Med Trop Sao Paulo 42: 87-94.

Tadei WP, Thatcher BD, Santos JM, Scarpassa VM, Rodrigues IB, Rafael MS 1998. Ecologic observations on anopheline vectors of malaria in the Brazilian Amazon. Am J Trop Med Hyg 59: 325-335.

Wirtz RA, Zavala F, Charoenvit Y, Campbell GH, Burkot TR, Schneider I, Esser KM, Beaudoin RL, Andre RG 1987. Comparative testing of monoclonal antibodies against Plasmodium falciparum sporozoites for ELISA development. Bull World Health Organ 65: 39-45.

Zapata MA, Cienfuegos AV, Quiros OI, Quiñones ML, Luckhart S, Correa MM 2007. Discrimination of seven Anopheles species from San Pedro de Uraba, Antioquia, Colombia, by polymerase chain reaction-restriction fragment length polymorphism analysis of its sequences. Am J Trop Med Hyg 77: 67-72.