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Detection of *Rickettsia* spp. in ticks (Acari: Ixodidae) of domestic animals in Colombia

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ABSTRACT

Rickettsiosis are emerging or re-emerging diseases, with a worldwide distribution associated to transmission by arthropod vectors. *Rickettsia* species belong to the spotted fever group (SFG) and are transmitted by hard ticks (Acari: Ixodidae) that may act as vectors and reservoirs. This study carried out a molecular detection of *Rickettsia* from 7 species of the family Ixodidae collected from domestic hosts by PCR amplification of fragments of the citrate synthase "*glt*A" gene and outer membrane protein "*omp*A" gene. Of the 204 samples analyzed, 11.3% (23) were positive for rickettsial infection. Three *Rickettsia* species belonging to the SFG were found, constituting the first reports of *Rickettsia rickettsii* in 2 departments of Colombia. Furthermore, we confirmed the first occurrence of *Candidatus* Rickettsia andeanae in Colombia, a species with an unknown pathogenic role in humans. These results raise awareness regarding the need to increase epidemiological control measures, as well as to consider new endemic regions in Colombia for Rocky Mountain spotted fever (RMSF).

1. Introduction

The genus *Rickettsia* encompasses strict intracellular bacteria that are transmitted by arthropods and mainly infect endothelial cells (Walker, 1982; Oteo et al., 2014). The genus is divided into four large groups: the typhus group (TG), the spotted fever group (SFG), the transitional group (TRG), and the ancestral group (AG) (Londoño et al., 2017). Rodents and ticks are the main reservoirs of *Rickettsia*; the initial infection of ticks with *Rickettsiae* can occur via the gut when bacteria-free ticks feed on rickettsemic hosts, or through transovarial or trans-stage transmission (Soares et al., 2012).

The first reported outbreak of tick-borne rickettsiosis in Colombia occurred between 1934 and 1936 in the municipality of Tobia, Department of Cundinamarca, and was named "Tobia spotted fever", which correspond to Rocky Mountain spotted fever (RMSF) caused by *Rickettsia rickettsii.* This disease affected 20% of the population and led to the death of 62 out of 65 patients (Patiño, 1941; Patiño et al., 1937). After a prolonged epidemiological silence, in 2003 and 2004, Hidalgo et al. (2007a) confirmed *R. rickettsii* as the causal agent of the death of two patients in the same region in Cundinamarca. Recently, three important SFG rickettsiosis outbreaks have occurred in Colombia, all caused by *R. rickettsii*: Necoclí – Department of Antioquia (2006), with five patients deceased out of 14 registered cases (Acosta et al., 2006); Los Córdobas – Department of Córdoba (2007), with 11 confirmed cases and the death of six patients (Hidalgo et al., 2007b), and Turbo – Antioquia (2008), with four patients deceased out of 15 reported cases (Pacheco et al., 2008).

Other important records for Colombia are: Faccini-Martínez et al. (2015) and Faccini-Martínez et al. (2016) isolated *R. rickettsii* and molecularly detected *Rickettsia amblyommii* from *Amblyomma patinoi* in the Department of Cundinamarca, and Londoño et al. (2014) registered specimens of *Amblyomma ovale* in the departments of Antioquia and Córdoba, infected by *Rickettsia* sp. strain Atlantic rainforest, which has shown to be pathogenic to humans. Gómez-Quintero et al. (2017) registered a probable case of a SFG rickettsial infection in a patient of the same region of the Colombian Orinoquía where Rivera-Páez et al.

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Table 1
Hard tick species and specimens analyzed – Detection and identification molecular of *Rickettsia* spp.

State	Municipality	Host	Tick species (number of specimens/ stage)	No. infected/No. tested (%)	Closest GenBank identity (gene: accession number)
Amazonas	**Leticia**	*Bos taurus*	*R. microplus* (14/adults)	0/14 (0)	
		B. taurus – Equus caballus	*D. nitens* (4/adults)	0/4 (0)	
		Canis lupus familiaris	*R. sanguineus* s. l. (6/adults)	2/6 (33.3)	100% *Candidatus* R. andeanae [*omp*A: KX158267]
Antioquia	**Medellín**	*C. lupus familiaris*	*R. sanguineus* s. l. (2/adults)	0/2 (0)	
Arauca	**Arauca**	*E. caballus – B. Taurus – Sus scrofa domesticus*	*D. nitens* (15/adults)	0/15 (0)	
		E. caballus – B. taurus-	*A. mixtum* (23/adults)	6/23 (26.1)	100% *Rickettsia rickettsii* [*omp*A: KJ735645]
		E. caballus – C. lupus familiaris	*R. sanguineus* s. l. (5/adults)	0/5 (0)	
		B. taurus	*R microplus* (2/adults)	0/2 (0)	
	Fortul	*B. taurus*	*R. microplus* (2/adults)	0/2 (0)	
	Saravena	*B. Taurus – C. lupus familiaris*	*R. microplus* (3/adults; 2 nymps)	0/5 (0)	
		C. lupus familiaris	*R. sanguineus* s. l. (2/adults)	0/2 (0)	
Bolívar	**San Jacinto**	*E. caballus- Equus asinus*	*D. nitens* (4/adults)	0/4 (0)	
		E. asinus	*R. microplus* (1/adults)	0/1 (0)	
Caldas	**Dorada**	*C. lupus familiaris*	*R. sanguineus* s. l. (2/adults)	0/2 (0)	
	Neira	*B. taurus*	*A. mixtum* (5/adults)	0/5 (0)	
	Norcasia	*C. lupus familiaris*	*A. maculatum* (2/adults)	0/2 (0)	
Casanare	**Nunchía**	*E. caballus – B. taurus* –Vegetation	*A. mixtum* (12/adults)	3/12 (25)	100% *Rickettsia rickettsii* [*omp*A: KJ735645]
		E. caballus	*D. nitens* (2/adults)	0/2 (0)	
	Yopal	*C. lupus familiaris – E. caballus*	*R. sanguineus* s. l. (6/adults)	0/6 (0)	
		E. caballus	*D. nitens* (4/adults)	0/4 (0)	
		E. caballus – E. asinus	*A. mixtum* (10/adults)	2/10 (20)	100% *Rickettsia rickettsii* [*omp*A: KJ735645]
		Vegetation	*A. mixtum* (2/nymps)	2/2 (100)	*Rickettsia* sp. strain Colombianensi 100% [*omp*A: KF691749]
		Vegetation	*A. dissimile* (1/nymps)	1/1 (100)	*Rickettsia* sp. strain Colombianensi 100% [*omp*A: KF691749]
Cundinamarca	**Puerto Salgar**	*C. lupus familiaris*	*R. sanguineus* s. l. (2/adults)	0/2 (0)	
Meta	**S. J. de Arama**	*B. taurus*	*A. mixtum* (1/adults)	0/1 (0)	
		B. taurus	*R. microplus* (2/adults)	0/2 (0)	
Tolima	**Ibagué**	*B. taurus*	*R. microplus* (7/adults)	0/7 (0)	
		B. taurus- C. lupus familiaris	*R. sanguineus* s. l. (12/adults)	0/12 (0)	
		C. lupus familiaris	*A. maculatum* (3/adults)	3/3 (100)	100% *Candidatus* R. andeanae [*omp*A: KX158267]
		C. lupus familiaris	*A. ovale* (8/adults)	4/8 (50)	100% *Candidatus* R. andeanae [*omp*A: KX158267]
		E. caballus – E. asinus	*D. nitens* (4/adults)	0/4 (0)	
	Saldaña	*C. lupus familiaris*	*R. sanguineus* s. l. (8/adults)	0/8 (0)	
		C. lupus familiaris	*A. maculatum* (14/adults)	0/14 (0)	
		C. lupus familiaris	*A. ovale* (2/adults)	0/2 (0)	
Valle del Cauca	**Cali**	*C. lupus familiaris*	*R. sanguineus* s. l. (2/adults; 3 nymps)	0/5 (0)	
	Restrepo	*C. lupus familiaris*	*R. sanguineus* s. l. (2/adults)	0/2 (0)	
		B. taurus	*R. microplus* (3/adults)	0/3 (0)	
			Total: 204 (196 adults: 110 males, 86 females) and 8 nymphs.	23/204 (11.3)	

(2016) found the presence of *Amblyomma mixtum*, a vector of *R. rickettsii*.

Considering the medical importance of the different species of *Rickettsia,* the aim of this study was the molecular detection of *Rickettsia* species associated with hard ticks collected from domestic hosts in 10 departments of Colombia.

2. Materials and methods

From August 2014 to May 2016, hard ticks were collected from domestic hosts, including cattle (*Bos taurus*), domestic dogs (*Canis lupus familiaris*), horses (*Equus caballus*), donkeys (*Equus asinus*), and one domestic pig (*Sus scrofa domesticus*). In addition to two tick samples were collected from the vegetation, in farms from 17 municipalities of 10 departments of Colombia (Rivera-Páez et al., 2018). Collected ticks were placed into plastic vials containing absolute ethanol and taken to the laboratory, where they were taxonomically identified based on current literature (Kohls, 1956; Jones et al., 1972; Estrada-Peña et al., 2005; Barros-Battesti et al., 2006; Martins et al., 2010; Nava et al., 2014, 2015). To confirm morphological identifications, some specimens of each tick species were subjected to molecular taxonomic identification by polymerase chain reaction (PCR) protocols to amplify fragments of at least one of the three following genes of the tick genome: targeting a ≈700-bp fragment of the mitochondrial cytochrome c oxidase subunit I gene (COI); targeting a ≈460-bp fragment of the mitochondrial 16S rDNA gene and a ≈1100-bp fragment that includes the entire second internal transcribed spacer (ITS2) region of the nuclear rRNA region (Rivera-Páez et al., 2018). Vouchers were deposited at the tick collection: "Coleção Nacional de Carrapatos Danilo Gonçalves Saraiva" – CNC (University of São Paulo, São Paulo, Brazil).

For the molecular detection and analysis of *Rickettsia* species, ticks were individually submitted to DNA extraction, using the DNeasy Blood and Tissue kit (Qiagen, Chatsworth, California), following the manufactureŕs protocol. Extracted DNA samples were tested by PCR, using primers CS-78 and CS-323, targeting a ~401-bp fragment of the citrate synthase gene (*glt*A) for presumably all *Rickettsia* species (Labruna et al., 2004). To avoid false negatives, the positive and negative samples for *glt*A were further tested by another PCR protocol,

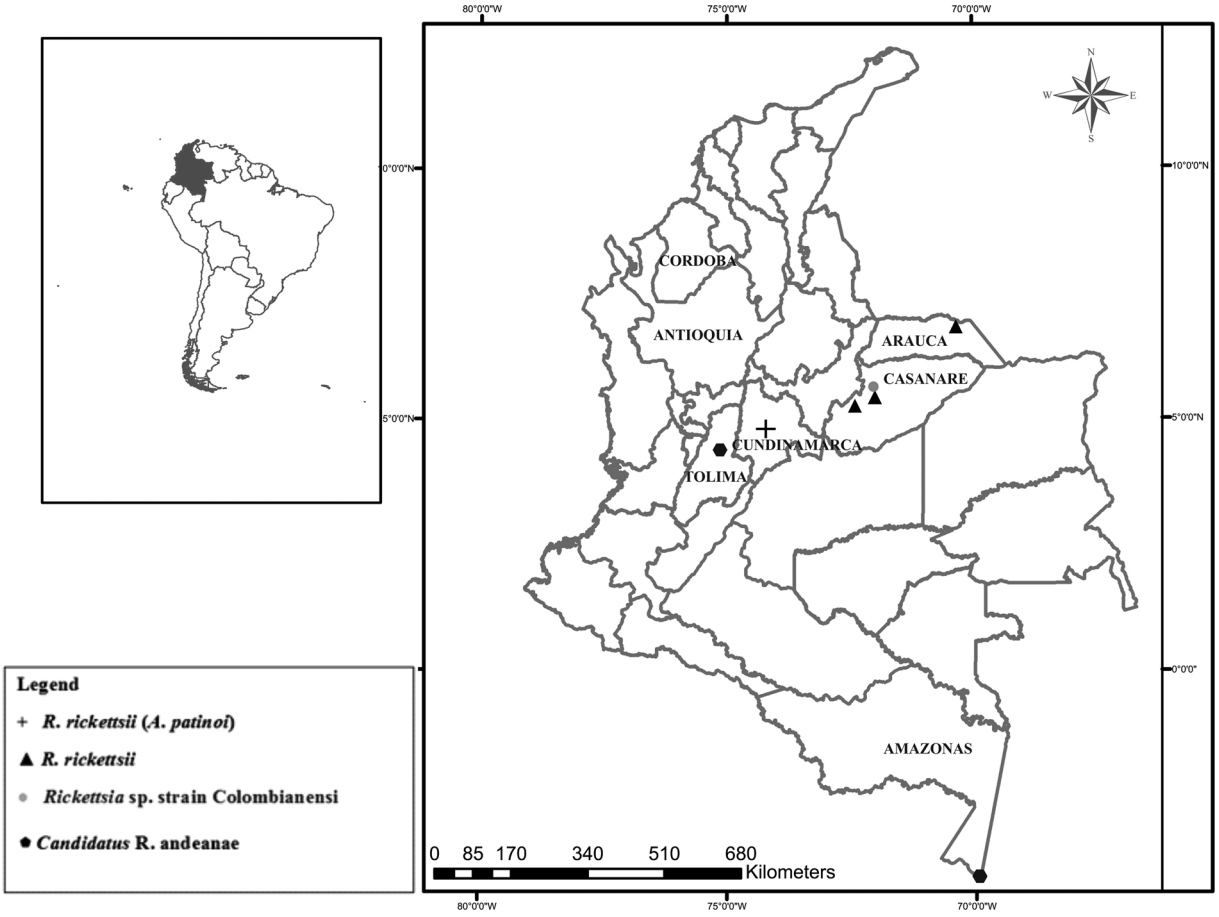

Fig. 1. Departments with registered *Rickettsia* species: (+) First reported outbreak of a tick-borne rickettsiosis in Colombia (Tobia- Department of Cundinamarca) – *R. rickettsii* (Patiño et al., 1937). Department of Antioquia (Acosta et al., 2006; Pacheco et al., 2008) and Department of Córdoba (Hidalgo et al., 2007b). (▲*R. rickettsia, Candidatus* R. andeanae and *Rickettsia* sp. strain Colombianensi) were reported during the present study.

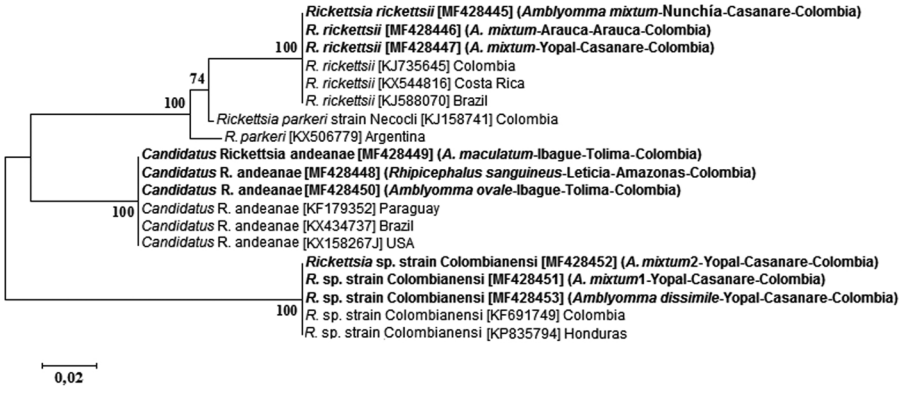

Fig. 2. Phylogenetic tree of *Rickettsia* species (detected in the present study in bold). Maximum Likelihood (ML) consensus tree using sequences of the 190-kDa outer membrane protein gene (*omp*A) present only in SFG *Rickettsia* species. Bootstrap values are only indicated for nodes with support greater than 70%. The sequence of *R. parkeri* was used as outgroup. Analysis model was accomplished in the program MEGA 7 and intraspecific nucleotide divergences were estimated using the Kimura 2-Parameter distance model, with the program MEGA 7.

using primers Rr190.70p and Rr190.602n, targeting a ~530-bp fragment of the 190-kDa outer membrane protein gene (*omp*A), present only in SFG *Rickettsia* species (Regnery et al., 1991). In each set of reactions, negative control tubes containing water and a positive control tube containing DNA of *Rickettsia parkeri* strain NOD were included.

PCR products were purified with the QIAquick PCR purification kit (Qiagen) and sent to the Universidad de Los Andes (Bogotá-Colombia) for DNA sequencing by the Sanger method. The *glt*A and *omp*A gene sequences were analyzed using Basic Local Alignment Search Tool (BLAST) (Altschul et al., 1990) to determine the closest similarities with other *Rickettsia* species. Species confirmation was carried out through a similarity analysis among gene *omp*A. Sequences obtained were evaluated and edited with the programs Geneious Trial v8.14 (Drummond et al., 2009). The sequences were search by MegaBlast against the public databases and to calculate sequence divergence, we downloaded sequences of closely related taxa available in GenBank database. Specifically, we obtained gene sequences corresponding to *R. rickettsia*, *Candidatus* Rickettsia andeanae and *Rickettsia* sp. strain Colombianensi; while, as outgroup, we used *Rickettsia parkeri*. The sequences were aligned using ClustalW (Thompson et al., 1997) included in the program MEGA version 7 (Tamura et al., 2013). Analysis model was accomplished in the program MEGA 7 (Tamura et al., 2013). Intraspecific nucleotide divergences were estimated using the Kimura 2-Parameter distance model (Kimura 2-parameter; Kimura, 1980), with the program MEGA 7. Species confirmation was carried out through a similarity analysis based on Maximum Likelihood (ML), with the K2P model and

1000 bootstrap replications, using the program MEGA 7.

3. Results

204 ticks were obtained, belonging to seven species of the family Ixodidae. Of these 196 were adults (110 males, 86 females) and 8 nymphs (Table 1). Of the 204 samples analyzed, 11.3% (23 ticks) were positive for rickettsial infection (Table 1). Three rickettsial agents were detected (Table 1, Fig. 1): i) *R. rickettsii* in *A. mixtum* (11 infected/53tested; 20.7% infection rate); ii) *Candidatus* Rickettsia andeanae in *Rhipicephalus sanguineus* sensu lato (2/52; 3.8%), *A. ovale* (4/10; 40%) and *A. maculatum* (3/19; 15.8%); and iii) *Rickettsia* sp. strain Colombianensi in *A. mixtum* (2/53; 3.8%) and *Amblyomma dissimile* (1/1; 100%).

The partial gene sequences for *glt*A and *omp*A of *R. rickettsii, Candidatus* Rickettsia andeanae and *Rickettsia* sp. strain Colombianensi were both 100% identical to the corresponding sequences available for each species in GenBank (Table 1, Fig. 2). GenBank nucleotide sequence accession numbers for the partial sequences generated in the present study are [MF428454-MF428463] for the *glt*A gene, and [MF428445-MF428453] for the *omp*A gene. This study demonstrated the presence of three SFG *Rickettsia* species based on PCR amplification of *glt*A and *omp*A gene fragments, in five municipalities of four departments of Colombia: *R. rickettsii*, in Arauca (Arauca), Yopal and Nunchía (Casanare); *Candidatus* R. andeanae, in Leticia (Amazonas) and Ibagué (Tolima); and *Rickettsia* sp. strain Colombianensi, in Yopal (Casanare).

4. Discussion

The new records of *Rickettsia* species in this work contribute to the knowledge of these infections in Colombia considering that only *R. rickettsii* has been the only species of the SFG identified in humans (Patiño et al., 1937). In addition, we found the presence of *R. rickettsii* in *A. mixtum* during active feeding on host, the second species of the *Amblyomma cajennense* complex recently registered for Colombia (Rivera-Páez et al., 2016) and a proven vector for *R. rickettsii*. Serological studies in human and animals conducted in the Colombian Orinoquía (departments of Arauca, Casanare, Guaviare, Meta, and Vichada) showed a high seroprevalence against SFGR (Miranda et al., 2011; Riveros-Pinilla et al., 2015) and, a probable case of infection by a *Rickettsia* species of the SFG (Gómez-Quintero et al., 2017). The molecular confirmation of *R. rickettsii* in three municipalities of the Orinoquía provides clear evidence that this region must be considered the third endemic region for rickettsiosis in Colombia, where only central (Department of Cundinamarca) and northwestern (Departments of Cordoba and Antioquia) regions of Colombia have constituted the two known endemic regions for rickettsiosis (Acosta et al., 2006; Hidalgo et al., 2007a, 2011).

Furthermore, the first record of *Candidatus* R. andeanae, in *R. sanguineus* s. l. in Leticia (Amazonas of Colombia), as well as in *A. ovale* and *A. maculatum* in Ibagué (Tolima), were found. However, for being captured ticks during active feeding on host, it is necessary show by real-life transmission experiments which ticks to be a vector of *Candidatus* R. andeanae. This *Rickettsia* species has been registered in Brazil, Peru, Argentina, USA, Chile and Paraguay, and its role as a human pathogen is unknown (Ferrari et al., 2013; Witter et al., 2016). In any case, the study of this species is relevant, since Paddock et al. (2015) reported that a high prevalence of *Candidatus* R. andeanae leads presumably to the exclusion of *R. parkeri* in adult *A. maculatum* in Kansas and Oklahoma (USA), an event that could be occurring in the populations of *A. maculatum* analyzed herein, where all ticks studied were adults.

Similarly, *Rickettsia* sp. strain Colombianensi was typed by Miranda et al. (2012), in *A. dissimile* ticks collected from iguanas, and also *Rhipicephalus microplus* from vegetation in Montería (Colombia); however, there has been no evidence suggesting that this *Rickettsia* species is transmissible to humans (Miranda and Mattar, 2014). The first records of *Rickettsia* sp. strain Colombianensi for the species *A. mixtum*, as well as the infection for *A. dissimile* in the department of Casanare raise the need for studies addressing the possible pathogenic potential of this *Rickettsia* species and to show by real-life transmission experiments which ticks to be a vector of *Rickettsia* sp. strain Colombianensi.

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