Wild and domestic animals likely involved in rickettsial endemic zones of Northwestern Colombia

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Résumé

Entre 2006 et 2008, trois épidémies de rickettiose humaine ont eu lieu dans le nord ouest de la Colombie (municipalités de Necoclí, Los Córdobas et Turbo), avec des taux de mortalité entre 27% et 54%. L’objectif de cet article était de déterminer l’exposition préalable des animaux sauvages et domestiques au groupe de rickettsies du foyer (GFR) rickettsiales par les méthodes sérologiques, pour détecter la présence de rickettsiales dans leurs ectoparasites, et analyser leur potentiel rôle dans l’épidémiologie des rickettsioses dans cette zone du pays. Une étude associée a été effectuée de 2010 à 2011. Des échantillons sanguins et d’ectoparasites ont été collectés chez des animaux domestiques et sauvages. Une association statistiquement significative (p < 0.05) a été observée entre les animaux séropositifs et les zones d’étude. En conclusion, cette étude a démontré l’occurrence de rickettsiales du groupe SFG dans les zones endémiques/synthéropiques, et suggère l’utilisation d’équidés et de chiens comme amplificateurs d’hostes pour les rickettsiales des zones enzootiques/endémiques.

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ABSTRACT

Between 2006 and 2008, three outbreaks of human rickettsiosis occurred in Northwestern Colombia (municipalities of Necoclí, Los Córdobas and Turbo), with case fatality rates between 27% and 54%. The aim of this study was to determine previous exposure of wild and domestic animals to spotted fever group (SFG) rickettsiae through serological tests, to detect rickettsial evidence in their ectoparasites, and to analyze their possible role in the epidemiology of rickettsial diseases in this zone of the country. A cross-sectional association study was performed from 2010 to 2011. Blood and ectoparasite samples were collected from domestic animals and small mammals. A statistically significant association (p < 0.05) between seropositive animals and the study zones was observed. A total of 2937 ticks, 672 fleas and 74 lice were collected and tested in pools by PCR. The minimum infection rate (MIR) of the positive pools was 5% in ticks, 4% in fleas, and 0% in lice. Phylogenetic analyses showed circulation of three 4. Rickettsia species: R. felis in fleas, and R. bellii and Rickettsia sp. strain Atlantic rainforest, both in Amblyomma oval ticks. In conclusion, this study demonstrated the occurrence of SFG rickettsiae in domestic, synanthropic and wild animals, and suggests the use of equines and canines as good sentinels of infection, in the study zone. We speculate that a transmission cycle exist involving rodents in the areas where these outbreaks have occurred. Tomes’ spiny rats (Proechimys semispinosus) and common opossums (Didelphis marsupialis) could be good candidates as amplifier hosts for SFG rickettsiae in enzootic/endemic zones.

1. Introduction

Rickettsia spp. are obligate intracellular bacteria transmitted by hematophagous arthropods such as fleas, mites, ticks and lice (Fourmier and Raoult, 2007). Most of these arthropods are thought to be vectors, but some may also act as reservoirs for some rickettsial human pathogens (Fourmier and Raoult, 2007). The genus Rickettsia has been divided into four groups (Typhus, TG; Spotted Fever, SFG; Transitional, TRG; and Ancestral, AG) (Gillespie et al., 2008), from which the SFG and TG have the most important public health concern (Fourmier and Raoult, 2007).

The first reported outbreak of a tick-borne rickettsiosis in Colombia occurred in 1934 in the town of Tobía (Department of Cundinamarca), when the disease was named “Tobia spotted fever”. This episode was caused by Rickettsia rickettsii, and produced 62 deaths from 65 cases (95% fatality rate) (Patiño, 1941; Patiño et al., 1937). In February 2006, an outbreak of human rickettsiosis occurred in the municipality of Necocli Colombia, five of the 14 individuals who fulfilled the case definition died (Acosta et al., 2006). This episode was followed by two more in the municipalities Los Córdobas where six of the 11 confirmed patients died (Hidalgo et al., 2007), and Turbo with four of the 15 in-
of these outbreaks occurred in neighboring towns in northwest Caribbean coast of Colombia and all of them were confirmed as caused by *R. rickettsii*.

Different studies have been performed in Colombia to establish the rickettsial transmission cycle involving humans and animals. In 2013, an eco-epidemiological study in Urbadá (Antioquia) reported 6 patients (3%) with active infection for SFG rickettsiosis shown by seroconversion. Researchers also found wild and synanthropic SFG-seropositive rodents and obtained a sequence of *Rickettsia* (gltA gene) from an *Amblyomma* sp. larva, which showed an identity of 99% with *Rickettsia tamaurae* in a Bayesian analysis (Quintero et al., 2013). Furthermore, a new species found in Colombia has been preliminarily called *Rickettsia* sp. strain colombianensis, and the circulation of *R. bellii* in ticks and of *R. felis* in fleas has also been described (Miranda et al., 2012; Miranda and Mattar, 2014; Ramirez-Hernandez et al., 2013).

The goal of this study was to assess rickettsial infection in wild and domestic animals and their ectoparasites in the three zones of Colombia where the last outbreaks occurred. Specifically, we show the presence of distinct species of *Rickettsia* and their possible reservoirs, likely amplifiers and vectors involved in the enzootic/endemic condition found in this region of the country.

2. Materials and methods

2.1. Study area

The study areas correspond to the villages of Alto de Mulatos (Municipality of Turbo, 08°08.272′N, 076°34.429′W) and Las Chagas (Municipality of Necocli, 08°32.892′N, 076°34.429′W); located in the Urabá region (Department of Antioquia) and the Contrapunto and Corea districts (Los Córdobas municipality 08°50.813′N, 076°20.294′W) in the Department of Córdoba. Hereafter we will call each sampling zone using the municipality name as either Turbo, Necocli or Los Córdobas. These three sites comprise part of the natural Caribbean region and have a hot dry climate characterized by a dry season from January to March and a rainy season from April to December (Correa, 2009).

Turbo is located at 400 m above sea level (MASL) and is characterized by heterogeneous agricultural areas, secondary vegetation, a small proportion of grassland pastures, as well as some patches of tropical rainforest. The average temperature is 28 °C, with an average annual precipitation of 2330 mm and a relative humidity of 86%. Necocli is located at 180 MASL in a tropical dry forest area with large deforested areas. It has a larger coverage of grassland pastures, heterogeneous agricultural areas, portions of secondary vegetation and a lower quantity of annual or transitory crops. The average temperature in the zone is 27 °C, the average annual precipitation is 1980 mm and the relative humidity is 86%. Los Córdobas has a greater proportion of grassland coverage, annual transitory crops and heterogeneous agricultural areas with small zones of secondary vegetation. This last municipality is located at 40 MASL in a dry tropical forest area and has an average temperature of 28 °C, an average annual precipitation of 2330 mm and a relative humidity of 85% (Correa, 2009) (Fig. 1).

2.2. Animal sampling

Blood samples were collected by venipuncture from canines, equines, bovines, and swine found in the various households during the house-to-house visits after obtaining the owner’s permission. Because the population of domestic animals in the study zones was unknown, a sample size was not determined. However, we were able to estimate the number of animals that lived with the people at each site.

Additionally, wild and synanthropic small mammals were captured and sampled over a period of 12 months for four nights per month using 100 Sherman live capture traps (8 × 9 × 23 cm, H.B. Sherman Trap Company, Tallahassee, FL, USA) and 30 Tomahawk traps (14 × 14 × 40 cm, Tomahawk Trap Company, Tomahawk, WI, USA). A mixture of peanut butter, oat flakes and vanilla extract was used as bait. Captured animals were taken to an open field and handled following biosecurity recommendations (Mills et al., 1998). All captured rodents were euthanized using an overdose of phenobarbital sodium (0.1 ml of 350 mg/ml Euthanex). The wild animals captured in the forest were principally marsupials; these animals were euthanized only when it was necessary for their identification. Lung, liver, kidney and spleen samples were obtained and stored in liquid nitrogen. Samples were transported from the field to the laboratory in liquid nitrogen and were subsequently stored in a −80 °C ultra-freezer. This study was performed under the approval of the University of Antioquia Animal Care and Use Committee (ACUC act No. 53 on June 30th, 2009) and the regional environmental corporation (CorpoUrabá, TDR: 200-03-20-02-1093-2010).

2.3. Classification of small mammals

Wild small animals were classified taxonomically using morphometric features. We recorded the weight of each captured animal and a description that principally referred to fur patterns, including the presence of spines and body and tail colorations. Skin was prepared, and carcasses and skulls were stored in 70% alcohol for species identification at the mammal laboratory’s collection at the University of Antioquia (Medellín, Colombia) (Emmons and Feer, 1997; Méndez, 1993).

2.4. Collection and handling of ectoparasites

Ectoparasites were collected from the domestic animals and small mammals by using tweezers. The adult ticks were collected from each animal in one or various dry vials (depending on the number collected per animal). All vials were transported in a poly styrene cooler with moist towels placed on the bottom to avoid desiccation of the ticks during transport to the laboratory. Nymphal and larval ticks and other types of ectoparasites (fleas, mites and lice) were stored in tubes with isopropanol.

The ectoparasites were identified using dichotomous keys (Barros-Battei et al., 2006; Furman and Catts, 1982; Linardi and Guimarães, 2000; Martins et al., 2010; Wenzel and Tipton, 1966). The ticks that arrived alive were disinfected for 10 min in 70% alcohol and then immediately submerged in sterile phosphate-buffered saline (PBS) and dried with absorbent paper. Each alive tick was split in half using sterile tweezers and fine-point scissors, individually stored and labeled. One half was frozen at −20 °C for nucleic acids purification and the second half at −80 °C for possible future bacterial isolation (beyond the scope of this study) (Sangioni et al., 2005). Dead ticks preserved in isopropanol were grouped according to stage, species, sex and host, in pools of five individuals. Lice and fleas were grouped in pools of 10 individuals at maximum.

2.5. Serological tests

Animals serum samples were tested for the presence of antibodies against SFG *Rickettsia* spp. by immunofluorescence assay (IFA), according to the method described by Pena et al. (2009) and Pinter et al. (2008) with some specific reagents. We used glass slides coated with *R. rickettsii* antigen donated by the National Institute of Health (Instituto Nacional de Salud, INS, Bogotá, Colombia). The slides were conjugated to detect IgG antibodies with one of the following: anti-mouse IgG (Cat. F4143), anti-dog IgG (Cat. F7884), anti-bovine IgG (Cat. F7887), anti-rat IgG (Cat. F6258), anti-pig IgG (Cat. F1638), anti-horse IgG (Cat. F7759) (all Sigma™ brand), or anti-opossum IgG produced by the Center for Control of Zoonosis (Centro de Controle de Zoonoses – CCZ, São Paulo city, Brazil) (Pena et al., 2009; Pinter et al., 2008).
2.6. Molecular assays

DNA was extracted from a fragment of lung of each small mammal, half of each of the ticks that arrived alive and were stored at −20 °C, and the ectoparasites preserved in isopropanol using the DNeasy Tissue & Blood kit (QIAGEN®). We followed the manufacturer’s protocol of DNA extraction from tissues. Some modifications were made to the protocol for the ectoparasites samples, i.e. the samples were frozen in conical tubes, and the conical portion was submerged in liquid nitrogen. The frozen ectoparasite was macerated with a plastic mortar, until totally triturated. DNA was eluted in 55 ul of buffer and stored at −80 °C. It is important to clarify that the ticks that arrived alive were processed individually, whereas the ticks that arrived dead were processed in a pool of five individuals at maximum from the same host. Lice and fleas were processed in pools of 10 individuals at maximum from the same host. For all cases in which pools were created, the males were always separated from the females.

Initially, a PCR control was generated for all ectoparasites with primers directed against a portion of the 16S mitochondrial subunit to rule out the presence of inhibitors (Mangold et al., 1998). Then, all samples were submitted to a screening test with the CS-78 and CS-323 primers for the gltA gene, which encodes the citrate synthase protein. These primers are Rickettsia-specific (Labruna et al., 2004). In the case of small mammals, only the screening test was performed. The gltA-positive samples were tested with primers Rr190.70p – Rr190.701, 120.M59–120.807 and CS 239–CS 1069 directed against fragments of the rickettsial genes ompA, ompB and gltA, respectively (Guedes et al., 2005). The commercial company Macrogen (Seoul, Rep. of South Korea) sequenced the PCR products. The sequences were assembled and edited with the Seqman program from the DNAstar packet (Lasergene®, Madison WI, USA), and a Bayesian phylogenetic analysis of rickettsial gltA gene was performed with the program MrBayes v3.1.2.

2.7. Statistical analysis

The description of the characteristics of the animals, and ectoparasites was formulated according to the type of variable. Specifically, the description of the qualitative variables was made according to frequencies and percentages. The prevalence of rickettsial infection according to different variables was described, along with their respective 95% confidence intervals. The Chi square test was used to compare proportions and the z test was used to compare means to determine whether significant differences existed between groups or categories. Differences with p-value less than 5% were considered statistically significant. Additionally, to establish the strength of the factors associated with the prevalence of rickettsial infection in animals, we estimated the Odds Ratio (OR) and the 95% confidence interval (CI). All other statistical analyses were performed using the SPSS 21.0, Stata 10.1 and EPIDAT 3.1 programs.

3. Results

The period for the fieldwork in the two regions did not coincide because of safety concerns. Consequently, there was a one-year disagreement between the schedules of Antioquia, September 2010 and October 2011, and Córdoba, June 2011 and June 2012. A total of 656 specimens were included in the study: 52% (n = 340) from domestic animals, 36% (n = 236) from synanthropic rodents (Rattus rattus and Mus musculus) and 12% (n = 80) from wild small mammals (marsupials and rodents). The animals were distributed in the following manner: 262 from Turbo, 199 from Necoclí, 195 from Los Córdobas (Table 1). The small mammal specimens were deposited, identified, preserved and catalogued by the curator of the University of Antioquia Mammal Collection (Colección Teriológica de la Universidad de Antioquia – CTUA) under the National Registry of Biological
Table 1 Description of hosts sampled in each location.

<table>
<thead>
<tr>
<th>Animal group</th>
<th>Host</th>
<th>Villages or districts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Neocoi n (%)</td>
</tr>
<tr>
<td>Domestic</td>
<td>Bovine</td>
<td>9 (18)</td>
</tr>
<tr>
<td></td>
<td>Equine</td>
<td>35 (26)</td>
</tr>
<tr>
<td></td>
<td>Canine</td>
<td>49 (35)</td>
</tr>
<tr>
<td></td>
<td>Porcine</td>
<td>8 (57)</td>
</tr>
<tr>
<td>Wild</td>
<td>D. marsupialis</td>
<td>1 (11.1)</td>
</tr>
<tr>
<td></td>
<td>H. of anomalus</td>
<td>3 (33.3)</td>
</tr>
<tr>
<td></td>
<td>M. robinioides</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>M. nudicaudatus</td>
<td>1 (33)</td>
</tr>
<tr>
<td></td>
<td>M. ischitica</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>Oligoryzomys spp.</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>P. semispinosus</td>
<td>8 (73)</td>
</tr>
<tr>
<td></td>
<td>T. talamancae</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>Z. cherrri</td>
<td>1 (3,5)</td>
</tr>
<tr>
<td>Synanthropic</td>
<td>M. musculus</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>R. rattus</td>
<td>84 (53)</td>
</tr>
</tbody>
</table>

* Didelphis marsupialis, Heteromyus of anomalus, Marmosa robinioides, Metachirus nudicaudatus, Marmosa ischitica, Proechimys semispinosus, Trasandinosyll talamancae, Zygodontomyx cherrri.

** Mus musculus, Rattus rattus.

Collections registry No. 167.

A total of 315 blood serum samples were obtained from 340 domestic animals. We were able to obtain samples from all of the wild and synanthropic animals. All samples were tested using IFA, and the samples that reacted to a dilution of 1:64 were considered positive (Penà et al., 2009; Pinter et al., 2008). For 100% of the samples obtained from the captured synanthropic rodents, the IFA results of the M. musculus were included but not the result of the R. rattus samples because of a non-specific reaction with the control negative (serum from a laboratory rat that had not been exposed to Rickettsia). This observation was corroborated by the laboratory of Dr. Marcelo Labruna of the University of Sao Pablo, who examined the blood serum samples of R. norvegicus laboratory rodents that had never been exposed to Rickettsia and found that these sera reacted non-specifically to R. rickettsii in the IFA.

No significant difference was found when results were analyzed by animal group (wild, synanthropic or domestic, Fig. 2), but a significant association was present (p < 0.05) when the positive animals were observed by the different study areas (Necoci, Turbo and Los Córdobas, Fig. 3). Accordingly, Necoci had the highest percentage of seropositive animals, with 65.4% (95% CI: 63.7–67.1), followed by Turbo with 56% (95% CI: 54.3–57.7), and Los Córdobas with 43% (95% CI: 41.5–44.7). Further analysis showed that positivity in ticks but not in equids was significant (p < 0.05) when ectoparasite positivity by the PCR assay are shown in Table 4. These variables were the following: stage of the ectoparasite, which was higher in adult stages with a 6% frequency of infection (95% CI: 4.6–6.7); ectoparasite species, which was higher in Amblyomma ovale ticks with an MIR of 14% (95% CI: 7.1–20.5), and the municipalities where they were collected, which was higher in Necoci with an 8% frequency of infection (95% CI: 6.1–9.9). When the distributions of positive ticks and fleas were analyzed separately in each of the zones, we found that positivity in ticks but not in fleas was significantly associated with their collection area. Specifically, the percent positivity was highest in Necoci at 9% (95% CI: 6.7–11.4), followed by Turbo at 5% (95% CI: 3.9–6.7) and finally Los Córdobas at 0.2% (95% CI: 0.0–0.8) (Figs. 5 and 6).

We were only able to analyze DNA sequences from A. ovale and C. felis. The quality of the sequences of A. cajennense sensu lato (s.l.), Amblyomma spp., Dermacentor nitens, Rhipicephalus microplus and R. sanguineus s.l. was insufficient to build contigs and performed phylogenetic analysis. Three species of Rickettsia were detected in the study areas; a sequence closely related to Rickettsia bellii (ancestral group) was generated from one A. ovale tick collected in the Necoci municipality, sequences related to R. felis (transitional group) were generated from C. felis fleas collected in the Necoci and Los Córdobas municipalities (accession numbers KP8701091 and KP870107.1 respectively, unpublished data), and sequences identical to the SFG R. parkeri-like agent, strain Atlantic rainforest (both by gkA and by the genes ompA and ompB) were generated from A. ovale ticks. Results of rickettsial infection in the A. ovale ticks collected in the present study were recently reported in another manuscript (Londoño et al., 2014) (Fig. 7).

4. Discussion

The serological results from domestic animals showed high percentages of seropositivity (between 40 and 60%). The frequency of seropositivity in domestic animals varied significantly according to the location.
sampling study area, with the highest positivity in Necoclí with an OR of 5.16 (95% CI: 2.83–9.41). The positivity was similarly associated with the animal species, with equines having a 46% seroreactivity (horses n = 47, donkeys n = 47 and mules n = 33) and canines having 41% (n = 135) IFA seropositivity. Bovines were all negative. Swines showed 60% seropositivity, but the sample size was too small (5 swine) to draw any conclusions (Fig. 4). These results show a high seropositivity in horses and canines, which are species that can act as important sentinels for pathogenic tick-borne SFG rickettsiae (Pinter et al., 2008; Sangioni et al., 2005). Moreover, one study performed in equines and canines in another endemic zone of Colombia (Villeta, Cundinamarca) showed seropositivities of 16% and 18%, respectively (Hidalgo et al., 2009). These results suggest the possibility that the ectoparasites of these animal species could be important vectors of the infection to humans and lead to the hypothesis that both equines and canines can be used as sentinels of SFG rickettsiae in the study areas.

The serological results of wild and synanthropic animals showed seropositivity values between 11% and 90% (Fig. 4). It is important to highlight that 8 out of 9 opossums (89%, D. marsupialis) were seropositive, the highest seropositivity detected in the study. In Brazil, a similar opossum species (Didelphis aurita) was incriminated as an efficient amplifier host of R. rickettsii to ticks (Horta et al., 2009). This finding justifies the hypothesis that D. marsupialis could fulfill the same role in the zones of this study. Moreover, a seropositivity of 36% was found in a sample of 11 individuals of the P. semispinosus (Tomes’ spiny rat). The same species was reported to have a 17% seropositivity in a sample of 18 individuals captured in the same zone (Quintero et al., 2013). Additionally, in this study we found that a pool of 5 A. ovale nymphs collected from one of these rats was PCR-positive for Rickettsia sp. that was identified as Rickettsia sp. strain Atlantic rainforest (Londoño et al., 2014). Although in our study, we did not find any sequence of Rickettsia sp. in lung tissue from Tomés spiny rats,

**Table 2**

<table>
<thead>
<tr>
<th>Tick species</th>
<th>Host species</th>
<th>Canine n(%)</th>
<th>Equine n(%)</th>
<th>Bovine n(%)</th>
<th>Porcine n(%)</th>
<th>R. ratti n(%)</th>
<th>Wild n(%)</th>
<th>M. musculus n(%)</th>
<th>Total N</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. cajennense s.l.</td>
<td>87 (7.7)</td>
<td>722 (64.1)</td>
<td>188 (16.7)</td>
<td>129 (11.5)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1126</td>
</tr>
<tr>
<td>A. ovale</td>
<td>148 (77.9)</td>
<td>9 (4.7)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>33 (17.4)</td>
<td>–</td>
<td>–</td>
<td>190</td>
</tr>
<tr>
<td>R. sanguineus s.l.</td>
<td>177 (100)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>177</td>
</tr>
<tr>
<td>R. microplus</td>
<td>–</td>
<td>39 (11.3)</td>
<td>306 (88.7)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>345</td>
</tr>
<tr>
<td>D. nitens</td>
<td>–</td>
<td>732 (100)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>50 (14.2)</td>
<td>–</td>
<td>–</td>
<td>732</td>
</tr>
<tr>
<td>Amblyomma spp</td>
<td>64 (18.2)</td>
<td>225 (64.1)</td>
<td>–</td>
<td>3 (0.9)</td>
<td>9 (2.6)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>351</td>
</tr>
<tr>
<td>O. puertoricensis</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>10 (62.5)</td>
<td>–</td>
<td>6 (37.5)</td>
<td>–</td>
<td>–</td>
<td>16</td>
</tr>
<tr>
<td>Total</td>
<td>476 (16.2)</td>
<td>1727 (58.8)</td>
<td>494 (16.8)</td>
<td>132 (4.5)</td>
<td>19 (0.7)</td>
<td>83 (2.8)</td>
<td>6 (0.2)</td>
<td>–</td>
<td>2937</td>
</tr>
</tbody>
</table>

* Amblyomma cajennense sensu lato, Amblyomma ovale, Rhipicephalus sanguineus sensu lato, Rhipicephalus microplus, Dermacentor nitens, Ornithodoros puertoricensis.
presence of antibodies and PCR-positive nymphs collected on those rats suggest that these wild mammals could be acting as amplifier host of SFG rickettsiae. However, further analyses are required to confirm this hypothesis.

In the present study, Rickettsia DNA was found in ticks and fleas, but not in lice. According to the collection area, ticks presented significant differences in PCR results, as tick pools obtained from Necoclí showed the highest positivity, 9% (95% CI: 6.7–11.4). This finding coincides with the positive serology result in animals, which also varied significantly according to their area of origin. Specifically, seropositivity was higher in animals from Necoclí, 55% (95% CI: 45.3–65.4) (Figs. 3 and 5).

This study was able to show evidence of three species of Rickettsia in the Urabá region (Colombian northwestern): Rickettsia sp. strain Atlantic rainforest, R. felis and R. bellii. The first of these strains belongs to the SFG and has been previously reported in Brazil as disease-causing in humans (Silva et al., 2011; Spolidorio et al., 2010). We suggest that this bacterium could be causing milder cases of disease and could even be protecting the population from more severe disease because previous infection with this bacterium might result in a much better response due to cross-immunity to infection with R. rickettsii (Portillo et al., 2017). R. felis corresponds to the transitional group and has been previously reported in the country, specifically in the Departments of Caldas (Ramirez-Hernandez et al., 2013) and Cundinamarca. In Cundinamarca, R. felis is thought to having been the cause of one clinical case (Paccini-Martínez et al., 2013), although its pathogenic potential is a matter of international controversy (Labruna and Walker, 2014). Finally, R. bellii has been classified in the ancestral group. R. bellii is not pathogenic and has been reported in different countries, including the United States, El Salvador, Costa Rica, Argentina, Brazil, Peru, and Colombia (Miranda and Mattar, 2014; Ogrzewalska et al., 2015; Parola et al., 2013).

In conclusion, this study demonstrates the occurrence of the SFG Rickettsia in domestic, synanthropic and wild animals in the study zone.
With regards to the ecology of the infection, we show evidence of other clinically important species of *Rickettsia* in the study area (*Rickettsia* sp. strain Atlantic rainforest), in addition to the circulation of *R. rickettsii*, which caused deaths in the 2006–2008 outbreaks. The use of equines and canines as good sentinels of infection of these agents has also been proposed for the detection of endemicity in surrounding areas. Finally, it is very important to continue these studies in order to establish whether Tomes’ spiny rats (*P. semispinosus*) and common opossums (*D. marsupialis*) could be playing any role in circulation of SFG rickettsiae in these zones.

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**Conflict of interest**

The authors have no conflict of interest to declare for the publication of the results presented in the manuscript.

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