Ticks and Tick-borne Diseases 8 (2017) 887–894

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

journal homepage: www.elsevier.com/locate/ttbdis

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

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ARTICLE INFO

Keywords:
Rickettsia
Colombia
Rodents
Canines
Equines

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 ovale* 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 (Fournier and Raoult, 2007). Most of these arthropods are thought to be vectors, but some may also act as reservoirs for some rickettsial human pathogens (Fournier 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 (Fournier and Raoult, 2007).

The first reported outbreak of a tick-borne rickettsiosis in Colombia occurred in 1934 in the town of Tobia (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 Necoclí 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 individuals who fitted the case definition died (Giraldo et al., 2008). All

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http://dx.doi.org/10.1016/j.ttbdis.2017.07.007
Received 1 August 2016; Received in revised form 23 April 2017; Accepted 17 July 2017
Available online 21 July 2017
1877-959X/

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 Urabá (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 tamurae* in a Bayesian analysis (Quintero et al., 2013). Furthermore, a new species found in Colombia has been preliminarily called *Rickettsia* sp. strain colombianensi, 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°33.009′W) and Las Changas (Municipality of Necoclí, 08°32.892′N, 076°34.429′W); located in the Urabá region (Deparment 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, Necoclí 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%. Necoclí 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 x 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 polystyrene 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-Battesti 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 specifics 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).

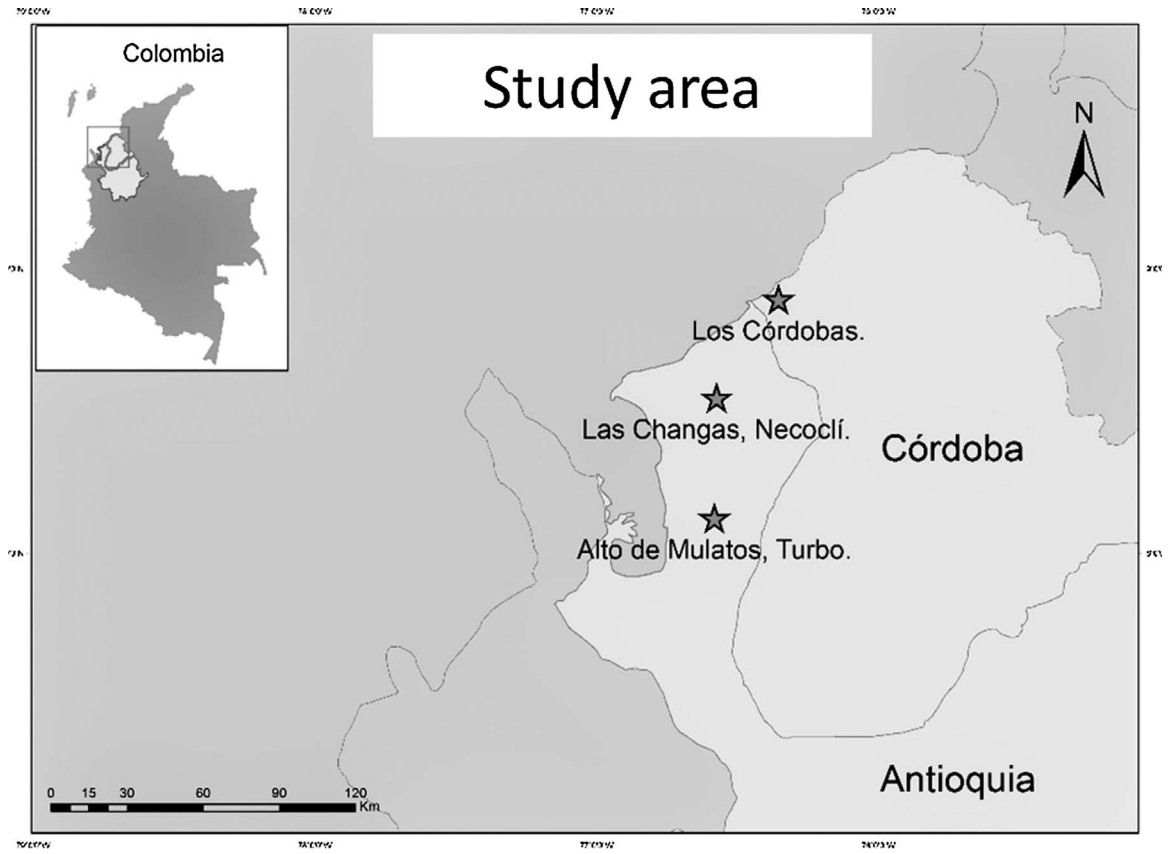

Fig. 1. Study area in Colombia Northwestern. Colombian map. The study zones are marked with stars. ArcGis 10.0. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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.

Animal group	Host	Villages or districts			
		Necoclí n (%) 199	Turbo n (%) 262	Los Córdobas n (%) 195	Total n 656
Domestic	Bovine	9 (18)	24 (49)	16 (33)	49
	Equine	35 (26)	62 (46)	39 (28)	136
	Canine	49 (35)	49 (35)	43 (30)	141
	Porcine	8 (57)	6 (43)	0 (0)	14
Wild*	*D. marsupialis*	1 (11.1)	8 (88.9)	0 (0)	9
	H. cf anomalus	3 (33.3)	3 (33.3)	3 (33.3)	9
	M. robinsoni	0 (0)	0 (0)	5 (60)	5
	M. nudicaudatus	1 (33)	2 (67)	0 (0)	3
	M. isthmica	0 (0)	3 (100)	0 (0)	3
	Olygoryzomys spp.	0 (0)	0 (0)	7 (100)	7
	P. semispinosus	8 (73)	1 (9)	2 (18)	11
	T. talamancae	0 (0)	4 (100)	0 (0)	4
	Z. cherrei	1 (3,5)	1 (3,5)	27 (93)	29
Synanthropic**	*M. musculus*	0 (0)	73 (95)	4 (5)	77
	R. rattus	84 (53)	26 (16)	49 (31)	159

* *Didelphis marsupialis*, *Heteromys cf anomalus*, *Marmosa robinsoni*, *Metachirus nudicaudatus*, *Marmosa isthmica*, *Proechimys semispinosus*, *Trasandinomys talamancae*, *Zygodontomys cherrei*.

** *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 (Pena 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 a non-specific reaction with the negative control (serum from a laboratory rat who 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 (Necoclí, Turbo and Los Córdobas, Fig. 3). Accordingly, Necoclí had the highest percentage of seropositive animals, with 55% (95% CI: 45.3–65.4), followed by Turbo with 33% (95% CI: 26.9–39.8) and Los Córdobas with 19% (95% CI: 12.4–25.9) (Fig. 3). After finding differences in the composition of species by zone ($p < 0.001$), a logistic regression of the prevalence of *Rickettsia* was performed by zone adjusted for animal species. The results were OR = 5.16 (95% CI: 2.83-9.41) for Necoclí and OR = 2.22 (95% CI: 1.28-3.85) for Turbo. Thus, the zone of origin for the animals had a significant effect on *Rickettsia* seropositivity that was independent of the animal species (data not shown). Regarding percentage of positivity for each animal species, we observed values that varied from 11% (in *H. anomalus*) to 89% (in *D. marsupialis*) (Fig. 4). When we compared seropositivity among different animal species, *D. marsupialis* showed antibodies more often than any other species (although the number of tested samples for this group was low compared to some of the domestic and wild mammals). *Didelphis* were followed by equids, canines and *M. musculus,* taking into consideration the total number of animals tested for each of these species (Fig. 4). On the other hand, DNA obtained from lung tissue of synanthropic and wild mammals did not amplify the expected fragment (401 bp) of the *gltA* gene from *Rickettsia*.

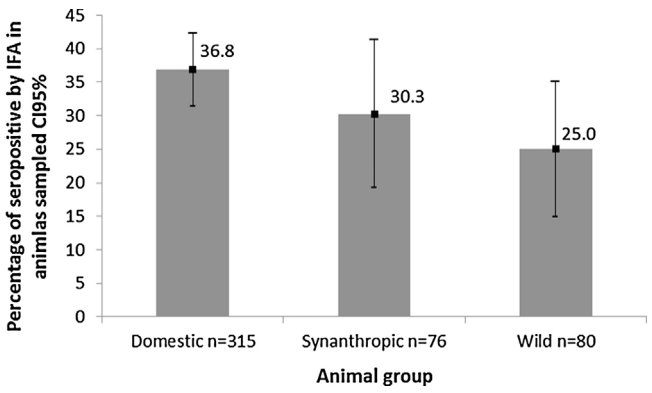

Fig. 2. Percentage of animals seropositive to spotted fever group rickettsiae by host group. P = 0.106.

A total of 2937 ticks were obtained from 264 animals: 58.8% (n = 1727) from 91 horses, followed by 16.8% (n = 494) from 22 bovines, 16.2% (n = 476) from 106 canines, 4.5% (n = 132) from 15 swine, 2.8% (n = 83) from 14 wild mammals, 0.7% (n = 19) from 14 *R. rattus* and 0.2% (n = 6) from 2*M. musculus* (Table 2).

We collected 672 fleas of two different genera: 24 *Pulex* sp. from 3 specimens of the rodent *H. anomalus*, and 648 *Ctenocephalides felis* from 92 canines. We also collected 74 lice from four different host species: 24 *Gyropus* sp. lice from 3 rodents of the *Proechimys semispinosus* species, 5 *Haematopinus asini* from one horse, 32 *Heterodoxus spiniger* from 7 dogs and 13 *Harrisonia uncinatanata* from 2 small mammals, *P. semispinosus* (Table 3).

In the case of the positive pools of the ectoparasites, we calculated the minimum infection rate (MIR), which conservatively assumed that only one individual was positive in each pool with a positive result. An MIR of 5% for ticks and 4% for fleas was found, whereas all lice were negative. The variables that were significantly associated ($p < 0.05$) with 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 Necoclí 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 Necoclí 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: 0–0.8) (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 Necoclí municipality, sequences related to *R. felis* (transitional group) were generated from *C. felis* fleas collected in the Necoclí 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 *gltA* 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

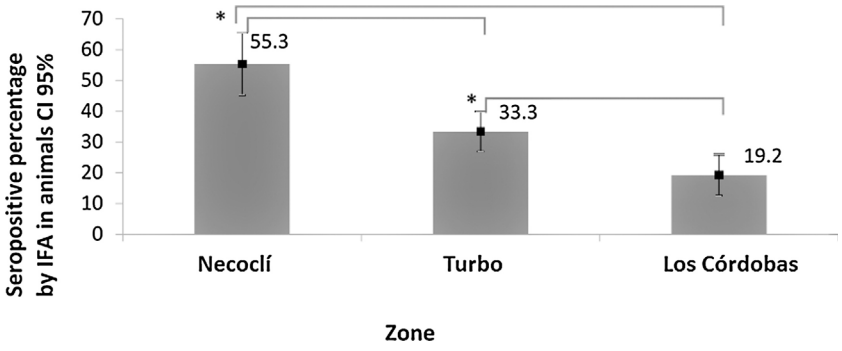

Fig. 3. Sero-prevalence to spotted fever group rickettsiae in animals by sampling areas. * P = 0.02.

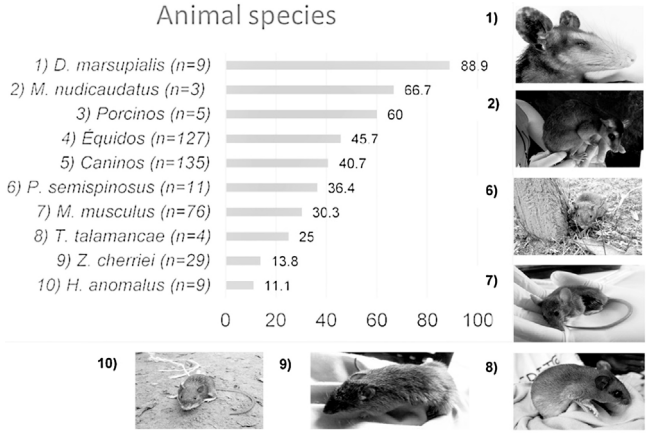

Fig. 4. Seropositivity to spotted fever group rickettsiae by animal species. Photos show the small mammal species that were positive by the serological test; numbers correspond to species. The following differences in the prevalence of antibodies were significant: *D. marsupialis* with canines (P = 0.01), *M. musculus* (P = 0.002), *Z. cherrei* (P = 0.0001) and *H. anomalus* (P = 0.004); equids with *M. musculus* (P = 0.04) and *Z. cherrei* (P = 0.003); canines with *Z. cherrei* (P = 0.01).

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.

Table 3
Number of sampled animals and number of fleas and lice collected from them.

Ectoparasite species (Animal species)		Number of hosts with parasites	Number of the fleas or lice
Flea species	*Pulex* sp. *(H. anomalus)*	3	24
	Ctenocephalides felis (Canines)	92	648
Total Fleas		**95**	**672**
Louse species	*Gyropus* sp. *(P. semispinosus)*	3	24
	Haematopinus asini (Equines)	1	5
	Heterodoxus spiniger (Canines)	7	32
	Harrisonia uncinatanata (P. semispinosus)	2	13
Total Lice		**13**	**74**

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, the

Table 2
Number of ticks by host species.

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

* *Amblyomma cajennense* sensu lato, *Amblyomma ovale*, *Rhipicephalus sanguineus* sensu lato, *Rhipicephalus microplus*, *Dermacentor nitens*, *Ornithodoros puertoricensis*.

Table 4
Percentage of the rickettsial infection in ectoparasites by biological features.

Factor		n (N)	%	CI95%	*p*-value*
Ectoparasite type	Tick	110 (2585)	4.26	(3.458–5.053)	0.8177
	Flea	30 (673)	4.46	(2.824–6.091)	
Ectoparasite stage	Adult	116 (2051)	5.66	(4.632–6.680)	0.001
	Nymph	13 (596)	2.18	(0.925–3.438)	0.789
	Larva	11 (611)	1.80	(0.664–2.936)	Ref
Ectoparasite species	*Amblyomma cajennense* s.l.	52 (873)	5.96	(4.329–7.584)	0.003
	Amblyomma ovale	16 (116)	13.79	(7.087–20.499)	0.001
	Amblyomma spp.	6 (272)	2.21	(0.277–4.135)	0.408
	Ornithodorus puertoricenses	0 (4)	0.00	–	–
	C. felis	30 (649)	4.62	(2.930–6.315)	0.017
	Dermacentor nitens	18 (750)	2.40	(1.238–3.562)	0.245
	Pulex irritans	0 (24)	0.00	–	–
	Rhipicephalus microplus	16 (340)	4.71	(2.308–7.104)	0.021
	Rhipicephalus sanguineus s.l.	2 (229)	0.87	(0.106–3.119)	Ref
Host	Environment	2 (143)	1.40	(0.170–4.961)	0.6756
	Bovine	21 (362)	5.80	(3.255–8.347)	
	Canine	47 (1037)	4.53	(3.218–5.847)	
	Didelphis marsupialis	0 (5)	0.00	–	
	Equine	60 (1354)	4.43	(3.298–5.564)	
	Feline	0 (4)	0.00	–	
	Heteromys anomalus	0 (43)	0.00	–	
	Human	1 (42)	2.38	(0.060–12.566)	
	Turkey	0 (3)	0.00	–	
	Porcine	7 (123)	5.69	(1.190–10.192)	
	Proechimys semispinosus	1 (27)	3.70	(0.094–18.971)	
	Rattus rattus	1 (9)	11.11	(0.281–48.250)	
	Transandinomys talamancae	0 (4)	0.00	–	
Municipality	Turbo	62 (1199)	5.17	(3.876–6.466)	< 0.001
	Los Córdobas	10 (1110)	0.9	(0.3–1.502)	Ref
	Necoclí	68 (850)	8.00	(6.117–9.883)	< 0.001

* Only factors with P values < 0.05 were discriminated. Ref: the reference variable.

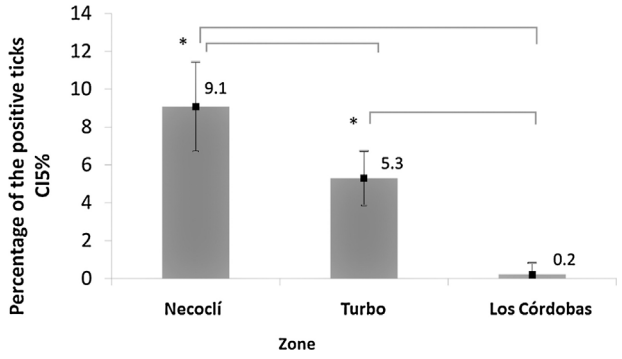

Fig. 5. Percentage of the positive ticks by PCR for rickettsiae and their distribution. * P < 0.001.

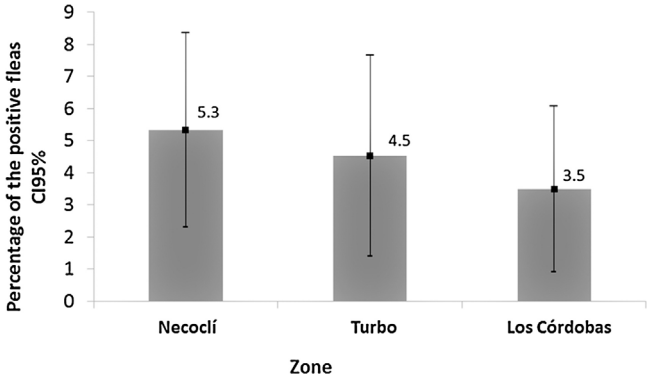

Fig. 6. Percentage of the positive fleas by PCR for rickettsiae and their distribution. P > 0.05.

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 (Faccini-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.

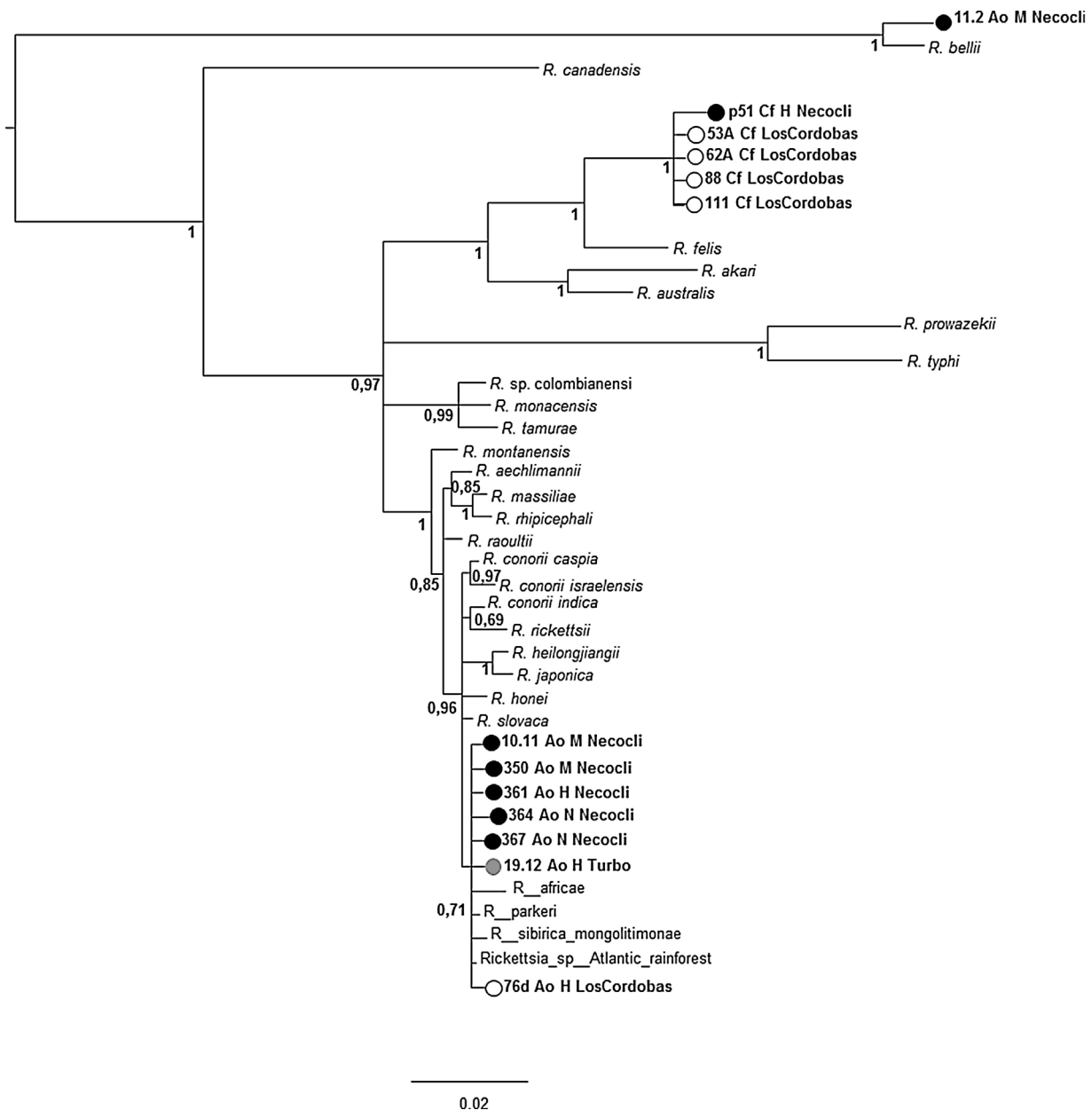

Fig. 7. Majority-rule consensus tree generated in the Bayesian phylogenetic analysis of rickettsial *gltA* gene performed with the program MrBayes v3.1.2., using the HKY + G substitution model. The analysis was run for 1,000,000 generations. The numbers near the nodes are posterior probabilities for the corresponding clade. The analysis involved 40 nucleotide sequences. There were a total of 350 positions in the final dataset. The tree was drawn in FigTree 1.4, and rooted with ancestral group (*R. bellii* and *R. canadensis*). The sequences from Necoclí are marked with black circles, Turbo with grey circles and Los Córdobas with white circles. Samples are identified by consecutive number, ectoparasite species, ectoparasite stage and municipality collection. Ao = *Amblyomma ovale*, Cf = *Ctenocephalides. felis*, F = Female, M = Male and N = Nymph.

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.

Funding

We thank Colciencias for their financial support through project No. 111549326228 [Ecological study of Rickettsia endemicity in Colombia] "Estudio ecologico de endemicidad por Rickettsia en Colombia". We are also grateful with the 2013/2014 University of Antioquia sustainability program for their financial support.

Conflict of interest

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

Acknowledgements

We would like to acknowledge the community leaders and health workers of Las Changas, Alto de Mulatos and Los Córdobas for their cooperation and help in obtaining the samples. We would also like to thank Daisy Gómez, Edwin Osorio and Laura Vargas from the

Mammalogy Group at the University of Antioquia (Grupo de Mastozoología y Colección Teriológica de la Universidad de Antioquia, CTUA) for their assistance in the identification of rodents; Luis Enrique Paternina from CENTAURO Group at the University of Antioquia for his help in the identification of fleas and lice; and the National Institute of Health (Instituto Nacional de Salud, INS, Bogotá, Colombia) for donation of glass slides coated with *R. rickettsii* antigen. Finally, we are indebted to Dr. Thomas Yuill (Emeritus professor from Wisconsin University), for his valuable corrections and feedback to this manuscript.

References

Acosta, J., Urquijo, L., Díaz, A., Sepúlveda, M., Mantilla, G., Heredia, D., González, M., Parra, E., Rey, G., Múnera, G., Hidalgo, M., Castañeda, E., Segura, O., Bolívar, J., Galeano, A., Giraldo, M.R., Rodríguez, E., Cedeño, N., Pérez, C., Bayard, V., Hernández, I., Villalobos, R., 2006. Brote de *Rickettsia rickettsii* en necoclí, antioquia, febrero-marzo, 2006. Inf. Quinc. Epidemiológico Nac. 11, 177–192.

Barros-Battesti, D., Arzua, M., Bechara, G., 2006. Carrapatos De Importancia Médico-veterinaria Da Regiao Neotropical: Um Guia Ilustrado Para Identificação De Espécies, Primeira. Ed. Integrated Consortium on Ticks and Tick-borne Diseases-ICTTD, São Paulo.

Correa, F.K., 2009. Geografía, generalidades. Monografia de Uraba. Imprenta Departamentalpp. 23–26.

Emmons, L., Feer, F., 1997. Neotropical Rainforest Mammals, second. ed. The University of Chicago Press Books, Chicago.

Faccini-Martínez, Á.A., Forero, E.G., Cortés, J.A., Polo, L.J., Jácome, J.H., Vargas, J.J., Valbuena, G., Hidalgo, M., 2013. Caso probable de fiebre manchada (*Rickettsia felis*) transmitida por pulgas. Biomédica 3333, 9–139. http://dx.doi.org/10.7705/biomedica.v33i0.723.

Fournier, P.-E., Raoult, D., 2007. Bacteriology, Taxonomy, and Phylogeny of Rickettsia, In: Rickettsial Diseases. Taylor & Francis Group, Boca Raton, pp. 1–14.

Furman, D.P., Catts, P., 1982. Manual of Medical Entomology, fourth. ed. Cambridge University Press, Cambridge.

Gillespie, J.J., Williams, K., Shukla, M., Snyder, E.E., Nordberg, E.K., Ceraul, S.M., Dharmanolla, C., Rainey, D., Soneja, J., Shallom, J.M., Vishnubhat, N.D., Wattam, R., Purkayastha, A., Czar, M., Crasta, O., Setubal, J.C., Azad, A.F., Sobral, B.S., 2008. *Rickettsia* phylogenomics: unwinding the intricacies of obligate intracellular life. PLoS One 3, e2018. http://dx.doi.org/10.1371/journal.pone.0002018.

Giraldo, M.R., Pacheco García, O.E., Galeano, A., Alvarez Vidal, H., Echeverri, E.I., Echavarría Rodríguez, L., Pacheco, C., 2008. Estudio sindrome febril en el muncipio de turbo, Corregimiento altos de mulatos enero 2008. Boletín Inf. para la acción 1–5.

Guedes, E., Leite, R.C., Prata, M.C.A., Pacheco, R.C., Walker, D.H., Labruna, M.B., 2005. Detection of *Rickettsia rickettsii* in the tick *Amblyomma cajennense* in a new Brazilian spotted fever-endemic area in the state of Minas Gerais. Memórias do Inst. Oswaldo Cruz. 100, 841–845 (/S0074-02762005000800004).

Hidalgo, M., Lizarazo, D., Ovalle, M.V., Castañeda, E., Heredia, D., Zambrano, P., Mantilla, G., Parra, E., Vera, M., Porras, A., Gaines, A., Munera, G., Carillo, P., Orejuela, L., Valbuena, G., 2007. Brote de rickettsiosis en Los Córdobas, departamento de Córdoba, febrero-marzo 2007. Inf. Quinc. Epidemiol. Nac. 12, 371–378.

Hidalgo, M., Vesga, J.F., Lizarazo, D., Valbuena, G., 2009. A survey of antibodies against *Rickettsia rickettsii* and *Ehrlichia chaffeensis* in domestic animals from a rural area of Colombia. Am. J. Trop. Med. Hyg. 80, 1029–1030.

Horta, M.C., Moraes-Filho, J., Casagrande, R.A., Saito, T.B., Rosa, S.C., Ogrzewalska, M., Matushima, E.R., Labruna, M.B., 2009. Experimental infection of opossums *Didelphis aurita* by *Rickettsia rickettsii* and evaluation of the transmission of the infection to ticks *Amblyomma cajennense*. Vector Borne Zoonotic Dis. 9, 109–118. http://dx.doi.org/10.1089/vbz.2008.0114.

Labruna, M.B., Walker, D.H., 2014. Rickettsia felis and changing paradigms about pathogenic rickettsiae. Emerg. Infect. Dis. 20, 1768–1769. http://dx.doi.org/10.3201/eid2010.131797.

Labruna, M.B., Whitworth, T., Horta, M.C., Bouyer, D.H., McBride, J.W., Pinter, A., Popov, V., Gennari, S.M., Walker, D.H., 2004. *Rickettsia* species infecting *Amblyomma cooperi* ticks from an area in the state of sao paulo, Brazil, where brazilian spotted fever is endemic. J. Clin. Microbiol. 42, 90–98. http://dx.doi.org/10.1128/JCM.42.1.90-98.2004.

Linardi, P., Guimarães, L., 2000. Sifonápteros do Brasil. Museu de Zoologia da Universidade de São Paulo, São Paulo.

Londoño, A.F., Díaz, F.J., Valbuena, G., Gazi, M., Labruna, M.B., Hidalgo, M., Mattar, S., Contreras, V., Rodas, J.D., 2014. Infection of Amblyomma ovale by Rickettsia sp. strain Atlantic rainforest, Colombia. Ticks Tick. Borne. Dis. 5, 672–675. http://dx.doi.org/10.1016/j.ttbdis.2014.04.018.

E. Méndez, 1993 Los roedores de Panamá, Primera. ed. Impresos Gráficos S.A., Panamá.

Mangold, A.J., Bargues, M.D., Mas-Coma, S., 1998. Mitochondrial 16S rDNA sequences and phylogenetic relationships of species of *Rhipicephalus* and other tick genera among Metastriata (Acari: ixodidae). Parasitol. Res. 84, 478–484.

Martins, T.F., Onofrio, V.C., Barros-Battesti, D.M., Labruna, M.B., 2010. Nymphs of the genus *Amblyomma* (Acari: ixodidae) of Brazil: descriptions, redescriptions, and identification key. Ticks Tick. Borne. Dis. 1, 75–99. http://dx.doi.org/10.1016/j.ttbdis.2010.03.002.

Mills, J., Childs, J., Ksiazek, T., Peters, C.J., 1998. Métodos Para Trampeo Y Muestreo De Pequeños Mamíferos Para Estudios Virológicos Centers for Disease Control and Prevention Atlanta.

Miranda, J., Mattar, S., 2014. Molecular detection of *Rickettsia bellii* and *Rickettsia* sp. strain colombianensi in ticks from Cordoba, Colombia. Ticks Tick. Borne. Dis. 5, 208–212. http://dx.doi.org/10.1016/j.ttbdis.2013.10.008.

Miranda, J., Portillo, A., Oteo, J.A., Mattar, S., 2012. Rickettsia sp. strain colombianensi (Rickettsiales: rickettsiaceae): a new proposed Rickettsia detected in Amblyomma dissimile (Acari: ixodidae) from iguanas and free-living larvae ticks from vegetation. J. Med. Entomol. 49, 960–965. http://dx.doi.org/10.1603/ME11195.

Ogrzewalska, M., Literák, I., Capek, M., Sychra, O., Calderón, V.Á., Rodríguez, B.C., Prudencio, C., Martins, T.F., Labruna, M.B., 2015. Bacteria of the genus *Rickettsia* in ticks (Acari: ixodidae) collected from birds in Costa Rica. Ticks Tick. Borne. Dis. 6, 478–482. http://dx.doi.org/10.1016/j.ttbdis.2015.03.016.

Parola, P., Paddock, C.D., Socolovschi, C., Labruna, M.B., Mediannikov, O., Kernif, T., Abdad, M.Y., Stenos, J., Bitam, I., Fournier, P.-E., Raoult, D., 2013. Update on tick-borne rickettsioses around the world: a geographic approach. Clin. Microbiol. Rev. 26, 657–702. http://dx.doi.org/10.1128/CMR.00032-13.

Patiño, L., Afanador, A., Paul, J.H., 1937. A spotted fever in tobia, Colombia. Am. J. Trop. Med. 17, 639–653.

Patiño, L., 1941. Nuevas observaciones sobre un tercer foco de fiebre petequial (Maculosa) en el hemisferio americano. Bol. Of. Sanit. Panam. 20, 1112–1124.

Pena, D.C.H., Mafra, C.L., Calic, S.B., Labruna, M.B., Milagres, B.S., Walker, D.H., Galvão, M.A.M., 2009. Serologic survey for antibodies to *Rickettsia* among domestic and wild animal populations in Brazil. Clin. Microbiol. Infect. 15 (Suppl 2), 243–244. http://dx.doi.org/10.1111/j.1469-0691.2008.02165.x.

Pinter, A., Horta, M.C., Pacheco, R.C., Moraes-Filho, J., Labruna, M.B., 2008. Serosurvey of Rickettsia spp. in dogs and humans from an endemic area for Brazilian spotted fever in the State of São Paulo, Brazil. Cad. Saude Publica 24, 247–252. 10.1590/S0102-311×2008000200003.

Portillo, A., de Sousa, R., Santibáñez, S., Duarte, A., Edouard, S., Fonseca, I.P., Marques, C., Novakova, M., Palomar, A.M., Santos, M., Silaghi, C., Tomassone, L., Zúquete, S., Oteo, J.A., 2017. Guidelines for the Detection of *Rickettsia* spp. Vector-Borne Zoonotic Dis. 17, 23–32. http://dx.doi.org/10.1089/vbz.2016.1966.

Quintero, J., Londoño, A., Agudelo-Floréz, P., Arboleda, M., Rodas, J.D., 2013. Ecoepidemiología de la infección por rickettsias en roedores, ectoparásitos y humanos en el noroeste de Antioquia. Colombia. Biomedica. 33 (S1), 2–9. http://dx.doi.org/10.7705/biomedica.v33i0.735.

Ramirez-Hernandez, A., Montoya, V., Martinez, A., Perez, J.E., Mercado, M., Ossa de la, A., Velez, C., Estrada, G., Correa, M.I., Duque, L., Ariza, J.S., Henao, C., Valbuena, G., Hidalgo, M., 2013. Molecular detection of *Rickettsia felis* in different flea species from caldas. Colombia. Am. J. Trop. Med. Hyg. 89, 453–459. http://dx.doi.org/10.4269/ajtmh.12-0698.

Sangioni, L.A., Horta, M.C., Vianna, M.C.B., Gennari, S.M., Soares, R.M., Galvão, M.A.M., Schumaker, T.T.S., Ferreira, F., Vidotto, O., Labruna, M.B., 2005. Rickettsial infection in animals and Brazilian spotted fever endemicity. Emerg. Infect. Dis. 11, 265–270. http://dx.doi.org/10.3201/eid1102.040656.

Silva, N., Eremeeva, M.E., Rozental, T., Ribeiro, G.S., Paddock, C.D., Ramos, E.A.G., Favacho, A.R.M., Reis, M.G., Dasch, G.A., de Lemos, E.R.S., Ko, A.I., 2011. Eschar-associated spotted fever rickettsiosis, Bahia. Brazil. Emerg. Infect. Dis. 17, 275–278. http://dx.doi.org/10.3201/eid1702.100859.

Spolidorio, M.G., Labruna, M.B., Mantovani, E., Brandao, P.E., Richtzenhain, L.J., Yoshinari, N.H., 2010. Novel spotted fever group rickettsiosis. Brazil. Emerg. Infect. Dis. 16, 521–523. http://dx.doi.org/10.3201/eid1603.091338.

Wenzel, R., Tipton, V., 1966. Ectoparasites of Panama. Field Museum of Natural History, Chicago.