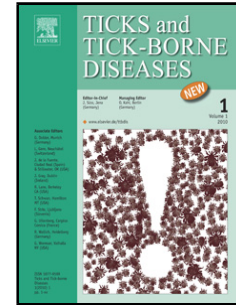


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Exposure of dogs to spotted fever group rickettsiae in urban sites associated with human rickettsioses in Costa Rica

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ABSTRACT

The zoonotic transmission cycles of *Rickettsia rickettsii* and other spotted fever group (SFG) rickettsiae in Latin America have usually been associated with rural or sylvatic environments, although domestic dogs can be implicated in more populated settings. In this study, exposure of dogs to SFG rickettsiae in the Greater Metropolitan Area of Costa Rica was investigated. Dogs from sites associated with human cases and from dog shelters were evaluated by indirect immunofluorescence assay (IFA) using antigen of SFG rickettsiae. *Rickettsia* spp. were detected in ectoparasites by polymerase chain reaction (PCR). A total 18.5% (31/168) of dogs associated with human cases and 6.8% (11/161) of dogs in shelters had IgG end titers ≥ 64 to *Rickettsia* spp. The odds of being seropositive were greater in dogs from areas associated with human cases when compared to shelters (OR: 3.2; 95% C.I: 1.5-5.6). *Rhipicephalus sanguineus* sensu lato (s. l.) was present in all sites associated with human cases. *Rickettsia felis* URRWXCal2 and *R. felis*-like RF2125 were detected in *Ctenocephalides felis*, and *Rickettsia* sp. IbR/CRC in *Ixodes boliviensis*. Results demonstrate that dogs from the main urban center of Costa Rica have been exposed to SFG rickettsiae, especially in areas with known human infection. Both human and animal health sectors must be aware of possible rickettsial diseases in urban areas, where dogs may also serve as sentinels for human infection.

Keywords: *Rickettsia*, dog, Rocky Mountain spotted fever, urban environment, Costa Rica

Introduction

Several species of *Rickettsia* (Rickettsiales: Rickettsiaceae) are known to cause disease in humans, as well as domestic and wild animals ([Parola and Raoult, 2001](#); [Parola et al., 2013](#)). *Rickettsia rickettsii*, the species responsible for Rocky Mountain spotted fever (RMSF), is the most virulent of all spotted fever group (SFG) rickettsiae, and it can affect several vertebrates, including humans and dogs ([Paddock et al., 2002](#); [Elchos and Goddard, 2003](#); [Piranda et al., 2008](#)). Although transmission cycles of *R. rickettsii* and other SFG rickettsiae in Latin America usually involve rodents or other wild animals and their ticks ([Souza et al., 2009](#)), contact with dogs may increase the risk of human infection. Evidence suggests that dogs may be implicated in the transmission cycles of *R. rickettsii* as victims and/or amplifying hosts ([Piranda et al., 2008, 2011](#); [Labruna et al., 2009](#)). There are case reports of parallel or subsequent *R. rickettsii* infection between dogs and their owners ([Paddock et al., 2002](#); [Elchos and Goddard, 2003](#)). Moreover, *Rhipicephalus sanguineus* sensu lato(s. l.), a common dog tick in urban areas, can be a competent vector of *R. rickettsii* and has been associated with outbreaks of RMSF in dogs ([Demma et al., 2005](#); [Piranda et al., 2011](#)). In addition to *R. rickettsii*, contact of dogs with other SFG rickettsiae in the Neotropical Region has been demonstrated in seroprevalence studies, which suggest exposure to *Rickettsia parkeri*, “*Candidatus Rickettsia amblyommii*”, *Rickettsia rhipicephali*, *Rickettsia bellii*, and *Rickettsia felis* or very similar species ([Labruna et al., 2007a](#); [Silva Fortes et al., 2010](#); [Spolidorio et al., 2010](#); [da Costa et al., 2015](#); [Lado et al., 2015](#)).

In Costa Rica, cases of rickettsiosis have been recognized since the 1950s, although *R. rickettsii* was first isolated in 1979 ([Fuentes, 1979](#)). Until recently, cases of RMSF were limited to the Northern and Caribbean areas of the country, and transmission was associated with rural or

sylvatic environments ([Hun-Opfer, 2008](#)). In April 2010, a fatal case of RMSF was diagnosed from the capital city, San Jose ([Arguello et al., 2012](#)), and at least two other cases with no history of travel outside the Greater Metropolitan Area were confirmed within the following two years. In addition to *R. rickettsii*, “*Candidatus R. amblyommii*”, *R. bellii*, *R. felis*, and an unnamed *Rickettsia* sp. (IbR/CRC) have been detected in the country ([Hun et al., 2011](#); [Trovo et al., 2014](#); [Ogrzewalska et al., 2015](#)). Considering that dogs and their ectoparasites are common in urban areas, the aim of this study was to evaluate the exposure of dogs to a possible urban transmission cycle of SFG rickettsiae in the Greater Metropolitan Area of Costa Rica, especially in areas associated with confirmed human cases of RMSF.

Materials and methods

Study sites and collection of samples

Two separate dog populations were selected for the study: dogs from sites in the Greater Metropolitan Area of Costa Rica associated with three confirmed human cases of RMSF during 2010-2012, and dogs from shelters in this same area. All procedures were approved by Universidad de Costa Rica’s Institutional Committee for the Use and Care of Laboratory Animals (number CICUA-09-12).

The three human RMSF cases were severe and clinically compatible with rickettsiosis, and *R. rickettsii* infection was confirmed at the Faculty of Microbiology of the University of Costa Rica by polymerase chain reaction (PCR) followed by DNA sequencing (2 cases, one of which was fatal), or by IgG seroconversion with a positive response after treatment with

doxycycline (1 case). Patients reported that they had not travelled outside the Greater Metropolitan Area or to other endemic areas previous to the onset of symptoms. The home of each patient at the time of disease onset was located, as well as other sites visited where transmission may have occurred. In total, five sites were identified: “Case #1”: San Rafael Arriba (SR), County of Desamparados (9°53’ N, 84°04’ W); “Case #2”: Nazareth (TR), County of La Union (9°55’ N, 83°59’ W), and Cocori (CO), County of Cartago (9°50’ N, 83°55’ W); and “Case #3”: San Miguel (SM), County of Desamparados (9°52’ N, 84°04’ W) and Higuito (HD), County of Desamparados (9°51’ N, 84°02’ W) (Figure 1 in supplementary material). At least 50 dogs were evaluated for each of the human cases, and they were selected within a radius of approximately 100 m from the patient’s home or specific place visited.

Three dog shelters with a population of at least 25 dogs and from the greater Metropolitan Area of Costa Rica were selected and coded as AS, CA, and PC. Shelters reported having a high adoption rate (most dogs usually stay < 3 months) and administering insecticidal and antiparasitic treatments regularly, but especially upon arrival of new animals.

Study sites were visited during 2012 and 2013. Consent was obtained from the dog owners or shelter administrators, and information such as age (juvenile, adult, senile), breed, sex, presence of ectoparasites, and site was noted for all dogs. Dogs were also evaluated for signs of disease, especially those compatible with rickettsial diseases such as fever, lethargy, anemia, ocular lesions, hemorrhage, edema, and neurologic signs ([Shaw et al., 2001](#); [Piranda et al., 2008](#)). A 1-3 ml blood sample was drawn from the cephalic vein of each dog, and the serum was separated and stored at -20°C until processed by serological analysis. A sample of the different ectoparasites present on each dog was collected with the aid of combs and forceps, usually up to 5-10 individuals (for fleas and ticks each). Ectoparasites were identified to species using

taxonomic keys (Hopkins and Rothschild, 1953; [Smit, 1958](#); Barros-Battesti et al., 2006; [Guzmán-Cornejo and Robbins, 2010](#)). In accordance with the total ectoparasites collected, pools of 1 to 10 specimens of the same species and from the same dog were prepared and stored at -80 °C until analyzed by PCR.

Indirect immunofluorescence assay (IFA)

Detection of IgG to spotted fever group (SFG) rickettsiae in dog sera was performed by IFA as previously described (Labruna et al., 2007a). For an initial screening of samples, serial two-fold dilutions were performed in phosphate-buffered saline (PBS), and a 1:32 dilution was tested using antigen of the species that have been isolated in Costa Rica: *R. rickettsii*, “*Candidatus R. amblyommii*”, and *R. felis* ([Hun et al., 2011](#); [Arguello et al., 2012](#)). Testing a 1:32 dilution allowed detection of low antibody titers that may be the result of past exposures, as well as establishment of a four-fold difference in endpoint titers of positive samples that had maximum detectable antibody at a 1:64 dilution (see below).

For the preparation of IFA slides, each *Rickettsia* was cultivated in Vero E6 cells (*R. rickettsii* and “*Candidatus R. amblyommii*”) or C6/36 cells (*R. felis*) and harvested when the infection rate reached 90%, as estimated by Gimenez staining (Labruna et al., 2007a; [Hun et al., 2011](#)). Cells were centrifuged at 500 g for 5 minutes and two microliters of the antigen was spotted onto 10 well slides, air dried, fixed in acetone for 10 minutes, and stored at 4°C until used.

In order to determine an endpoint titer, serial two-fold dilutions of each preliminary positive sample were further tested by IFA using slides prepared with antigen of the three species

mentioned above, as well antigen of *R. parkeri*, *R. rhipicephali*, and *R. bellii* (provided by Dr. Marcelo Labruna, University of Sao Paulo). *R. parkeri* and *R. rhipicephali* have not been reported in Costa Rica, but they are present in the Neotropical Region and seroreactivity in dogs has been documented (Labruna et al., 2007a; [Labruna et al., 2007b](#); Melo et al., 2011; [Barbieri et al., 2012](#); [Lado et al., 2015](#)). Samples with an endpoint titer ≥ 64 to any of the six species analyzed were confirmed as positive, indicating exposure of the corresponding dogs to SFG rickettsiae or *R. bellii*. When serum showed an end point titer at least four-fold higher for one *Rickettsia* species than that observed for any other, it was considered as probably homologous to the species with the highest titer, or to a very closely related species (unknown or not tested) ([Horta et al., 2004](#)).

In each slide, a non-reactive dog serum (negative control) and a reactive dog serum (positive control) were included at a 1:64 dilution. FITC-labeled anti-dog IgG produced in rabbit (Sigma-Aldrich) was used in all IFAs.

PCR and DNA sequencing

Ectoparasites in each pool were washed 3 times in 0.1% iodine and 70% ethanol solution and 3 times in sterile distilled water prior to maceration. Genomic DNA was extracted from macerated ectoparasite pools using the NucleoSpin[®] Tissue kit (Macherey-Nagel), following the manufacturer's instructions. *Rickettsia*-specific DNA fragments were detected by single-step PCR using primers CS-78 and CS-323 that amplify a fragment of the *gltA* gene (401 bp product), as previously described ([Labruna et al., 2004](#)). For each PCR run, a positive control (*R. rickettsii* isolate), and a negative control (Vero E6 or C6/36 cells) were included. To identify the species

present in positive pools, amplified fragments were purified using ExoI/FastAP (Thermo Scientific) and sent to Macrogen (Korea) for sequencing. Sequences were edited using BioEdit, versión 7.1.3.0 and sequence homology searches in GenBank were performed using the Basic Local Alignment Search Tool (BLAST).

Statistical analyses

The association between exposure to SFG rickettsiae (binary variable), demonstrated by seroreactivity at end point titers ≥ 64 , and sex, age (juvenile, adult, senile), breed (mix-breed or pure breed), ectoparasites (present or absent), and location (shelters or sites associated with human cases) were analyzed by binary logistic regression. Odds ratio (OR) and 95% confidence intervals (CI) were determined for each significant variable. The binary logistic regression analysis was conducted in SPSS software version 11.5 (SPSS Inc., Chicago, IL, USA), using the “Enter” method.

Results

A total of 168 dogs from five sites associated with the three human RMSF cases and 161 dogs from three shelters were analyzed (Table 1). Dogs seroreactive (IgG) to SFG rickettsiae were present in all sites associated with human RMSF cases, as well as in two of the three dog shelters evaluated. Of the dogs from sites associated with human cases, 18.5% (31/168) were confirmed as positive (titers ≥ 64), and seroreactivity varied from 4.9% (3/61) to 45.8% (11/24) (Table 1). Antibodies to *R. rickettsii* and “*Candidatus R. amblyommii*” were detected more

frequently and with higher end titers: two dogs had end titers of 2046 (SR06 and SM133) and one dog had an end titer of 512 (SR47) (Table 2). Of note, dog SR06 showed end point titers of 2046 to both *R. rickettsii* and “*Candidatus R. amblyommii*”, and had belonged to one of the human cases of RMSF (Arguello et al. 2011). Seroreactivity to *R. felis*, *R. rhipicephali*, and *R. parkeri* was also detected in dogs from sites associated with human cases of RMSF, although with much lower end titers than for “*Candidatus R. amblyommii*” and *R. rickettsii* in the same dog. Overall, probable exposure could be attributed to *R. rickettsii* or very similar species in six dogs, and to “*Candidatus R. amblyommii*”, *R. felis*, and *R. rhipicephali* in one dog each. However, highest antibody titers to *R. rickettsii* and *R. felis* were only 64 in these cases.

Of dogs from sites associated with human cases, 73.8% (124/168) had ectoparasites. *Ctenocephalides felis*, *Pulex simulans*, and *Rhipicephalus sanguineus* s. l. were identified. *C. felis* (56.7%) and *R. sanguineus* s. l. (18.5%) were the most common, and they were collected from all sites (Table 2). Of a total 155 pools of ectoparasites analyzed by PCR, *Rickettsia* DNA was detected only in pools of *C. felis*, specifically in 14.3% (16/112). Of these, 14 sequences were most similar ($\geq 99.3\%$ partial sequence homology) to *R. felis* strain URRWXCal2 (accession number CP000053) and Clone 6-OP-2-1 (accession number JN982948), which had been reported in Costa Rica (Troyo et al., 2012a). The two other sequences were from the only two positive flea pools from Nazareth, and they were most similar ($\geq 99.2\%$ partial sequence homology) to *R. felis*-like RF2125 (accession number AF516333) and Clone 4-G/JP-10-2 (accession number JN982949), which was also reported from Costa Rica (Troyo et al., 2012a).

In the shelters evaluated, 6.8% (11/161) of dogs were seroreactive (end titers ≥ 64) with titers ranging from 64 to 512, although it was not possible to determine a specific exposure to any one of the species tested (Tables 1 and 2). Ectoparasites were detected in 28.6% (46/161) of dogs:

the most frequent were *C. felis* and *R. sanguineus* s. l., although *P. simulans* and *Ixodes boliviensis* were also identified (Table 1). *Rickettsia* spp. DNA was detected in 18 of 55 pools analyzed (32.7%), specifically in 100% (6/6) of *I. boliviensis*, 38.5% (10/26) of *C. felis*, and 9.1% (2/22) of *R. sanguineus* s. l. Sequences in *I. boliviensis* were identified as an undescribed *Rickettsia* sp. of the *Rickettsia monacensis* group, which was named IbR/CRC (Troyo et al., 2014). All sequences of *Rickettsia* detected in *C. felis* pools were most similar ($\geq 99.3\%$ partial sequence homology) to *R. felis* strain URRWXCal2 (accession number CP000053) and Clone 6-OP-2-1 from Costa Rica (accession number JN982948) (Troyo et al., 2012a). After several attempts, it was not possible to obtain a sequence of the fragment amplified from pools of *R. sanguineus*. Presence of the *ompA* gene was also evaluated in positive tick pools as described previously (Troyo et al., 2014), but there was no DNA amplification from *R. sanguineus* s. l. pools.

The statistical analysis showed that in the Greater Metropolitan Area of Costa Rica, the odds of dogs being seropositive to *Rickettsia* spp. (titer ≥ 64) in areas where cases of RMSF had been reported were approximately 3.2 times the odds of seropositivity in shelters (OR: 3.2; 95% C.I: 1.5-5.6; $p = 0.002$). Seroreactivity to *Rickettsia* spp. was not significantly associated with sex, age, breed, or presence of ectoparasites.

Discussion

The present study shows that dogs from the Greater Metropolitan area of Costa Rica are exposed to rickettsiae of the SFG, and exposure is significantly higher in dogs from sites associated with recent cases of human RMSF. In Costa Rica, the only published study concerning

exposure of dogs to rickettsiae was carried out in the 1980s and reported antibodies to SFG rickettsiae in 61% (22/36) of dogs from endemic areas ([Fuentes, 1986](#)). At that time, end titers of positive samples suggested *R. rickettsii* infection, although the only species tested from the SFG were *R. rickettsii*, *R. conorii*, and *R. akari*. Although several rickettsiae can cause symptomatic infections in dogs and human cases of RMSF are diagnosed sporadically in Costa Rica ([Hun, 2013](#)), no cases have been reported in dogs.

End titers of anti-*Rickettsia* spp. IgG antibodies in seroreactive dogs suggest that most of them were exposed to species that have been previously detected in Costa Rica (*R. rickettsii*, “*Candidatus R. amblyommii*”, and *R. felis*) ([Hun et al., 2011](#); [Troyo et al., 2012a](#); [Ogrzewalska et al., 2015](#)). Although cross-reactivity makes it difficult to determine which species was involved in each case, it is likely that at least one dog was exposed to “*Candidatus R. amblyommii*” and one dog to *R. rhipicephali*. Moreover, it is possible that one dog was exposed to *R. felis* and 6 dogs to *R. rickettsii* or very similar species, but end titers in these cases were only 64, which would not indicate a recent infection. Circulation of *R. rickettsii* may have occurred in the past, especially considering the high antibody end titers (2048) in a dog that belonged to one of the confirmed human cases of *R. rickettsii* infection ([Arguello et al., 2012](#)). Even though the presence of *R. rhipicephali* has not been reported in the country, it is present in North and South America, it is associated with *Rhipicephalus* spp. and other dog ticks, and previous studies have detected seroreactivity in dogs ([Labruna et al., 2007a, 2007b](#); [Melo et al., 2011](#); [Parola et al., 2013](#)).

Exposure of dogs to “*Candidatus R. amblyommii*” and *R. felis* is not surprising, since both are common in ectoparasites of dogs and other domestic animals in Central America and the rest of the Neotropical Region ([Bermúdez et al., 2009, 2011](#); [Hun et al., 2011](#); [Troyo et al., 2012b](#)). Even though “*Candidatus R. amblyommii*” is not currently recognized as a pathogen,

there is evidence of infection in dogs and humans ([Apperson et al., 2008](#); [Bermúdez et al., 2011](#); [Barrett et al., 2014](#)). Moreover, experimental infections in guinea pigs with strains from Texas and Costa Rica have shown that infection with “*Candidatus R. amblyommii*” generates a cross-protective immune response against pathogenic *R. rickettsii* strains ([Blanton et al., 2014](#); [Rivas et al. 2015](#)). Conversely, *R. felis* is a recognized human pathogen, but isolates from a vertebrate host have not been made ([Hun and Troyo, 2012](#)). Serologic evidence of exposure has been demonstrated in dogs, as well as detection of *R. felis* DNA in blood by PCR ([Richter et al., 2002](#); [Silva Fortes et al., 2010](#); [Hii et al., 2011](#); [Wei et al., 2014](#)). Despite reports of high prevalence of *R. felis* in *C. felis*, disease in dogs is infrequent but has been suggested in at least one study that described a PCR-positive dog with fatigue, vomiting, and diarrhea ([Oteo et al., 2006](#)). The dog that was probably exposed to *R. felis* in this study may have been immunocompromised, considering that it was a senile adult. Therefore, the outcomes of infection with these species of rickettsiae should be further evaluated in dogs, especially in immunocompromised individuals.

R. sanguineus s. l. and *C. felis* were the only ectoparasites infesting dogs in all study sites associated with human RMSF cases, whereas *P. simulans* and *I. boliviensis* were found sporadically. Ectoparasites were less frequent in dogs from shelters, probably due to regular washing, application of insecticide treatments, and cleaning of kennels. A high prevalence of *R. sanguineus* s. l. and *C. felis* and has been described previously on dogs from Costa Rica ([Álvarez et al., 2006](#); [Troyo et al., 2012b](#); [Rojas et al., 2014](#)). It was not surprising to find *R. felis* and a *R. felis*-like RF2125 in *C. felis*, since both have been reported in Costa Rica ([Hun et al., 2011](#); [Troyo et al., 2012a](#)); however, *R. felis* was the most common species in fleas of the Greater Metropolitan Area, which contrasts with previous studies in other areas of the country and confirms a different geographical distribution of these bacteria in Costa Rica.

R. rickettsii was not identified in the ectoparasites analyzed, but *R. sanguineus* s. l. (or another less frequent tick) may have been responsible for transmission of rickettsiae in these urban areas. *R. sanguineus* s. l. is the most common dog tick in Costa Rica ([Álvarez et al., 2006](#); [Troyo et al., 2012b](#); [Rojas et al., 2014](#)), although two species of this group are present in the Neotropical Region (Nava et al., 2015). Studies have demonstrated that *R. sanguineus* s. l. can transmit *R. rickettsii*, and it has been associated with outbreaks ([Demma et al., 2005](#); [Eremeeva et al., 2011](#); [Piranda et al., 2011](#)). Moreover, the present study was conducted a year or more after the case reports, and prevalence of virulent rickettsiae in ticks may be low ([Socolovschi et al., 2009](#)). In addition, experimental studies have demonstrated low *R. rickettsii* infection rates in immature stages and low filial infection rates in *R. sanguineus* s. l. ([Piranda et al., 2011](#)). Although the PCR assay used should be specific for *Rickettsia* spp., the fragment amplified from *R. sanguineus* s. l. could not be sequenced. Other species of the Neotropical Region that can be found in *R. sanguineus* s. l. include *R. massiliae*, *R. rhipicephali*, “*Candidatus R. amblyommii*”, *R. akari*, and *R. felis* ([Zavala-Castro et al., 2009](#); [Labruna et al., 2011](#); [Parola et al., 2013](#)).

The high antibody prevalence to SFG rickettsiae in dogs from some of the urban sites in the Greater Metropolitan Area of San José suggests contact and possible infection, which may indicate a higher risk of exposure in humans. Given that seroprevalence of dogs from areas associated with human cases was higher than that of dogs from shelters, this study further supports the role of dogs as important sentinel hosts for human infection ([Paddock et al., 2002](#); [Pinter et al., 2008](#); [Hii et al., 2011](#)). In addition, a confirmed human case was directly linked to a seropositive dog (with a high end titer), suggesting that both dog and human were in contact with the same vector species. Furthermore, investigations are required to determine the potential involvement of dogs and their ectoparasites in maintaining urban transmission cycles in Costa

Rica and similar settings within Latin America. Veterinary health professionals should also explore the possibility of mild to severe disease caused by several SFG rickettsiae that can infect dogs ([Grasperge et al., 2012](#); [Levin et al., 2014](#); [Solano-Gallego et al., 2015](#)), since rickettsioses may go undetected or misdiagnosed due to other tick-borne diseases that respond to the same antibiotic treatment and are common in Costa Rica, like ehrlichiosis and anaplasmosis ([Romero et al., 2011](#); [Rojas et al., 2014](#)). The presence of several SFG rickettsiae in the most important urban area of Costa Rica warrants coordination of both animal and human health sectors, which should be aware of rickettsial diseases and coordinate efforts to prevent them.

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Table 1

Seroreactivity of dogs to spotted fever group (SFG) rickettsiae (titer ≥ 64) and frequency of ectoparasites identified on dogs from sites associated with human cases of Rocky Mountain spotted fever and from shelters in the Greater Metropolitan Area of Costa Rica.

Collection site		Dogs evaluated	SFG <i>Rickettsia</i> seroreactive dogs (%)	Dogs with ectoparasites (%)			
				<i>C. felis</i>	<i>P. simulans</i>	<i>R. sanguineus</i> s. l.	<i>I. boliviensis</i>
Case #1	San Rafael	61	3 (4.9)	39 (63.9)	0 (0)	10 (16.4)	0 (0)
Case #2	Nazareth	35	4 (11.4)	21 (60.0)	3 (8.6)	1 (2.9)	0 (0)
	Cocori	18	5 (27.8)	9 (50.0)	0 (0)	4 (22.2)	0 (0)
Case #3	San Miguel	30	8 (26.7)	19 (63.3)	1 (3.3)	13 (43.3)	0 (0)
	Higuito	24	11 (45.8)	24 (100.0)	8 (33.3)	3 (12.5)	0 (0)
All case sites		168	31 (18.5)	112 (56.7)	12 (7.1)	31 (18.5)	0 (0)
Shelters	AS	105	8 (7.6)	26 (24.8)	1 (1.0)	20 (19.0)	6 (5.7)
	CA	34	3 (8.8)	0 (0)	0 (0)	2 (5.9)	0 (0)
	PC	22	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
All shelters		161	11 (6.8)	26 (16.1)	1 (0.6)	22 (13.7)	6 (3.7)

Table 2

Results of immunofluorescence assay (IFA) for *Rickettsia* species in seroreactive dogs (titer ≥ 64) from sites associated with human cases of Rocky Mountain spotted fever and from shelters in the Greater Metropolitan Area of Costa Rica.

Site	ID	Age	Sex	Breed	IgG endpoint titer*						PAIHR	Ectoparasites
					<i>R. rickettsii</i>	<i>R. amblyommii</i>	<i>R. felis</i>	<i>R. parkeri</i>	<i>R. rhipicephali</i>	<i>R. bellii</i>		
San Rafael	SR06	Adult	Male	Miniature Schnauzer	2048	2048	512	512	1024	NR		no
	SR47	Adult	Female	Mix-breed	512	512	NR	128	128	NR		<i>C. felis</i>
	SR56	Adult	Male	Cocker spaniel	64	64	NR	NR	NR	NR		no
Nazareth	TR82	Adult	Female	Mix-breed	64	64	NR	NR	NR	NR		no
	TR83	Adult	Female	Mix-breed	64	64	NR	NR	NR	NR		no
	TR86	Adult	Female	Miniature Schnauzer	128	64	NR	NR	NR	NR		no
	TR92	Adult	Female	Mix-breed	64	32	NR	NR	NR	NR		<i>C. felis</i>
Cocori	CO98	Adult	Female	Mix-breed	64	32	NR	NR	NR	NR		<i>C. felis</i>
	CO104	Adult	Female	Miniature Schnauzer	64	128	NR	NR	NR	NR		no
	CO113	Adult	Male	Mix-breed	64	32	NR	NR	NR	NR		<i>C. felis</i>
	CO114	Adult	Female	Mix-breed	64	NR	NR	NR	NR	NR	<i>R. rickettsii</i>	<i>R. sanguineus</i>
	CO115	Adult/senile	Male	Mix-breed	NR	NR	64	NR	NR	NR	<i>R. felis</i>	<i>R. sanguineus</i>
San Miguel	SM120	Adult	Female	French poodle	64	NR	NR	NR	NR	NR	<i>R. rickettsii</i>	<i>R. sanguineus</i>
	SM131	Adult/senile	Female	French poodle	128	128	64	NR	NR	NR		<i>C. felis</i> - <i>R. sanguineus</i>
	SM133	Adult	Male	Mix-breed	128	2048	256	NR	64	NR	<i>R. amblyommii</i>	<i>C. felis</i>
	SM134	Adult	Male	Mix-breed	64	64	NR	NR	NR	NR		<i>R. sanguineus</i>
	SM135	Adult	Male	Mix-breed	64	NR	NR	NR	NR	NR	<i>R. rickettsii</i>	no
	SM141	Adult	Female	Mix-breed	128	32	NR	NR	128	NR		<i>C. felis</i> - <i>R. sanguineus</i>
	SM144	Adult	Male	Chihuahua	64	NR	NR	NR	NR	NR	<i>R. rickettsii</i>	<i>C. felis</i>
SM145	Adult	Female	Siberian Husky	64	NR	NR	NR	NR	NR	<i>R. rickettsii</i>	<i>C. felis</i>	
Higuito	HD146	Adult	Male	Beagle	64	128	NR	NR	64	NR		<i>C. felis</i>
	HD148	Adult/senile	Male	Mix-breed	256	256	128	NR	64	NR		<i>C. felis</i>
	HD151	Adult	Male	Mix-breed	32	NR	NR	NR	64	NR		<i>C. felis</i>
	HD152	Adult	Male	Mix-breed	64	NR	NR	NR	NR	NR	<i>R. rickettsii</i>	<i>C. felis</i>
	HD153	Adult	Female	Mix-breed	64	64	NR	NR	64	NR		<i>C. felis</i>
	HD155	Adult/senile	Male	Miniature Schnauzer	128	32	32	64	NR	NR		<i>C. felis</i>
	HD156	Adult	Male	Mix-breed	64	64	NR	NR	64	NR		<i>C. felis</i> - <i>P. simulans</i>

	HD157	Juvenile	Female	Mix-breed	128	128	256	64	256	NR		<i>C. felis</i>
	HD164	Adult	Male	Cocker spaniel	64	64	NR	NR	<u>256</u>	NR	<i>R.</i> <i>hipicephali</i>	<i>C. felis</i>
	HD165	Adult/senile	Male	Mix-breed	32	NR	NR	NR	64	NR		<i>C. felis</i>
	HD168	Adult	Male	Mix-breed	NR	NR	128	64	64	NR		<i>C. felis - P. simulans</i>
Shelters	AS 19	Adulto	Female	Mix-breed	128	128	32	NR	NR	NR		no
	AS 47	Adulto	Female	Mix-breed	32	32	NR	NR	64	NR		<i>R. sanguineus</i>
	AS 67	Adulto	Female	Mix-breed	32	64	NR	NR	64	NR		<i>C. felis</i>
	AS 70	Adulto	Female	Mix-breed	64	64	NR	NR	NR	NR		no
	AS 72	Adulto	Male	Mix-breed	NR	NR	32	NR	64	NR		no
	AS 87	Senil	Female	Mix-breed	64	64	32	NR	NR	NR		no
	AS 89	Adulto	Female	Mix-breed	512	512	128	128	32	NR		<i>C. felis - R. sanguineus</i>
	AS 97	Senil	Female	Mix-breed	32	32	NR	NR	NR	64		<i>C. felis</i>
	CA 16	Adulto	Female	Mix-breed	128	128	64	NR	NR	NR		no
	CA 18	Adulto	Female	Mix-breed	128	128	NR	NR	NR	NR		no
	CA 30	Adulto	Female	Mix-breed	128	128	NR	NR	NR	NR		no

* Underlined values indicate a four-fold or greater difference in the end titer with this antigen when compared to all others.

NR: Not reactive at 1:32 dilution.

PAIHR: Possible antigen involved in a homologous reaction.