Ectoparasite Infestations and Canine Infection by *Rickettsiae* and *Ehrlichiae* in a Semi-Arid Region of Northeastern Brazil

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Abstract

This study investigated the prevalence of *Rickettsia* spp. and *Ehrlichia canis* infection in dogs and their ectoparasites from rural and urban areas of two municipalities, Petrolina and Juazeiro, within a semiarid region (Caatinga biome) of northeastern Brazil, by immunofluorescence assay (IFA) and polymerase chain reaction (PCR). Overall, 12.1% (61/504) and 23.0% (116/504) of canine plasma samples had antibodies reactive to *Rickettsia* spp. and *E. canis*. *E. canis* DNA was detected by PCR in 8.3% (42/504) of canine blood samples, whereas no blood sample was positive for *Rickettsia* spp. The infection by *E. canis* was determined by PCR in 4.9% (14/285) *Rhipicephalus sanguineus* sensu lato (s.l.) ticks and by *Rickettsia felis* in 1.1% (3/285) and 40.6% (74/182) ticks and fleas, respectively. Multivariate regression analyses revealed that canine seropositivity to *Rickettsia* spp. was associated statistically with the variables “to reside in Petrolina” and “presence of ectoparasites.” Our results indicate that canine infection by *E. canis* might be endemic in the Caatinga biome as it is in other Brazilian biomes. Although no previous serosurvey for *Rickettsia* spp. has been conducted on dogs from the Caatinga biome, our values are much lower than the ones reported for rural dogs from other Brazilian biomes. These differences are likely related to the semiarid climate of the Caatinga biome, which minimizes the exposure of rural dogs to *Amblyomma* spp. ticks, the most common vectors of *Rickettsia* spp. in Brazil. Considering that dogs are excellent sentinels for human exposure to *Rickettsia* spp., we can infer that the risks of human acquiring tick-borne rickettsiosis in the Caatinga region of the present study are low. The rickettsial infection rates in fleas and ticks were not related to canine seropositivity; *i.e.*, areas with higher *Rickettsia* infection rates in fleas had the lowest canine seroreactivity to *Rickettsia* spp.

Key Words: *Rickettsia*—*Ehrlichia canis*—Dogs—Fleas—Ticks—Caatinga.

Introduction

DURING THE LAST TWO DECADES, there has been a substantial improvement in our knowledge regarding vector-borne agents that infect domestic dogs in Brazil (Soares et al. 2014). On the other hand, most of the studies have been conducted traditionally with canine populations from more densely populated areas of Brazil, such as the southeastern and southern regions, and coastal areas of the

northeastern region (Horta et al. 2007, Pinter et al. 2008, Saito et al. 2008, Ramos et al. 2010, Vieira et al. 2011, Silva et al. 2012, Demoner et al. 2013, Santos et al. 2013, Vieira et al. 2013). Conversely, very few studies have been done with dogs living within the Caatinga biome, a semiarid region of northeastern Brazil, where environmental conditions are drastically different from the remaining Brazilian biomes, namely Amazonia, Atlantic forest, Cerrado (Savannah), Pantanal, and southern open fields (Pampas). The primary

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characteristic that differs Caatinga from these other biomes is the much lower rainfall regimen, typically ≈ 500 mm annually, although occurrences of droughts sometimes lasting for longer than 1 year is also a characteristic of the region (Batista et al. 2007). Consequently, a distinct vegetation composition, adapted to xeric conditions, is typical of the Caatinga biome (Andrade-Lima 1981). Such environmental conditions should have an important role on the prevalence of vector-borne diseases within the Caatinga biome. However, only two serosurvey studies, both encompassing canine hospital populations of the same locality, have been conducted in this Brazilian biome (Azevedo et al. 2011, Tanikawa et al. 2013).

On the basis of this assumption, the present study aimed to evaluate the occurrence of the infection by two groups of

vector-borne bacteria, *Rickettsia* spp. and *Ehlichia* spp., in domestic dogs and their ectoparasites from a region within the Caatinga biome of northeastern Brazil.

Materials and Methods

Study site and sample collection

This study was conducted in urban and rural areas of the municipalities of Petrolina, Pernambuco state ($09^{\circ}23'55''\text{S}$, $40^{\circ}30'03''\text{W}$), and Juazeiro, Bahia state ($09^{\circ}24'42''\text{S}$, $40^{\circ}29'55''\text{W}$), both located in the São Francisco Valley, a semiarid region of northeastern Brazil (Fig. 1).

Between August, 2009, and May, 2012, blood samples were collected from 504 domestic dogs, 252 per municipality. In each municipality, 126 dogs were sampled in the urban

FIG. 1. Localization of the two municipalities (Petrolina and Juazeiro) in which dogs were sampled for the present study.

area, whereas other 126 dogs were sampled in the rural area. Each dog was sampled in its own household; during sample collection, all dogs were apparently healthy with no signs of illness, although no clinical examination was performed. Blood collection was performed from the cephalic vein in vacuum tubes with ethylenediamine tetraacetic acid (EDTA). After centrifugation ($3000 \times g$, 15 min), plasma and whole blood with EDTA were stored into 1.5-mL microtubes at -20°C until processing. During blood collection, dogs were examined for the presence of ectoparasites (ticks, fleas, lice), which were collected in tubes containing absolute ethanol and identified taxonomically following guidelines of Guimarães et al. (2001) and Nava et al. (2015).

Serological analyses

Canine plasma samples were tested by the indirect immunofluorescence assay (IFA) using crude antigens derived from five *Rickettsia* isolates from Brazil (*R. rickettsii* strain Taiaçu, *R. parkeri* strain At24, *R. amblyommii* strain Ac37, *R. rhipicephali* strain HJ5, and *R. bellii* strain Mogi), as previously described (Labruna et al. 2007). Briefly, plasma were diluted in two-fold increments with phosphate-buffered saline (PBS), starting from a 1:64 dilution. Slides were incubated with fluorescein isothiocyanate (FITC)-labeled rabbit anti-dog immunoglobulin G (IgG; Sigma, St. Louis, MO). For each sample, the end point IgG titer reacting with each of the five *Rickettsia* antigens was determined. In each slide, a serum previously shown to be nonreactive (negative control) and a known reactive serum (positive control) derived from the study of Piranda et al. (2008) were tested at the 1:64 dilution.

Plasma samples were also tested by IFA using *Ehrlichia canis*-infected DH82 cells as antigen, performed with the São Paulo strain of *E. canis* from Brazil as previously described (Aguar et al. 2007a, 2008). Plasma was considered to contain antibodies reactive to *E. canis* if it displayed a reaction at the 1:40 dilution (McBride et al. 2001). In each slide, a serum previously shown to be nonreactive (negative control) and a known reactive canine serum (positive control) derived from the study of Aguiar et al. (2007a) were tested at the 1:40 dilution. Samples that reacted at the screening dilution (1:40) were titrated using serial two-fold dilutions to determine end point titers.

Molecular analyses

DNA extraction from 150 μL of whole blood samples and ticks were performed using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI) according to manufacturer's recommendations. Fleas and lice were individually submitted to DNA extraction using a previously validated protocol (Horta et al. 2007). Briefly, samples were washed with 50 μL of TE buffer (10 mM Tris HCl, 1 mM EDTA, pH 8.0), cut in pieces with sterile 18-gauge needles, subjected to boiling (100°C , 20 min), and then stored at -20°C until testing.

DNA samples were tested by two PCR protocols. One protocol, targeting a 401-bp fragment of the *gltA* gene of *Rickettsia* spp. consisted of primers CS-78 and CS-323, as previously described (Labruna et al. 2004). The other protocol consisted of a heminested PCR targeting a fragment of the *dsb* gene of *Ehrlichia* spp, using primers DSB-330

(5'-GAT GAT GTT TGA AGA TAT SAA ACA AAT-3') and DSB-720 (5'-CTA TTT TAC TTC TTA AAG TTG ATA WAT C-3') in the first reaction (amplicon size, 401 bp), and primers DSB380 (5'-ATT TTT AGR GAT TTT CCA ATA CTT GG-3') and DSB-720 in the second reaction (349-bp), as previously described (Almeida et al. 2013).

In each set of reactions, negative control tubes containing water and a positive control tube containing DNA of *R. parkeri* or *Ehrlichia chaffeensis* were included. All PCR products of the expected size were purified with ExoSap (USB, Cleveland, OH) and DNA-sequenced in an ABI automated sequencer (Applied Biosystems/Thermo Fisher Scientific, model ABI 3500 Genetic Analyzer, Foster City, CA) with the same primers used for PCR. The sequences generated were submitted to BLAST analysis (www.ncbi.nlm.nih.gov/blast) to infer closest similarities available in GenBank.

Statistical analyses

The proportion of dogs infested by ectoparasites or infected by *Rickettsia* spp or *E. canis* were compared between animals from urban and rural areas by the chi-squared test. Values were considered significantly different when $p < 0.05$.

For each sampled dog, a questionnaire was given to the dog owner with the purpose of gaining information about independent variables that could be associated with seroreactivity to *Rickettsia* spp. or *E. canis*, or ehrlichial DNA in canine blood (dependent variables). An additional independent variable, "presence of ectoparasites," was also considered in the association analyses. In this case, "presence of ectoparasites" was inferred from our direct observations of ticks, fleas, or lice on the dogs during our field work. The data obtained from epidemiological questionnaires were analyzed using the chi-squared or Fisher exact test. Variables that presented $p \leq 0.20$ were selected for multivariate analysis using logistic regression (Hosmer and Lemeshow 2000). The final fit of the model was verified with the Hosmer and Lemeshow test, whereby a p value ≥ 0.5 indicated satisfactory fit. All analyses were performed at a 5% significance level and using SPSS for Windows (1999) software.

This study was previously approved by the Human and Animal Ethic Commission of the Federal University of São Francisco Valley (protocol number 18061035).

Results

Overall, 64.3% of the dogs (324/504) were infested by ectoparasites, 61.1% in Petrolina and 67.5% in Juazeiro. In Petrolina, 47.6% of the dogs were infested with ticks, 21.0% with fleas, and 3.2% with lice. In Juazeiro, infestation rates were 62.7% for ticks, 13.1% for fleas, and 0.8% for lice. Generally, rural dogs presented higher tick and flea infestations than urban dogs, at either Petrolina or Juazeiro (Table 1). Lice infestations were always low regardless of dog origin (rural or urban). All ticks collected from dogs were identified as *Rhipicephalus sanguineus* sensu lato (s.l.); all fleas were *Ctenocephalides felis felis*, and all lice were identified as *Heterodoxus spiniger*. Infestation abundance was not calculated because these infestations were not quantified accurately; i.e., not all specimens present on dogs were collected.

Overall, 12.1% of the dogs (61/504) were seropositive to at least one *Rickettsia* species, with canine seroprevalence values significantly higher ($p < 0.05$) in Petrolina than in

TABLE 1. ECTOPARASITE INFESTATIONS ON DOMESTIC DOGS LIVING IN THE MUNICIPALITIES OF PETROLINA AND JUAZEIRO, NORTHEASTERN BRAZIL

Municipality	Area	No. dogs with ectoparasites/ no. examined dogs (%)	No. dogs with ticks (%)	No. dogs with fleas (%)	No. dogs with lice (%)
Petrolina	Rural	92/126 (73.0)a	64 (50.8)a	46 (36.5)a	7 (5.5)a
	Urban	62/126 (49.2)b	56 (44.4)a	7 (5.5)b	1 (0.8)a
Juazeiro	Rural	101/130 (77.7)a	95 (73.1)b	23 (17.7)c	0 (0)a
	Urban	69/122 (56.5)b	63 (51.3)a	10 (8.2)b	2 (1.6)a
Total		324/504 (64.3)	278 (55.1)	86 (17.1)	10 (2.0)

Numbers followed by different letters in the same column indicate statistically significant differences ($p < 0.05$).

Juazeiro (Table 2). Within each of these municipalities, seroprevalence values were greater in rural than urban areas, albeit not statistically different ($p > 0.05$). Canine end point titers to *Rickettsia* spp. varied from 64 to 8192 in both municipalities. Considering the overall results for each of the five *Rickettsia* as antigens, 47 (9.3%) dogs were reactive to *R. rhipicephali* (range of end point titers, 64–2048; mean, 290.0), 43 (8.5%) to *R. rickettsii* (64–1024; mean, 299.2), 33 (6.5%) to *R. amblyommii* (64–8192; mean, 663.3), 29 (5.7%) to *R. bellii* (64–8192; mean, 794.5), and 26 (5.1%) to *R. parkeri* (64–256; mean, 150.2). Serologically, cross-reactivity was observed frequently, because 51 (83.6%) out of the 61 seropositive dogs reacted to two or more *Rickettsia* species. Thirty-five dog samples cross-reacted to *R. rhipicephali* and *R. rickettsii*; 23 to *R. rhipicephali* and *R. parkeri*; and 31 to *R. rhipicephali* and *R. amblyommii* antigens. Finally, 23 dogs reacted to both *R. bellii* (a non-spotted fever group [SFG] agent) and at least one SFG agent (*R. parkeri*, *R. rickettsii*, *R. rhipicephali*, or *R. amblyommii*). Generally, cross-reactivity was characterized by similar end point titers, usually with less than four-fold differences.

Overall, 23.0% of the dogs (116/504) were seropositive to *E. canis* antigen, with similar ($p > 0.05$) seroprevalence values between urban and rural dogs, and between dogs from Petrolina and Juazeiro (Table 2). Canine end point titers to *E. canis* varied from 64 to 81,920; at least 62.9% (73/116) of the *E. canis*-reactive dogs had high end point titers, ≥ 1280 . PCR on DNA samples extracted from canine blood revealed the presence of ehrlichial DNA in 8.3% (42/504) of the dogs (Table 3). PCR products from seven of these samples were

sequenced, resulting in sequences 100% identical to partial sequences of the *E. canis dsb* gene (AF403710). There was no amplification of *Rickettsia* DNA in the blood of the dogs.

Among the ectoparasites tested by PCR, ehrlichial DNA was detected in 4.9% (14/285) *R. sanguineus* s.l. ticks, with similar ($p > 0.05$) infection rates in ticks from rural and urban dogs between Petrolina and Juazeiro (Table 3). PCR products of four ticks were submitted randomly to DNA sequencing, resulting in sequences 100% identical to partial sequences of the *E. canis dsb* gene (AF403710). Among the ectoparasites tested by PCR targeting *Rickettsia* spp., only 1.1% (3/285) and 40.6% (74/182) of ticks and fleas, respectively, resulted in amplification of rickettsial DNA (Table 3), with similar values ($p > 0.05$) between ticks from Petrolina and Juazeiro. On the other hand, fleas from Juazeiro had significantly higher rickettsial infection rates than fleas from Petrolina (Table 3). DNA sequences were generated from the PCR products of three ticks, 10 fleas from Petrolina, and 13 fleas from Juazeiro, resulting in all cases in sequences identical to each other and 100% identical to corresponding *gltA* partial sequences of *R. felis* strain URRWXC2 (CP000053).

Through univariate analysis, canine seropositivity to *Rickettsia* spp. was statistically coupled ($p < 0.20$) with the following independent variables: “to reside in Petrolina” ($p < 0.001$); “older age” ($p = 0.029$); and “presence of ectoparasites” ($p = 0.079$). When these three independent variables were subjected to multivariate analysis, only the variables “to reside in Petrolina” (odds ratio [OR] = 4.58; 95% interval confidence [CI] 2.40–8.74) and “presence of ectoparasites” (OR = 2.05; 95% CI 1.09–3.84) were significantly associated ($p < 0.05$).

Other independent variables tested in this study, but without significant association to rickettsial seropositivity, were access to rural area, gender, breed (pure or crossbred), access to streets, contact with other animals, and recent use of acaricides. No significant association was observed between the independent variables and canine seropositivity to *E. canis* or canine-positive PCR for *Ehrlichia*.

Discussion

The present study evaluated the seroprevalence for *E. canis* among urban and rural dogs from the Caatinga, the Brazilian semiarid biome. Although our serological analysis alone does not exclude the possibility that seropositive dogs have been infected by an *Ehrlichia* species different from *E. canis* (McBride et al. 2001), canine infection was confirmed by molecular methods, which revealed *E. canis* DNA in both canine blood and their ticks, *R. sanguineus* s.l. This tick

TABLE 2. RESULTS OF SEROLOGICAL ANALYSIS BY IMMUNOFLUORESCENCE ASSAY WITH ANTIGENS OF *RICKETTSIA* SPP. AND *EHRlichia CANIS* FOR DOMESTIC DOGS LIVING IN THE MUNICIPALITIES OF PETROLINA AND JUAZEIRO, NORTHEASTERN BRAZIL

Municipality	Area	No. dogs reactive to <i>Rickettsia</i> spp./no. tested dogs (%)	No. dogs reactive to <i>E. canis</i> /no. tested dogs (%)
Petrolina	Rural	27/126 (21.4)a	33/126 (26.2)a
	Urban	21/126 (16.7)a	23/126 (18.2)a
Juazeiro	Rural	9/130 (6.9)b	26/130 (20)a
	Urban	4/122 (3.3)b	34/122 (27.9)a
Total		61/504 (12.1)	116/504 (23.0)

Numbers followed by different letters in the same column indicate statistically significant differences ($p < 0.05$).

TABLE 3. RESULTS OF POLYMERASE CHAIN REACTIONS FOR *RICKETTSIA* SPP. AND *EHRLICHIA* SPP. ON BLOOD SAMPLES AND ECTOPARASITES COLLECTED FROM DOMESTIC DOGS LIVING IN THE MUNICIPALITIES OF PETROLINA AND JUAZEIRO, NORTHEASTERN BRAZIL

Municipality	Area	Canine blood: No. infected/ no. tested (%)		Ticks: No. infected/ no. tested (%)		Fleas: No. infected/ no. tested (%)
		Rickettsia	Ehrlichia	Rickettsia	Ehrlichia	Rickettsia
Petrolina	Rural	0/126 (0)a	7/126 (5.5)a	0/69 (0)a	3/69 (4.3)a	5/63 (7.9)a
	Urban	0/126 (0)a	10/126 (7.9)a	1/69 (1.4)a	4/69 (5.8)a	9/29 (31)b
Juazeiro	Rural	0/130 (0)a	17/130 (13.1)a	0/75 (0)a	3/75 (4.0)a	31/48 (64.6)c
	Urban	0/122 (0)a	8/122 (6.5)a	2/72 (2.8)a	4/72 (5.5)a	29/42 (69)c
Total		0/504 (0)	42/504 (8.3)	3/285 (1.1)	14/285 (4.9)	74/182 (40.6)

Numbers followed by different letters in the same column indicate statistically significant differences ($p < 0.05$).

species is recognized as the only natural vector of *E. canis* (Vieira et al. 2011).

To the best of our knowledge, only two previous studies evaluated canine seroprevalence for *E. canis* in the Caatinga biome (Azevedo et al. 2011, Tanikawa et al. 2013). In contrast to the present study, these two previous studies evaluated canine hospital populations, both from the same area of the state of Paraíba, which had 69.4–72.5% of the dogs seroreactive to *E. canis*. Conversely, the present study reports only 18.3–27.9% canine seroreactivity to *E. canis*. Although these differences could be related to unknown local factors, indeed they are related to the canine populations, namely hospital populations in the two previous studies versus healthy dogs in the present study. Therefore, the present results are more realistic to the actual canine prevalence of *E. canis* among dogs from the Caatinga.

In other Brazilian biomes, where canine healthy populations were also sampled, *E. canis* seroprevalence values were 42.5–44.7% in the Cerrado biome (Costa et al. 2007, Silva et al. 2010), 15.7–37.9% in the Amazonian biome (Aguiar et al. 2007b, Spolidorio et al. 2013), 70.9% in the Pantanal biome (Melo et al. 2011), and 35.6–44.9% in the Atlantic forest biome (Souza et al. 2010, Vieira et al. 2013). Therefore, the 18.3–27.9% *E. canis* seroprevalence values of the present study are nearly within the overall range that has been reported in other Brazilian phytogeographic regions, suggesting that canine infection by *E. canis* might be endemic in the Caatinga biome as it is in other Brazilian biomes. One exception is the Pampas biome (extreme southern Brazil), where two seroprevalence studies have reported only 4.4–4.8% *E. canis*-seroreactive dogs (Saito et al. 2008, Krawczak et al. 2012).

One factor commonly related to these Brazilian biomes (except for the Pampas biome) is the widespread presence of the tropical lineage of *R. sanguineus* s.l. ticks, the natural vector of *E. canis* (Moraes-Filho et al. 2011). This exotic tick species, introduced in Brazil possibly from sub-Saharan Africa, has spread to all Brazilian biomes, except for the Pampas biome (Aragão 1936, Moraes-Filho et al. 2011). The main characteristic of this tick species is that its off-host developmental stages take place primarily inside cracks and crevices of human dwellings; i.e., its survival does not depend directly on vegetation composition (Labruna and Pereira 2001). This characteristic makes this tick typically an urban ectoparasite of dogs, although it is relatively frequent on dogs from rural areas, where human dwellings allow its

establishment (Labruna et al. 2001, Szabó et al. 2001). Due to its dependence on human dwellings, *R. sanguineus* s.l. might not have encountered obstacles to get established among canine populations from semiarid regions of the Caatinga biome, where mean annual temperatures are very similar to other Brazilian biomes, such as the Amazonia and the Atlantic forest of northeastern Brazil (Andrade-Lima 1981, Batista et al. 2007).

We report an overall 12.1% canine seroprevalence for *Rickettsia* spp, with a highest seroprevalence value of 21.4% for rural dogs living in Petrolina municipality. Although no previous serosurvey for *Rickettsia* spp. has been conducted on dogs from the Caatinga biome, our values are much lower than the ones reported for rural dogs from other Brazilian biomes, such as 87% for Amazonia (Minervino et al. 2015), 75.6% for Pantanal (Melo et al. 2011), 67.3% for Atlantic forest (Barbieri et al. 2014), and 42.4% for Pampas (Saito et al. 2008). One distinct characteristic of the dogs from these other biomes is that many of them were infested by *Amblyomma* spp. ticks, native to the respective biomes, and usually found to be infected by *Rickettsia* spp. (Labruna et al. 2011).

Comparative studies comprising dogs from urban and rural areas of Brazil have shown that urban dogs are infested almost solely by *R. sanguineus* s.l. ticks, whereas rural dogs are infested by different *Amblyomma* species, and also by *R. sanguineus* s.l. (Szabó et al. 2001, Melo et al. 2011, Costa et al. 2013). This scenario contrasts to the present study, where rural dogs were found infested solely by *R. sanguineus* s.l. ticks. Indeed, this result is related to the semiarid climate and vegetation composition of the Caatinga biome, which minimizes the exposition of rural dogs to *Amblyomma* spp. ticks, the most common vectors of *Rickettsia* spp. in Brazil (Labruna et al. 2011). Considering that dogs are excellent sentinels for human exposure to *Rickettsia* spp. (Sangioni et al. 2005, Barbieri et al. 2014), we can infer that the risks of human acquiring tick-borne rickettsiosis in the Caatinga region of the present study are low.

Because canine seropositivity to *Rickettsia* spp. was strongly associated with “presence of ectoparasites” (OR=2.05), and “to reside in Petrolina” (OR=4.58), this higher seropositivity could be biologically associated with fleas, since the rural dogs of Petrolina had significantly higher flea infestation rate (Table 1) and rickettsial reactivity (Table 2), when compared to the remaining dogs. At first sight, this statement could be explained by the fact that 7.9% of the fleas collected from rural dogs in Petrolina were

infected by *Rickettsia* (Table 3), including *R. felis*, as confirmed by DNA sequencing of some PCR-positive fleas. On the other hand, an argument against this assumption is that previous studies showed that canine exposure to *R. felis*-infected fleas did not induce seroconversion to *Rickettsia* spp. (Horta et al. 2007, Pinter et al. 2008). In addition, our findings for Juazeiro dogs support these previous studies, because the highest *Rickettsia* infection rates (64.6–69.0%) were found for fleas collected from Juazeiro dogs (Table 3), these dogs had the lowest seroprevalence values for *Rickettsia* spp. (3.3–6.9%) (Table 2). Alternatively, the statistical association of canine seropositivity to *Rickettsia* spp. with “presence of ectoparasites” could be an indirect evidence of less hygiene conditions, making the dogs more exposed to other invertebrate animals potentially infected by unknown *Rickettsia* spp.

Interestingly, the highest mean end point titer to *Rickettsia* spp. of the dogs of the present study was for *R. bellii* (mean, 794.5), which is considered a nonpathogenic agent that is genetically classified in a basal group of the genus *Rickettsia* (Perlman et al. 2006). A recent study showed that this basal group is composed by a vast array of *R. bellii*-like genotypes, most of them associated with free-living invertebrate hosts, such as nonhematophagous arthropods (Perlman et al. 2006). Therefore, we cannot discount that some of the dogs of the present study became seropositive to *Rickettsia* spp. after accidental contact with nonhematophagous arthropods. In addition, it is also possible that some of the dogs seroconverted to *Rickettsia* spp. after having been infested by *Amblyomma auricularium* ticks. Whereas this tick species was not found in the present study, *R. amblyommii*-infected *A. auricularium* ticks were recently reported on armadillos from the rural area of Petrolina (Saraiva et al. 2013), where we found the highest rickettsial seropositivity value (21.4%) of the present study. This possibility is supported by the high antibody titers to *R. amblyommii* (mean, 663.3) among sampled dogs, which was the second highest mean titer in the present study.

Finally, whereas we found that *R. sanguineus* s.l. ticks are highly prevalent on dogs from Petrolina and Juazeiro, either on rural or urban areas, this tick is possibly not directly related to rickettsial transmission to these dogs. This assumption relies on our findings for rural dogs of Juazeiro, where the highest infestation rate by *R. sanguineus* s.l. (73.1%), and at the same time, the lowest rickettsial seroreactivity value (3.3%) were found. On the other hand, this tick species undoubtedly has a great veterinary importance in the study area, where infection by *E. canis* seems to be prevalent among dogs. The semiarid weather, typical of the Caatinga biome, does not seem to elicit a significant negative effect on the establishment of *R. sanguineus* ticks, in contrast to native *Amblyomma* ticks, for which off-host developmental stages take place at outdoor conditions. This situation might minimize the chances of domestic dogs, and consequently humans, to become infected by tick-borne rickettsiae in the Caatinga biome.

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Author Disclosure Statement

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