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Serologic and molecular survey of *Rickettsia* spp. in dogs, horses and ticks from the Atlantic rainforest of the state of Bahia, Brazil

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Abstract:
Tick-borne spotted fever in Brazil is known to be caused by two agents, *Rickettsia rickettsii* and *Rickettsia parkeri*. Nothing was known about these agents in one area of the Atlantic rainforest biome of Bahia state, where during March to June 2016, 356 rural dogs and 69 horses were sampled and their sera were processed through indirect immunofluorescence assay against antigens of *R. rickettsii, R. parkeri, Rickettsia amblyommatis* and *Rickettsia bellii*. Ticks collected from these dogs and horses were molecularly tested for the presence of rickettsial DNA. Overall, 16.4% (58/356) dogs and 24.6% (17/69) horses were seroreactive to *Rickettsia* spp. Five tick species, *Rhipicephalus sanguineus* sensu lato (s.l.), *Amblyomma ovale, A. sculptum, R. microplus*, and *A. naponense*, were collected from dogs, whereas horses were infested by *A. sculptum* and *Dermacentor nitens*. A total of 242 ticks from dogs and 62 from horses were analyzed by PCR targeting rickettsiae, which were detected in only 4/27 (14.8%) *A. ovale*. Fragments of the rickettsial *gltA* and *ompA* genes from these four ticks were 100% identical to the Atlantic rainforest strain of *R. parkeri*. The presence of *A. ovale* on dogs was positively associated with local canine seroreactivity to *R. parkeri*. Our results provide evidence for the transmission of *R. parkeri* strain Atlantic rainforest from *A. ovale* to domestic dogs within the rural area of Ilhéus, similarly to other areas of the Atlantic rainforest biome of Brazil, where human cases of *R. parkeri*-caused spotted fever have been reported.

Keywords Dogs · Horses · *Rickettsia parkeri* · *Amblyomma ovale* · Northeast Brazil

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Introduction

The genus *Rickettsia* (Rickettsiaceae) contains gram-negative, obligatory intracellular bacteria that have been genetically classified into the following groups: (i) the spotted fever group (SFG), composed by tick-associated agents, including the human pathogens *Rickettsia rickettsii* and *Rickettsia parkeri*; (ii) the typhus group, composed by *Rickettsia prowazekii* and *Rickettsia typhi*, associated with lice and fleas; (iii) the transitional group, composed by agents associated mainly with fleas, mites and ticks; and (iv) the basal group, which includes agents associated with a variety of hosts such as ticks (e.g., *Rickettsia bellii*), leeches, insects, protozoa, and plants (Murray et al. 2016).

Rickettsia rickettsii, the most pathogenic SFG species, is the agent of Rocky Mountain spotted fever (RMSF) in the USA and Brazilian spotted fever (BSF) in Brazil (Parola et al. 2013). Human cases of BSF have been confirmed only in the Brazilian southeastern and southern regions, where *R. rickettsii* is transmitted to humans chiefly by *Amblyomma sculptum* (formerly *A. cajennense*) (Szabó et al. 2013a). Besides *R. rickettsii*, the only other SFG pathogenic agent known to occur in Brazil is *R. parkeri*, which is represented by two strains in the country: *R. parkeri* sensu stricto, associated with *Amblyomma triste* and *A. tigrinum* ticks, and *R. parkeri* strain Atlantic rainforest, primarily associated with *A. ovale* (Faccini-Martínez et al. 2018; Nieri-bastos et al. 2018). Recent reports indicate that *R. parkeri* strain Atlantic rainforest is an emerging agent of spotted fever in Brazil, with three laboratory-confirmed cases, each in the states of São Paulo (Spolidorio et al. 2010), Bahia (Silva et al. 2011), and Santa Catarina (Krawczak et al. 2016a). These three cases were epidemiologically associated with *R. parkeri*–infected *A. ovale* ticks. Moreover, recent reports of *R. parkeri*–infected *A. ovale* ticks in several other regions of Brazil suggest that human cases caused by this agent might have gone undiagnosed (Faccini-Martínez et al. 2018).

Since horses and dogs are major domestic hosts for *A. sculptum* and *A. ovale* in rural areas of Brazil, these two animals are considered suitable sentinels for the infection by *R. rickettsii* and *R. parkeri*, respectively (Sangioni et al. 2005; Barbieri et al. 2014). Herein, we performed a serosurvey in horses and dogs of one area of the Atlantic rainforest biome of Brazil, where no studies on tick-borne diseases had been done. In addition, we collected ticks from these domestic animals and tested them for rickettsial infection. Our results highlight a new endemic area for the Atlantic rainforest strain of *Rickettsia parkeri* in Brazil.

Material and methods

Study area

A survey was conducted from March to June, 2016 in the rural area of Ilhéus municipality, state of Bahia, within the Central Corridor of the Atlantic rainforest biome in the Brazilian coast (Fig. 1). Ilhéus is located in the meso-region of southern Bahia. It has a humid tropical climate and is located in the ecoregion of the coastal forests of Bahia in the Atlantic rainforest Biome. This region has an annual rainfall ranging from 1000 to 2700 mm and an average annual temperature of 25 °C. Besides the urban area, Ilhéus is divided into 10 rural administrative areas that are known as districts.

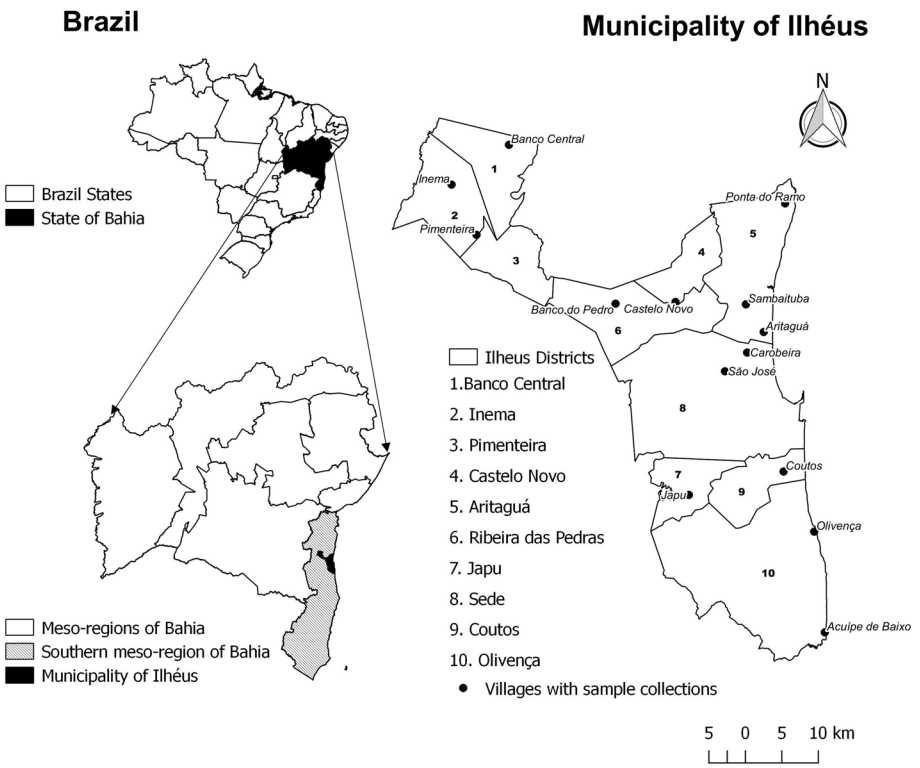


Fig. 1 Location of the municipality of Ilhéus, Bahia state, Brazil and the districts and villages that were investigated in the present study from March to June 2016.

Dog and horse sampling

Sourceforge® software was used to calculate the canine sample taking into account the canine population as 10% of the rural population, totaling 2895 animals (Cifuentes 1988), 95% confidence interval, a 50% prevalence of seropositive dogs, and 5% error. Based on these premises, we sampled 12% dogs in each of the 10 districts, totaling 356 owned dogs, regardless of age, breed or gender. Horse sampling was done by convenience due to the resistance of the owners to participate in the survey. Handling of dogs and horses was approved by the animal research ethics committee of the State University of Santa Cruz, state of Bahia, protocol 038/2015.

Blood samples were collected by vein puncture of dogs and horses in 4.0 mL-vacuum tubes without anticoagulant and disposable needles. The tubes were kept under refrigeration (− 4 °C) for 1 day, then centrifuged at 2500 rpm/15 min to obtain the sera, which were conditioned in microtubes and stored at − 20 °C until tested.

Each sampled dog was carefully inspected for 10 min for the presence of attached ticks, which were removed with tweezers, placed in vials containing absolute ethanol and kept at − 20 °C until taxonomic identification in the laboratory. Ticks were identified according to Clifford and Anastos (1960) for larvae, Martins et al. (2010) for nymphs, and Barros-Battesti et al. (2006) and Martins et al. (2016) for adults.

Serological analysis

Serum samples from dogs and horses were processed through indirect immunofluorescence assay (IFA) using antigens of four rickettsial isolates from Brazil: three SFG species, *R. rickettsii* strain Taiaçu (Pinter and Labruna 2006), *R. parkeri* strain At24 (Silveira et al. 2007), *Rickettsia amblyommatis* (published as *Rickettsia amblyommii*) strain Ac37(Labruna et al. 2004); and one basal group species, *Rickettsia bellii* strain Mogi (Pinter and Labruna, 2006), as previously described (Labruna et al. 2007). Briefly, Sera were diluted in PBS buffer (0.1 M, pH 7.2) starting from the 1:64 dilution. The slides were incubated with anti-dog or anti-horse IgG secondary antibody (Sigma Diagnostics, St. Louis, MO, USA) coupled with fluorescein isothiocyanate at 1:1000 and 1:1200 dilutions for sera from dogs and horses, respectively. Sera reacting at the 1:64 dilution were titrated at two-fold increments to determine the endpoint titer to each of the four *Rickettsia* antigens. Sera showing antibody titers to a *Rickettsia* species at least fourfold higher than those observed for the other *Rickettsia* species were supposed to be homologous to the first *Rickettsia* species or to a very closely related genotype (Piranda et al. 2008; Saito et al. 2008; Ueno et al. 2016). In each slide, previously tested non-reactive serum (negative control) and a known positive serum (positive control) from the studies of Piranda et al. (2008) and Ueno et al. (2016) were tested at the 1:64 dilution.

Molecular analysis of rickettsiae in ticks

One tick of each species per animal was individually processed for DNA extraction using the guanidine isothiocyanate technique (Sangioni et al. 2005) and tested by the polymerase chain reaction (PCR) technique using the primers CS-78 and CS-323, which amplify a 401-bp fragment of the citrate synthase gene (*gltA*), common to the genus *Rickettsia* (Labruna et al. 2004b). Positive samples for the *gltA* gene were subjected to a new PCR using primers Rr190.70p and Rr190.701n, which amplify a ≈ 532-bp fragment of the rickettsial 190-kDa outer membrane protein gene (*ompA*), common to *Rickettsia* species of the spotted fever group (Regnery et al. 1991). Each PCR run included negative (ultrapure water) and positive (*R. parkeri* strain NOD) controls. Amplicons generated from the tested ticks were purified with PureLink® kit (Invitrogen Life Technologies) and submitted to DNA sequencing in an ABI automated sequencer (Applied Biosystems/Thermo Fisher Scientific, model ABI 3500 Genetic Analyzer, Foster City, California, USA) with the same primers used for PCR. The sequences obtained were analyzed with the ChromasPro 1.5 (TechnelysiumPtyLtd., Tewantin, Qld, Australia) software, and analyzed for similarity by Blast analysis (www.ncbi.nlm.nih.gov/blast).

Data analysis

The 10 districts were categorized in a dichotomous level for the presence or absence of *A. ovale* ticks in the sampled dogs. Then, we analyzed the association of canine seroreactivity to *Rickettsia* spp. with the presence of *A. ovale* in the sampled district by the chi-square test ($\alpha = 0.05$). Canine serological results were analyzed at four conditions: sera reactive to *Rickettsia* spp. at the 1:64 dilution; sera reactive to *R. parkeri* at the 1:64 dilution;

sera reactive to *R. bellii* at the 1:64 dilution; or sera reactive to *R. parkeri* with endpoint titer ≥ 256. Analyses were performed using the Minitab Release 18 program.

Results

Serological analysis

A total of 356 dogs from 10 districts (13 to 75 dogs per district) were tested by IFA for four *Rickettsia* species. Overall, 16.4% (58/356) of the dogs were seroreactive (titer ≥ 64) to at least one *Rickettsia* species. A total of 34 (9.6%), 34 (9.6%), 20 (5.6%), and 19 (5. 3%) dogs were seroreactive to *R. parkeri, R. bellii, R. amblyommatis*, and *R. rickettsii*, respectively (Table 1). Sixteen (4.5%) canine sera from five districts showed titers to *R. parkeri* at least fourfold higher than those to any of the other three antigens. The antibody titers in these 16 dogs were considered to have been stimulated possibly by *R. parkeri* (Table 1). Based on these criteria, seven sera were considered to have been stimulated by *R. bellii* or a closely related agent, and one serum was considered to have been stimulated possibly by *R. amblyommatis*. For the remaining 34 seroreactive dogs, it was not possible to discriminate the infection agent, because they displayed similar titers (< fourfold difference) for two or

Table 1 Results of indirect IFA for four *Rickettsia* species in dogs from 10 districts of Ilhéus Municipality, state of Bahia, Brazil

District	No. sera tested	No. seroreactive dogs to each of the *Rickettsia* species (% seroreactivity per district)				PAIHR (No. dogs)
		R. rickettsii	*R. parkeri*	*R. amblyommatis*	*R. bellii*	
Arituguá	74	3 (4.05)	4 (5.4)	1 (1.35)	1 (1.35)	*R. parkeri* (1)
Banco Central	20	0 (0)	0 (0)	0 (0)	3 (15.0)	*R. bellii* (2)
Japu	16	2 (12.5)	4 (25.0)	3 (18.75)	4 (25.0)	*R. parkeri* (2)
Inema	19	0 (0)	0 (0)	0 (0)	2 (10.5)	*R. bellii* (1)
Pimenteiras	13	0 (0)	1 (7.7)	0 (0)	2 (15.4)	*R. parkeri* (1) *R. bellii* (2)
São José	56	4 (7.14)	12 (21.43)	5 (8.9)	8 (14.3)	*R. parkeri* (8) *R. bellii* (2)
Olivença	75	8 (10.6)	11 (14.6)	7 (9.3)	5 (6.6)	*R. parkeri* (4)
Rio do Braço	32	0 (0)	0 (0)	0 (0)	5 (15.62)	0
Castelo Novo	25	2 (8.0)	2 (8.0)	4 (16.0)	2 (8.0)	*R. amblyommatis* (1)
Coutos	26	0 (0)	0 (0)	0 (0)	2 (13.0)	0
Total	356	19 (5.33)	34 (9.55)	20 (5.61)	34 (9.55)	*R. parkeri* (16) *R. bellii* (7) *R. amblyommatis* (1)

The likelihood of a homologous reaction was determined when an endpoint titer to a *Rickettsia* species was at least fourfold higher than those observed for the other *Rickettsia* species. In this case, the *Rickettsia* species involved in the highest endpoint titer was considered the PAIHR

PAIHR possible antigen involved in a homologous reaction

more *Rickettsia* species or had a single titer of 64 for a single *Rickettsia* species. IFA titers for either *R. parkeri* or *R. bellii* varied from 64 to 1024, whereas IFA titers for either *R. rickettsii* or *R. amblyommatis* varied from 64 to 512.

A total of 69 horses from 10 districts (four to 14 horses per district) were tested by IFA for four *Rickettsia* species. Overall, 24.6% (17/69) of the horses were seroreactive (titer ≥ 64) to at least one *Rickettsia* species. A total of 15 (21.7%), six (8.7%), four (5.8%), and three (4. 3%) horses were seroreactive to *R. bellii, R. parkeri, R. rickettsii*, and *R. amblyommatis*, respectively (Table 2). Six (8.7%) horse sera from five districts showed titers to *R. bellii* at least fourfold higher than those to any of the other three antigens. The antibody titers in these six horses were considered to have been stimulated by *R. bellii* or a very closely related agent (Table 2). One serum was considered to have been stimulated possibly by *R. parkeri*. The remaining ten seroreactive horses did not show ≥ fourfold difference in titers between agents, precluding a discrimination of the possible involved agent. IFA titers ranged from 128 to 512 for *R. parkeri*, 128 to 256 for *R. amblyommatis*, and from 64 to 512 for either *R. rickettsii* or *R. bellii*.

Ticks and molecular analyses

Among 356 examined dogs, 242 (68.0%) were found infested by ticks. A total of 1344 ticks (616 males, 552 females, 169 nymphs, seven larvae) were collected from dogs, and were identified into five species: *Rhipicephalus sanguineus* sensu lato (s.l.) (1133 adults,

Table 2 Results of indirect IFA for four *Rickettsia* species in horses from 10 districts of Ilhéus Municipality, state of Bahia, Brazil

District	No. sera tested	No. seroreactive horses to each of the *Rickettsia* species (% seroreactivity per district)				PAIHR (No. horses)
		R. rickettsii	*R. parkeri*	*R. amblyommatis*	*R. bellii*	
Arita guá	5	0 (0)	0 (0)	0 (0)	0 (0)	0
Banco Central	7	0 (0)	0 (0)	0 (0)	0 (0)	0
Japu	5	0 (0)	0 (0)	0 (0)	0 (0)	0
Inema	5	1 (20.0)	1 (20.0)	0 (0)	3 (60.0)	0
Pimenteiras	6	0 (0)	0 (0)	0 (0)	1 (16.7)	*R. bellii* (1)
São José	4	0 (0)	1 (25.0)	1 (25.0)	1 (25.0)	*R. bellii* (1)
Olivença	7	0 (0)	0 (0)	0 (0)	2 (28.6)	*R. bellii* (2)
Rio do Braço	9	1 (11.1)	1 (11.1)	0 (0)	2 (22.2)	*R. bellii* (1)
Castelo Novo	14	2 (14.3)	2 (14.3)	1 (7.1)	2 (14.3)	*R. parkeri* (1) *R. amblyommatis* (1)
Coutos	7	0 (0)	1 (14.3)	1 (14.3)	4 (57.1)	*R. bellii* (1)
Total	69	4 (5.8)	6 (8.7)	3 (4.3)	15 (21.7)	*R. parkeri* (1) *R. bellii* (6) *R. amblyommatis* (1)

The likelihood of a homologous reaction was determined when an endpoint titer to a *Rickettsia* species was at least fourfold higher than those observed for the other *Rickettsia* species. In this case, the *Rickettsia* species involved in the highest endpoint titer was considered the PAIHR

PAIHR possible antigen involved in a homologous reaction

134 nymphs, three larvae), *A. ovale* (27 adults, one nymph), *A. sculptum* (two adults, 29 nymphs), *Rhipicephalus microplus* (six adults, four nymphs), and *Amblyomma naponense* (one nymph). Four larvae could not be identified to species level and were retained as *Amblyomma* spp. *Rhipicephalus sanguineus* s.l. was the most frequent canine tick, found on 46.1 to 93.7% of the dogs of the 10 districts (Table 3). The second most frequent tick species was *A. sculptum*, present in eight districts with prevalence values ranging from 1.3 to 15.8%. *Amblyomma ovale* was found in six districts, where 5.3 to 14.6% of the dogs were infested. The remaining two tick species, *R. microplus* and *A. naponense*, were found in dogs of only 2 and 1 districts, respectively (Table 3).

Among 69 examined horses, 62 (89.9%) were found infested by ticks. A total of 398 (393 adults, five nymphs) were collected from horses, and were identified into three species: *A. sculptum* (363 adults, one nymph), *Dermacentor nitens* (29 adults, four nymphs), and *A. ovale* (one adult). *Amblyomma sculptum* was the most frequent tick, found on 75.0 to 100% of the horses of the 10 districts (Table 4). The second most frequent tick species was *D. nitens*, present in eight districts with prevalence values ranging from 7.1 to 50.0%. *Amblyomma ovale* was found only on one horse (Table 4).

A total of 242 ticks from dogs were analyzed by PCR targeting rickettsiae. This sample consisted of at least one individual tick per every infested dog, and included 239 adults (two *A. sculptum*, 27 *A. ovale*, 210 *R. sanguineus*), and 3 nymphs (two *R. microplus*, one *A. naponense*). Rickettsiae were detected in only 4/27 (14.8%) adults of *A. ovale*. The *gltA* (350-bp) and *ompA* (511-bp) partial sequences of the four *A. ovale* ticks matched 100% identity with corresponding sequences of *R. parkeri* strain Atlantic rainforest from GenBank (JQ906783 and MF536975, respectively). A total of 72 ticks (62 *A. sculptum*, 10 *D. nitens*) from horses were tested by PCR; however, no rickettsial DNA was detected.

Statistical associations

The presence of *A. ovale* in the district was positively associated with local canine seroreactivity to *R. parkeri*, either at the 1:64 serum dilution ($P = 0.001$) or at endpoint titer ≥ 256 ($P < 0.001$). There was no significant association between *A. ovale* presence and canine seroreactivity to *Rickettsia* spp. ($P = 0.34$) or *R. bellii* ($P = 0.51$).

Discussion

Dogs living in all 10 rural districts of Ilhéus were sampled in the present study. Our serological results indicate that at least 16 dogs from five districts have been exposed to *R. parkeri* infection, a condition corroborated by our findings that 14.8% of the *A. ovale* ticks from dogs were infected by *R. parkeri* strain Atlantic rainforest. Moreover, canine seroreactivity to *R. parkeri* was significantly associated with the presence of *A. ovale* on dogs in the district. Altogether, our results provide evidence for the transmission of *R. parkeri* strain Atlantic rainforest from *A. ovale* to domestic dogs within the rural area of Ilhéus, just like it has been reported in several other areas of the Atlantic rainforest biome of Brazil, where human cases of *R. parkeri*-caused spotted fever have been reported (Szabó et al. 2013b; Barbieri et al. 2014; Nieri-bastos et al. 2016). Therefore, the present study appoints to a clear likelihood of the occurrence of human cases of spotted fever in the rural area of

Table 3 Ticks of dogs from 10 districts of Ilhéus Municipality, state of Bahia, Brazil

Districts (no. examined dogs)	No. infested dogs (% infested dogs) – total no. ticks collected					
	Amblyomma ovale	*A. sculptum*	*A. naponense*	*Rhipicephalus sanguineus* s.l.	*R. microplus*	*Amblyomma* spp
Aritaguá (74)	8 (10.8) – 15	0 (0) – 0	0 (0) – 0	46 (62.1) – 177	2 (2.7) – 7	0 (0) – 0
Banco Central (20)	0 (0) – 0	2 (10.0) – 2	0 (0) – 0	15 (75.0) – 83	0 (0) – 0	0 (0) – 0
Japu (16)	2 (12.5) – 2	1 (6.2) – 1	0 (0) – 0	15 (93.7) – 102	0 (0) – 0	1 (6.2) -1
Inema (19)	0 (0) – 0	3 (15.8) – 3	0 (0) – 0	14 (73.7) – 66	1 (5.2) – 1	0 (0) – 0
Pimenteiras (13)	0 (0) – 0	2 (15.4) – 7	0 (0) – 0	6 (46.1) – 20	1 (7.7) – 2	0 (0) – 0
São José (56)	3 (5.3) – 8	1 (1.8) – 1	0 (0) – 0	42 (75.0) – 64	0 (0) – 0	0 (0) – 0
Olivença (75)	11 (14.6) – 27	1 (1.3) – 2	0 (0) – 0	57 (76) – 304	0 (0) – 0	0 (0) – 0
Rio do Braço (32)	1 (3.1) – 1	5 (15.6) – 10	1 (3.1) -1	22 (68.7) – 185	0 (0) – 0	1 (3.1) – 3
Castelo Novo (25)	2 (8.0) – 2	2 (8.0) – 5	0 (0) – 0	12 (46.1) – 63	0 (0) – 0	0 (0) – 0
Coutos (26)	0 (0) – 0	0 (0) – 0	0 (0) – 0	12 (46.1) – 60	0 (0) – 0	0 (0) – 0
TOTAL (356)	26 (7.3) – 28	17 (4.7) – 31	1 (0.3) – 1	241 (67.7) – 1270	4 (1.1) – 10	2 (0.5) – 4

Table 4 Ticks of horses from 10 districts of Ilhéus Municipality, state of Bahia, Brazil

Districts (no. examined horses)	No. infested horses (% infested horses) – total no. ticks collected		
	Amblyomma sculptum	*Dermacentor nitens*	*A. ovale*
Aritaguá (5)	5 (100) – 33	1 (20) – 12	0 (0) – 0
Banco Central (7)	6 (85.7) – 33	1 (14.3) – 1	0 (0) – 0
Japu (5)	5 (100) – 29	0 (0) – 0	0 (0) – 0
Inema (5)	5 (100) – 29	1 (20) – 6	0 (0) – 0
Pimenteiras (6)	6 (100) – 22	1 (16.6) – 1	0 (0) – 0
São José (4)	3 (75) – 13	2 (50) – 4	0 (0) – 0
Olivença (7)	6 (100) – 48	2 (28.5) – 5	0 (0) – 0
Rio do Braço (9)	8 (88.8) – 33	1 (11.1) – 2	0 (0) – 0
Castelo Novo (14)	11 (78.5) – 83	1 (7.1) – 2	1 (7.1) – 1
Coutos (7)	7 (100) – 41	0 (0) – 0	0 (0) – 0
TOTAL (69)	62 (89.8) – 364	10 (14.5) – 33	1 (1.4) – 1

Ilhéus, a condition that must be informed to local health authorities, since spotted fever has never been reported in the Ilhéus region.

One of the three confirmed cases of *R. parkeri*-caused spotted fever that have been reported in Brazil were in Bahia state, in an Atlantic rainforest fragment ≈ 400 Km distant from Ilhéus (Silva et al. 2011; Nieri-bastos et al. 2016). Interesting, an earlier clinical case, based primarily on seroconversion to SFG antigens, was reported 40 years ago in another area of Bahia state, ≈ 200 Km distant from Ilhéus (Plank et al. 1979). While the *Rickettsia* species was not confirmed in this earlier case, the clinical profile was compatible with *R. parkeri* rickettsiosis, namely the presence of an inoculation eschar at the tick bite site, mild fever, myalgia, and rash. Thus, it is highly possible that human cases of *R. parkeri* spotted fever have been under reported in the Bahia state, where the epidemiological scenario of its Atlantic rainforest biome is permissive for human infection via the bite of *A. ovale*, a proven vector of this rickettsial agent (Krawczak et al. 2016b).

The most frequently reported spotted fever illness of Brazil is BSF, caused by *R. rickettsii* and transmitted chiefly by *A. sculptum* (Szabó et al. 2013a). While *A. sculptum* was the most common tick on horses in the present study, none of these animals presented serological evidence for exposure to *R. rickettsii*-infected ticks. Considering that horses are the most suitable domestic sentinel for BSF in areas of transmission by *A. sculptum* (Sangioni et al. 2005), our results are indicative that the rural area of Ilhéus did not present favorable conditions for the occurrence of BSF.

The SFG agent *R. amblyommatis* was reactive to much less dogs and horses than *R. parkeri* was, suggesting that these domestic animals were not commonly exposed to *R. amblyommatis*-infected ticks in the study area. Even though one dog and one horse presented serological evidence of *R. amblyommatis* exposure, this agent was not found in the tested ticks. In previous studies in which *R. amblyommatis* was detected in ticks from dogs and horses, many of these animals presented serological evidence of *R. amblyommatis* exposure(Bermúdez et al. 2011; Costa et al. 2017). Therefore, the low seropositivity rate to *R. amblyommatis* in this study could be related to a possible low infection rate of this rickettsia in ticks of Ilhéus, which could include any of the tested tick species or even a tick species that was not found during our sampling period.

A high number of dogs and horses presented serological evidence of *R. bellii* exposure. While *R. bellii* has been reported as the most common *Rickettsia* species infecting ticks in Brazil (Labruna 2011; Krawczak et al. 2018), it was not found in any of our tested ticks, indicating that the high rates of *R. bellii* seroreactivity in the present study is not associated with tick transmission. Since *R. bellii* represents a rickettsial basal group that includes a variety of closely related agents infecting, leeches, insects, protozoa, or even plants (Murray et al. 2016), we cannot exclude the possibility that any of these other organisms could be related to the *R. bellii* high seropositivity in the present study, a condition yet to be investigated.

Conclusions

The results of this study, based on serology of sentinel animals and detection of rickettsial DNA in human-biting tick species, indicate that the rural area of Ilhéus is endemic for spotted fever caused by *R. parkeri*, but not by *R. rickettsii*. Human cases of *R. parkeri*-caused spotted fever have probably been unreported in the area.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interests.

Ethical approval All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted (CEUA/UESC protocol nº 038/2015).

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