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Original article

Epidemiology of *Rickettsia* sp. strain Atlantic rainforest in a spotted fever-endemic area of southern Brazil

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ABSTRACT

The present study was performed in Vila Itoupava, an area of the state of Santa Catarina, southern Brazil, in which a tick-borne spotted fever illness has been endemic since 2003. Notably, both the etiological agent and the vector of these spotted fever cases remain unknown. During January 2011, humans, domestic dogs, and their ticks were sampled in households that are typically surrounded by highly preserved Atlantic rainforest fragments. Ticks collected from dogs were *Amblyomma ovale* (34% prevalence), *Amblyomma aureolatum* (18.9%), and *Rhipicephalus sanguineus* (3.8%). A total of 7.8% (6/77) *A. ovale* and 9.3% (4/43) *A. aureolatum* were infected by *Rickettsia* sp. strain Atlantic rainforest, a *Rickettsia parkeri*-like agent recently shown to cause spotted fever illness in southeastern Brazil. Overall, 67.3% (35/52) of the dogs were seroreactive to spotted fever group rickettsiae, mostly with highest endpoint titers to *R. parkeri*. Among humans, 46.7% (7/15) reacted serologically to rickettsiae at low to moderate endpoint titers. Because canine seroreactivity to *R. parkeri* was strongly associated with frequent contact with forests (the preferred habitat for *A. ovale* and *A. aureolatum*), it is concluded that sampled dogs have been infected by strain Atlantic rainforest through the parasitism of these tick species. The present study provides epidemiological evidence that the spotted fever in the study area has been caused by *Rickettsia* sp. strain Atlantic rainforest, transmitted to humans by either *A. ovale* or *A. aureolatum*. Further studies encompassing direct diagnostic methods on clinical specimens from patients are needed to confirm the above epidemiological evidence.

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Introduction

Tick-borne rickettsioses are emerging or reemerging diseases caused by bacteria from to the spotted fever group of the genus *Rickettsia*, with worldwide distribution (Parola et al., 2013). Until 2004, the species *Rickettsia rickettsii*, the agent of Rocky Mountain spotted fever (RMSF) or Brazilian spotted fever (BSF), was the only tick-borne rickettsia known to occur in South America, where clinical cases are characterized by high fever, severe hemorrhagic manifestations, and high fatality rates (Labruna, 2009). During 2004–2008, the pathogen *Rickettsia parkeri* was reported in *Amblyomma triste* ticks in Uruguay, Brazil, and Argentina (Venzal et al., 2004; Silveira et al., 2007; Nava et al., 2008). Human cases of *R. parkeri*-caused spotted fever were subsequently reported in Uruguay and Argentina; these cases differed clinically from RMSF by presenting mild fever, inoculation eschar (*tache noire*), lymphadenopathy, and no fatalities (Conti-Díaz et al., 2009; Romer et al., 2011; Portillo et al., 2013).

In 2009, a novel rickettsial agent, *Rickettsia* sp. strain Atlantic rainforest, was reported causing illness in a patient in an Atlantic rainforest area of the state of São Paulo, southeastern Brazil (Spolidorio et al., 2010). In the subsequent year, a second clinical case caused by this novel agent was reported in the state of Bahia, northeastern Brazil (Silva et al., 2011). These two cases were characterized by mild fever, inoculation eschar, and lymphadenopathy, very similar to the clinical cases attributed by *R. parkeri* in Uruguay and Argentina. In fact, genetic analysis of strain Atlantic rainforest revealed it to be closest related to *R. parkeri, Rickettsia africae*, and *Rickettsia sibirica*, which are known to cause very similar clinical entities in different parts of the world (Parola et al., 2013). Two

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studies with ticks collected in Atlantic rainforest areas, one in the state of São Paulo (Sabatini et al., 2010), and one in the state of Santa Catarina, southern Brazil (Medeiros et al., 2011), reported strain Atlantic rainforest infecting the ticks *Amblyomma ovale, Amblyomma aureolatum*, and *Rhipicephalus sanguineus* collected from the environment and from domestic dogs. More recently, an epidemiological study performed in the same area of the strain Atlantic rainforest-caused human index case reported by Spolidorio et al. (2010) revealed that *A. ovale* was the most abundant tick species with 12.9% infection rate by strain Atlantic rainforest (Szabó et al., 2013). These authors incriminated *A. ovale* as the main vector of strain Atlantic rainforest in the state of São Paulo.

Clinically severe cases of BSF (caused by *R. rickettsii*) have been confirmed in the southeastern states of Brazil, with fatality rates varying from 20% to 44% (Del Fiol et al., 2010; Barros e Silva et al., 2014). In contrast, the state of Santa Catarina in southern Brazil had 270 cases of spotted fever from 2003 to 2012, with no fatality (Del Fiol et al., 2010; Barros e Silva et al., 2014). All these cases were laboratory-confirmed by seroconversion through the analysis of acute and convalescent serum samples tested against *R. rickettsii* antigens. Because serologic cross-reaction usually occurs between different SFG *Rickettsia* species (Parola et al., 2013), the *Rickettsia* species responsible for these clinical cases of Santa Catarina have remained undetermined. Angerami et al. (2009) performed a comparative study on the clinical cases of spotted fever in the state of São Paulo (where BSF-caused *R. rickettsii* is known to occur), and the state of Santa Catarina; the authors found marked clinical differences between the two states, suggesting that the milder cases of Santa Catarina were caused by an unknown spotted fever agent different from *R. rickettsii.*

Notably, the state of Santa Catarina accounted for the highest overall incidence (≈0.5 cases/100,000 habitants) of spotted fever rickettsiosis among all Brazilian states during the 2007–2012 year-period (Barros e Silva et al., 2014); however, both the *Rickettsia* species and the tick vector remain unknown in the endemic areas of Santa Catarina. In the present study, we visited an area of Santa Catarina that has been endemic for spotted fever during the last 10 years. Domestic animals and asymptomatic humans were blood sampled, and their ticks were examined for rickettsial infection.

Materials and methods

Study site

During January 2011, we visited a location called "Vila Itoupava" in Blumenau municipality, state of Santa Catarina, southern Brazil. Vila Itoupava is a suburban village inhabited by ≈1700 inhabitants, most of them living in houses surrounded by Atlantic rainforest areas (Fig. 1). From 2003 to 2010, 12 cases of spotted fever were confirmed in residents from Vila Itoupava (Godoy, 2011). Due to the irregular landscape of the region, livestock or crop activities are not common, what have precluded deforestation of the Atlantic rainforest cover of the region. Dogs encompass the most common (most of the time the sole) domestic animals in the area.

Sample collection

A total of 53 dogs from 25 households (mean: 2.1 dogs per household; range: 1–6) were examined for ticks, which were collected and brought alive to the laboratory for taxonomic identification following Barros-Battesti et al. (2006). All but one of these dogs were subjected to blood collection, from which serum was separated by centrifugation and stored frozen at −20 °C until serologic tests. From the same households of the sampled dogs, blood serum samples were obtained from 15 humans that agreed to be sampled. None of these 15 humans had a history of spotted fever illness.

Isolation and molecular characterization of rickettsiae from ticks

Right after arriving in the laboratory, live ticks were initially tested by the hemolymph test with Gimenez staining (Burgdorfer, 1970), and stored frozen at −80 °C. Ticks presenting *Rickettsia*-like organisms within hemocytes were processed by isolation of rickettsiae in Vero cell culture by the shell vial technique, as previously described (Labruna et al., 2004). A rickettsial isolate was considered established in Vero cell culture after at least three passages, each infecting >90% of the cells, determined by Gimenez staining as previously described (Labruna et al., 2004). In this case, a sample of infected cells from the third passage was submitted to DNA extraction and tested by a battery of PCRs to amplify fragments of the rickettsial genes citrate synthase (*gltA*) (primers CS-78, CS-323, CS-239, CS-1069), 17-kDa protein *(htrA*) (primers 17k-3, 17k-5), outer membrane protein (*ompB*) (primers 120-M59, 120–807) and *ompA* (primers Rr190.70p, Rr190.602n), as described (Regnery et al., 1991; Roux and Raoult, 2000; Labruna et al., 2004). PCR products were sequenced in an automatic sequencer (Applied Biosystems/Thermo Fisher Scientific, model ABI 3500 Genetic Analyser, Foster City, California) and subjected to BLAST analyses (www.ncbi.nlm.nih.gov/blast) to infer closest similarities to other *Rickettsia* species available in Genbank. The remaining ticks were thawed and submitted to DNA extraction by the guanidine isothiocyanate-phenol technique, and tested by the above mentioned-PCR protocols targeting the rickettsial genes *gltA* and *ompA*. The same procedure was done with remnants of ticks processed by the shell vial technique.

Serological analyses

Canine and human sera were tested by the indirect immunofluorescence assay (IFA) using crude antigens derived from 6 *Rickettsia* isolates from Brazil (*R. bellii* strain Mogi, *R. felis* strain Pedreira, *R. amblyommii* strain Ac37, *R. rhipicephali* strain HJ5, *R. rickettsii* strain Taiaçu, and *R. parkeri* strain At24), as previously described (Labruna et al., 2007). Briefly, sera were diluted in 2-fold increments with phosphate-buffered saline (PBS), starting from the 1:64 dilution. Slides were incubated with fluorescein isothiocyanate-labelled rabbit anti-dog IgG, and goat anti-human IgG (Sigma, St Louis, MO, USA) for canine, and human sera, respectively. For each sample, the endpoint IgG titer reacting with each of the 6 *Rickettsia* antigens was determined. An endpoint titer at least 4-fold higher for a *Rickettsia* species than that observed for any other *Rickettsia* species was considered probably homologous to the first *Rickettsia* species or to a very closely related species (Labruna et al., 2007). In each slide, a serum previously shown to be non-reactive (negative control) and a known reactive serum (positive control) were tested at the 1:64 dilution.

Data analysis

Prevalence and mean intensity of tick infestation on main host species were determined according to the method of Bush et al. (1997). 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. For statistical analysis, seroreactivity to *Rickettsia* spp. was analyzed qualitatively at two dichotomous levels (0: non-reactive at the 1:64 dilution or non-reactive at the 1:1024 dilution; 1: endpoint titer ≥64 or endpoint titer ≥1024, respectively). First, the independent variables were subjected to univariate analysis, and those with statistical association ($P < 0.20$, Chi-square or Fisher

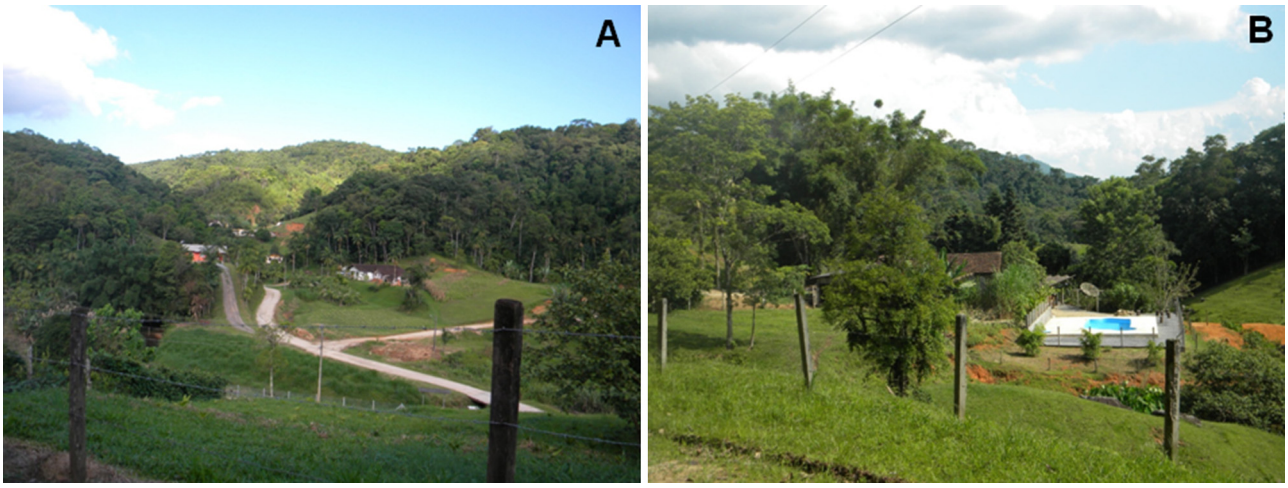


Fig. 1. Typical households in the suburban of Vila Itoupava, southern Brazil, where the irregular landscape is covered by Atlantic rainforest fragments. (A) Households where dogs were sampled in the present study. (B) A household where a person had a laboratory-confirmed case of spotted fever.

exact test) were selected to be tested in the multivariate model by the stepwise forward method. The variables were included in the multivariate model if they displayed statistical significance of $P < 0.05$. The odds ratio (OR) was calculated for the independent variable that showed statistical significance ($P < 0.05$) in the multivariate analysis. The same procedure was repeated considering canine seroreactivity solely to *R. parkeri*. All analyses were performed using SPSS for Windows 16.0 (Bakersfield, CA, USA).

Ethical statements

This work has been previously approved by the Ethical Committee of Animal Use of the Faculty of Veterinary Medicine of the University of São Paulo (protocol 1975/2010), and the Ethical Committee of Human Research of the Institute of Biomedical Sciences of the University of São Paulo (protocol 984/CEP-2010).

Results

Ticks and rickettsial infection

A total of 153 adult ticks (no subadult tick was found) were collected from dogs. *Amblyomma ovale* was the most prevalent species (34% prevalence), which also showed the highest mean intensity and mean abundance values (Table 1). *Amblyomma aureolatum* was the second most prevalent (18.9%), followed in a much lesser extent by *R. sanguineus* (3.8%). Rickettsial infection was evaluated individually in 125 ticks (77 *A. ovale*, 43 *A. aureolatum*, 5 *R. sanguineus*).

Table 1
Ticks collected from dogs in Vila Itoupava, state of Santa Catarina, southern Brazil.

	Amblyomma ovale	*Amblyomma aureolatum*	*Rhipicephalus sanguineus*
Total no. ticks	95	52	6
No. infested dogs (prevalence %)	18 (34.0)	10 (18.9)	2 (3.8)
Mean intensity	5.3	5.2	3.0
Mean abundance	1.8	1.0	0.06
No. ticks infected with *Rickettsia*[+]	6	4	0
Prevalence of rickettsial infection (%)[*]	7.8	9.3	0

[+] Infection by *Rickettsia* sp. strain Atlantic rainforest.
[*] Prevalence of *Rickettsia* sp. strain Atlantic rainforest was based on 77, 43, and 5. *A. ovale*, *A. aureolatum*, and *R. sanguineus* ticks, respectively that were tested by PCR.

Because the remaining 28 ticks arrived dead in the laboratory, they were not processed and were deposited as voucher specimens in the tick collection "Coleção Nacional de Carrapatos" under the accession number CNC-1781. A total of 6 (7.8%) *A. ovale* and 4 (9.3%) *A. aureolatum* were infected by rickettsiae. In all cases, DNA sequencing of PCR products revealed that these ticks were infected by *Rickettsia* sp. strain Atlantic rainforest, since their *gltA* and *ompA* partial sequences matched 100% with corresponding sequences of strain Atlantic rainforest (GenBank accession numbers GQ855235 and JQ906785, respectively).

Among the above PCR-positive ticks, attempts to isolate rickettsiae by the shell vial technique were performed on 4 *A. ovale* and 4 *A. aureolatum*, which had showed by the hemolymph test to contain *Rickettsia*-like organisms within their hemocytes. While rickettsiae were successfully isolated from all of them, six isolates were lost during first or second passage due to contamination with fungi or extracellular bacteria. Therefore, only two isolates (from two *A. aureolatum* ticks) were successfully established in the laboratory with several passages, each one reaching >90% infection of the cells. These isolates, designated as strain Aa46 and strain Aa47, have been cryopreserved in our rickettsial collection. DNA extracted from the third passage of strains Aa46 and Aa47 were processed by PCR, resulting in partial sequences of the rickettsial genes *gltA* (1077-bp), *htrA* (497-bp), *ompB* (789-bp), and *ompA* (590-bp). By BLAST analyses, the *gltA*, *ompB*, and *ompA* sequences matched 100% to corresponding sequences of *Rickettsia* sp. strain Atlantic rainforest (GQ855235, JQ906785, and JQ906784, respectively). Because there is no *htrA* corresponding sequence of strain Atlantic rainforest available in GenBank, the present *htrA* sequences, identical to each other, matched closest (99%) to corresponding sequences of various spotted fever group rickettsiae, including *R. rickettsii* (493/497-bp; CP003305), *R. parkeri* (492/497-bp; CP003341), *R. africae* (491/497-bp; CP001612), and *R. sibirica* (492/498-bp; AF445384). The *gltA*, *htrA*, *ompA*, and *ompB* partial sequences generated in the present study have been deposited in GenBank under the accession numbers KJ855083–KJ855086.

Serology

Overall 67.3% (35/52) of the dogs from 20 households reacted to at least 1 rickettsial antigen. Generally, canine endpoint titers were highest against *R. parkeri*, with 19 animals displaying endpoint titers ≥1024, and 2 dogs with endpoint titer 32,768. It was found that 15 dogs had endpoint titers to *R. parkeri* at least 4-fold

Table 2
Results of indirect immunofluorescence assay for six *Rickettsia* species in 52 dogs and 15 humans from Vila Itoupava, southern Brazil.

Dogs ($N=52$) Humans ($N=15$)	*Rickettsia* antigen[+]						
	Rp	*Rr*	*Ra*	*Rrh*	*Rf*	*Rb*	*R* spp
No. reactive dogs (%)	30 (57.7)	26 (50.0)	25 (48.1)	23 (44.2)	14 (26.9)	13 (25.0)	35 (67.3)
No. dogs with endpoint titer ≥1024 (%)	19 (36.5)	14 (27.0)	5 (9.6)	8 (15.3)	0 (0)	0 (0)	22 (42.3)
No. dogs with PAIHR (%)[*]	15 (28.8)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	
No. reactive humans (%)	4 (26.7)	4 (26.7)4	3 (20.0)	6 (40.0)	2 (13.3)	1 (6.7)	7 (46.7)

[+] *Rp: R. parkeri; Rr: R. rickettsii; Ra: R. amblyommii; Rrh: R. rhipicephali; Rf: R. felis; Rb: R. bellii; R* spp*; Rickettsia* spp.
[*] A homologous reaction was determined when an endpoint titer to a *Rickettsia* species was at least 4-fold higher than those observed for the other *Rickettsia* species. In this case, the *Rickettsia* species (or a very closely related species) involved in the highest endpoint titer was considered the possible antigen involved in a homologous reaction (PAIHR).

Table 3
Dichotomous categories of each independent variable used in the univariate analysis with seroreactivity to *Rickettsia* spp or solely *Rickettsia parkeri*, and their corresponding *P* values for statistical association by the χ^2 test or Fisher's Exact test.

Independent variables	Code 0 [No. dogs (%)]	Code 1 [No. dogs (%)]	*P* values for seroreactivity to *Rickettsia* spp.		*P* values for seroreactivity to *Rickettsia parkeri*	
			Titer ≥ 64	Titer ≥ 1024	Titer ≥ 64	Titer ≥ 1024
Canine age	≤2 years old [23 (44.2)]	>2 years old [29 (55.8)]	0.140[*]	0.123[*]	0.065[*]	0.102[*]
Canine breed	Crossbreed [42 (80.8)]	Pure [10 (19.2)]	0.062[*]	>0.20	>0.20	>0.20
Time living in the area	≤12 months [22 (42.3)]	>12 months [30 (57.7)]	0.093[*]	0.014[*]	0.126[*]	0.046[*]
Tick parasitism (past and present)	No [19 (36.5)]	Yes [33 (63.5)]	0.087[*]	0.019[*]	0.004[*]	0.002[*]
Frequent contact with forests	No [34 (65.4)]	Yes [18 (34.6)]	0.002[*]	<0.001[*]	0.001[*]	<0.001[*]

[*] Variable selected for the multivariate analysis (see text and Table 4).

higher than those observed for the other five *Rickettsia* species, indicating a possible homologous reaction to *R. parkeri* or closely related species (Table 2). Among humans, 46.7% (7/15) of the samples from 5 households reacted to at least 1 rickettsial antigen, with highest endpoint titer varying from 256 to 512 for *R. parkeri, R. rickettsii*, and *R. amblyommii;* no human serum showed ≥4-fold difference between the highest endpoint titers, precluding any inference on a possible homologous reaction.

Data analysis

By univariate analysis, the dependent variable canine seroreactivity for *Rickettsia* spp. or solely for *R. parkeri* (with endpoint titer either ≥64 or ≥1024) was statistically coupled ($P<0.20$) with all independent variables (except for "canine breed" in some cases) listed in Table 3. However, when these independent variables were submitted for multivariate analysis, only the variable "frequent contact with forests" was significantly associated ($P<0.05$) with canine seroreactivity for *Rickettsia* spp. or solely for *R. parkeri* (Table 4). Dogs that had frequent contact with forests were 15.1 times or 19.3 times more likely to be seroreactive for *Rickettsia* spp. at endpoint titers ≥64 or ≥1024, respectively, than dogs that had no frequent contact with forests. Similarly, dogs that had frequent contact with forests were 11.4 times or 16.3 times more likely to be seroreactive for *R. parkeri* at endpoint titers ≥64 or ≥1024, respectively, than dogs that had no frequent contact with forests. The r^2 values in these analyses ranged from 26.5 to 40.7% (Table 4). In each analysis, the r^2 value means the percentage of seropositive dogs that are explained by the independent variable; in all cases, the frequent contact with forests.

Discussion

The present study was performed in Vila Itoupava, an area of the state of Santa Catarina that has been endemic for tick-borne spotted fever since 2003, possibly under the highest spotted fever incidence of Brazil; 70.8 cases/10,000 habitants (Godoy, 2011). Notably, both the etiological agent and the vector of these cases remain unknown. Despite that the spotted fever cases of Santa Catarina were laboratory confirmed through seroconversion (between acute

Table 4
Results of multivariate analysis of canine seroreactivity to *Rickettsia* spp. or *Rickettsia parkeri* (endpoint titers ≥64 or ≥1024) with the independent variables selected in the univariate analysis ($P<0.20$), as shown in Table 3.

Independent variable significant ($P<0.05$) in the final model	r^2	*P*-value	OR (odds ratio)	95% confidence interval	
				Lower	Upper
	Dependent variable: seroreactivity to *Rickettsia* spp (titer ≥64)				
Frequent contact with forests	26.5%	0.012	15.110	1.803	126.682
	Dependent variable: seroreactivity to *Rickettsia* spp (titer ≥1024)				
Frequent contact with forests	43.0%	<0.001	19.286	4.335	85.789
	Dependent variable: seroreactivity to *R. parkeri* (titer ≥64)				
Frequent contact with forests	28.2%	0.003	11.429	2.260	57.801
	Dependent variable: seroreactivity to *R. parkeri* (titer ≥1024)				
Frequent contact with forests	40.7%	<0.001	16.333	3.954	67.473

and convalescent sera) to *R. rickettsii* antigens, because of the clinical mildness (no fatality) and the occurrence lymphadenopathy in 40–50% of the cases, it has been proposed that the disease in Santa Catarina is caused by a unknown spotted fever group agent different from *R. rickettsii* (Angerami et al., 2009; Godoy, 2011).

In the present study, it was observed that the houses of Vila Itoupava are surrounded by highly preserved Atlantic rainforest fragments, where livestock are generally not raised, and domestic dogs represent the most abundant (most of the times the sole) domestic animals. This scenario has favored infestation of dogs with *A. ovale* and *A. aureolatum* ticks within the forest environment, ideal for developmental of non-parasitic stages and with an abundant host supply, passerines and small mammals for immature ticks, and carnivores for adult ticks (Ogrzewalska et al., 2012; Szabó et al., 2013). Of course, to allow infestation, it is needed that domestic dogs enter the forest frequently, a condition reported for at least 34.6% of the sampled dogs (Table 3). We found that 7.8–9.3% of the *A. ovale* and *A. aureolatum* ticks collected from dogs were infected by *Rickettsia* sp. strain Atlantic rainforest, a novel pathogen very closely related to *R. parkeri* (Spolidorio et al., 2010). Because canine seroreactivity to *R. parkeri* was strongly associated with frequent contact with forest, it is concluded that these dogs have been infected by strain Atlantic rainforest through the parasitism by *A. ovale* and/or *A. aureolatum* infected ticks.

Amblyomma ovale has been implicated as the vector of strain Atlantic rainforest to humans in the state of São Paulo, where the index case of the spotted fever illness caused by this agent was described from an area in which 12.9% of the *A. ovale* ticks collected from dogs were found to be infected (Szabó et al., 2013). Herein, besides *A. ovale*, strain Atlantic rainforest was found infecting *A. aureolatum* ticks. Similar results were previously reported for other areas of Santa Catarina (Medeiros et al., 2011). Interestingly, the infection by strain Atlantic rainforest was detected in *A. aureolatum* only when this tick occurred together with *A. ovale* on the same canine population (Medeiros et al., 2011; present study). While this rickettsial agent is found in *A. ovale* ticks from areas that lack *A. aureolatum*, it was never reported in *A. aureolatum* ticks from areas where *A. ovale* was absent (Sabatini et al., 2010; Medeiros et al., 2011; Ogrzewalska et al., 2012; Szabó et al., 2013). A similar scenario has been observed for *R. sanguineus*, which was infected by strain Atlantic rainforest only when the dogs were also infested by *A. ovale* ticks infected by this agent (Sabatini et al., 2010; Medeiros et al., 2011; Szabó et al., 2013). Indeed, these findings indicate that *A. ovale* is the primary vector of strain Atlantic rainforest; however, other tick species occurring on *A. ovale*-infested dogs could also play an important role as vectors. Finally, these findings suggest that there is horizontal transmission of strain Atlantic rainforest between different tick species while feeding on the same domestic dogs.

Clinical signs attributed to the infection by strain Atlantic rainforest on humans in the states of São Paulo and Bahia included fever, rash, myalgia, an eschar at a tick-bite location (tache noire), clinical resolution without complications, and no hospitalization (Spolidorio et al., 2010; Silva et al., 2011). While enlargement of the superficial lymph node draining the eschar was reported in the case of Bahia (Silva et al., 2011), regional lymphadenopathy could not be observed in the case of São Paulo because the lymph nodes draining the eschar were abdominal; i.e., not superficial (Spolidorio et al., 2010). These clinical cases are very similar to the dozens of cases reported for the state of Santa Catarina, in which fever, myalgia, rash, and lymphadenopathy are among the most frequently reported clinical signs (Angerami et al., 2009; Barros e Silva et al., 2014). In addition, only 14 (7.7%) out of 180 confirmed cases of Santa Catarina have required hospitalization, with no fatalities (Barros e Silva et al., 2014). Indeed, such a mild acute disease can be underdiagnosed or misdiagnosed with other mild acute diseases. This situation could have occurred to some of seroreactive persons of the present study, who did not recall a history of spotted fever.

While the inoculation eschar has not been officially reported to any of the clinical cases of Santa Catarina (Angerami et al., 2009; Barros e Silva et al., 2014), during our field work we had the opportunity to talk to two persons (one of them lived in the household shown in Fig. 1B) that had a serologically-confirmed case of spotted fever three to four years before; the two persons still had a visible healed eschar at the original tick-biting site, which according to them, was an eruptive lesion during the febrile period (data not shown). Because spotted fever is a nationally notified disease in Brazil, clinical data of notified cases must be submitted to the National Information System of the Brazilian Health Ministry. In these official files, clinical signs are usually registered by starring them within a list of clinical signs that can be found in acute febrile hemorrhagic syndromes. As eschar or tick bite lesion is not included in this list, and because the literature reports of spotted fever in Santa Catarina have relied solely on the compilation of the clinical data marked in these official files (Angerami et al., 2009; Godoy, 2011; Barros e Silva et al., 2014), absence of eschar is not a surprise in these reports.

The present study provides epidemiological evidence that the spotted fever in a highly endemic area of Santa Catarina has been caused by *Rickettsia* sp. strain Atlantic rainforest, transmitted to humans by either *A. ovale* or *A. aureolatum*. While these two species are recognized human biting ticks (Guglielmone et al., 2006), human infestation are indeed enhanced by unrestrained domestic dogs, which typically get infested in the forest, and carry infected ticks to households, as reported for other Atlantic rainforest areas of Brazil in which *Rickettsia* sp. strain Atlantic rainforest or *R. rickettsii* infects persons (Ogrzewalska et al., 2012; Szabó et al., 2013). Further studies encompassing direct diagnostic methods on clinical specimens from patients of Santa Catarina are needed to confirm the above epidemiological evidence.

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