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Seroprevalence of *Rickettsia bellii* and *Rickettsia felis* in dogs, São José dos Pinhais, State of Paraná, Brazil

Soroprevalência de *Rickettsia bellii* e *Rickettsia felis* em cães, São José dos Pinhais, Paraná, Brasil

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Abstract

Brazilian spotted fever (BSF) is a vector-borne zoonosis caused by *Rickettsia rickettsii* bacteria. Dogs can be host sentinels for this bacterium. The aim of the study was to determine the presence of antibodies against *Rickettsia* spp. in dogs from the city of São José dos Pinhais, State of Paraná, Southern Brazil, where a human case of BSF was first reported in the state. Between February 2006 and July 2007, serum samples from 364 dogs were collected and tested at 1:64 dilutions by indirect immunofluorescence assay (IFA) against *R. rickettsii* and *R. parkeri*. All sera that reacted at least to one of *Rickettsia* species were tested against the six main *Rickettsia* species identified in Brazil: *R. rickettsii*, *R. parkeri*, *R. bellii*, *R. rhipicephali*, *R. amblyommii* and *R. felis*. Sixteen samples (4.4%) reacted to at least one *Rickettsia* species. Among positive animals, two dogs (15.5%) showed suggestive titers for *R. bellii* exposure. One sample had a homologous reaction to *R. felis*, a confirmed human pathogen. Although *Rickettsia* spp. circulation in dogs in the area studied may be considered at low prevalence, suggesting low risk of human infection, the present data demonstrate for the first time the exposure of dogs to *R. bellii* and *R. felis* in Southern Brazil.

Keywords: Brazilian spotted fever, *Rickettsia rickettsii*, sentinels, indirect immunofluorescence assay.

Resumo

A febre maculosa brasileira (FMB) é uma zoonose veiculada por carrapatos e causada pela bactéria *Rickettsia rickettsii*, podendo os cães ser hospedeiros sentinelas para essa bactéria. O objetivo do estudo foi determinar a presença de anticorpos contra *Rickettsia* spp. em cães de São José dos Pinhais, estado do Paraná, Sul do Brasil. Entre fevereiro de 2006 e julho de 2007, amostras séricas de 364 cães foram coletadas e testadas na diluição de 1:64 por Reação de Imunofluorescência Indireta (RIFI) contra *R. rickettsii* e *R. parkeri*. Todos os soros reagentes para pelo menos uma espécie de *Rickettsia* foram testados contra as seis principais espécies de *Rickettsia* identificadas no Brasil: *R. rickettsii*, *R. parkeri*, *R. bellii*, *R. rhipicephali*, *R. amblyommii* e *R. felis*. Dezesesseis amostras (4,4%) reagiram para pelo menos uma espécie de *Rickettsia*. Dos animais positivos, dois cães (15,5%) apresentaram títulos sugestivos de exposição a *R. bellii*. Uma amostra apresentou reação homóloga frente à *R. felis*, um agente patogênico confirmado para seres humanos. Muito embora os resultados demonstrem uma baixa prevalência de *Rickettsia* spp. em cães, sugerindo um baixo risco de infecção humana, este estudo relatou pela primeira vez a evidência de exposição a *R. bellii* e *R. felis* em cães no Sul do Brasil.

Palavras-chave: Febre maculosa brasileira, *Rickettsia rickettsii*, sentinelas, reação de imunofluorescência indireta.

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Introduction

Rickettsia spp. are obligate intracellular bacteria, members of the Rickettsiales order and Rickettsiaceae family (RAOULT; ROUX, 1997). The genus *Rickettsia* was recently divided into four distinct groups: the spotted fever group (SFG), the typhus group (TG), the transitional group (TRG), and the ancestral group (AG) (GILLESPIE et al., 2007). In Brazil at least seven species of *Rickettsia* have been described: *Rickettsia rickettsii*, *Rickettsia parkeri*, *Rickettsia rhipicephali*, and *Rickettsia amblyommii*, all of them from the SFG transmitted through ticks; *Rickettsia felis*, of TRG, and *Rickettsia typhi*, of TG, both transmitted through fleas; and *Rickettsia bellii*, of AG, transmitted through ticks (LABRUNA, 2009).

Although virulence of bacterial genotypes may vary (PAROLA et al., 2009), *R. rickettsii* pathogenicity has been reported in humans and dogs as Rocky Mountain spotted fever (RMSF), a severe clinical disease, also named Brazilian spotted fever (BSF) (LABRUNA et al., 2009). *Rickettsia parkeri* pathogenicity has also been described in one case of human infection in the United States, and probably in Uruguay (PADDOCK, 2005); moreover, occurrence of human infection has been suggested in Brazil (LABRUNA, 2009). *Rickettsia felis* and *R. typhi* are also pathogenic in humans, although *R. rickettsii* infection is relatively more severe (PAROLA et al., 2005). Other rickettsial species described in Brazil (*R. bellii*, *R. rhipicephali* e *R. amblyommii*) have never been associated to human disease; however, *R. amblyommii* has been recently incriminated as the causative agent of febrile disease in humans in the United States (APPERSON et al., 2008).

Ticks of the *Amblyomma* genus are the main vectors of BSF, notably *Amblyomma cajennense* and *Amblyomma aureolatum* (PINTER; LABRUNA, 2006). In general, this genus is characterized by low host specificity, particularly in their immature stages. Some domestic animals, such as horses and dogs, are important amplifier hosts of ticks and serve as sentinels for Rickettsiae species (SANGIONI et al., 2005). Domestic dogs may play an important epidemiological role in the urban cycle of this disease due to household carriage of infected ticks and closer contact with their owners (PIRANDA et al., 2008).

Serological studies in several Brazilian States have indicated previous exposure of dogs to *R. rickettsii*, sentinel hosts for bacterial dissemination (HORTA et al., 2004; SANGIONI et al., 2005; LABRUNA et al., 2007a). Information based on serological surveys for *R. rickettsii* in sentinel animals may identify new endemic areas of BSF where the population can be properly warned about the risk of this disease (PADDOCK et al., 2002).

The occurrence of BSF by *R. rickettsii* has been reported in Brazil since the 1920s. The disease has been classically reported in the southeastern region, presenting a severe form which may evolve to death when patients are left untreated or receive late treatment. BSF is currently considered a reemerging zoonosis with high prevalence in the States of Minas Gerais and São Paulo (PINTER et al., 2008). Recently the disease has been first reported in humans in all three Brazilian Southern States. However, different from the 18 to 30% lethality found in southeastern Brazil (ANGERAMI et al., 2006; CALIC et al., 2005), no deaths were seen in human cases reported in the Southern region. In

addition, local epidemiological differences such as absence of *A. cajennense* in most Southern States (ESTRADA-PENÁ et al., 2004; EVANS et al., 2000) suggest that the etiological agent of BSF in southern region of Brazil may be other than *R. rickettsii*.

The first case in humans in the State of Paraná was reported in 2005 in the city of São José dos Pinhais in the suburban area of the state capital of Curitiba. A recent serum surveillance study on cart horses used for carrying recyclable material has demonstrated the presence of antibodies against *R. rickettsii* (FREITAS et al., 2010). Although dogs are considered an important sentinel for BSF, no previous study has been conducted on dogs in that area.

The aim of the present study was to examine dogs received by the Zoonosis Control Center of São José dos Pinhais (ZCC/SJP) for the presence of antibodies against *Rickettsia* spp.

Material and Methods

1. Area of study

The present study was conducted in the municipality of São José dos Pinhais (Figure 1), located in the metropolitan area of Curitiba, Paraná State capital, Southern Brazil (25° 32' 05" S and 49° 12' 23" W). The city has an altitude of 906 meters above sea level and an area of 946 km² of which about 40% is protected native Atlantic forest. The estimated population is about 263,622 residents (demographic density of 276 inhab/km²), 90% of which living in the urban area. The weather is described as humid subtropical with annual average temperatures of 16 °C (IBGE, 2007).

2. Animals

From February 2006 to July 2007 a total of 364 dogs, of which 192 from 34 urban areas, 129 from 31 rural areas and 43 with unknown origin were received at the Zoonosis Control

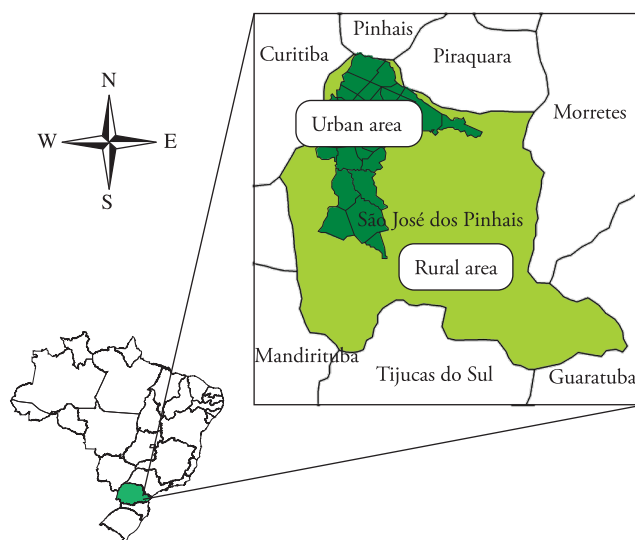


Figure 1. Rural and urban areas in the city of São José dos Pinhais, State of Paraná, Southern Brazil.

Center (ZCC) of São José dos Pinhais. Of all, 222 were stray dogs captured on streets, and 142 were domiciled dogs whose owners have requested them to be removed. The animals were physically restrained and sedated with ketamine chloride (10 mg.kg⁻¹) and xylazine chloride (1 mg.kg⁻¹) intramuscularly. Following initial sedation, 12.5 mg.kg⁻¹ of sodium thiopental was intravenously administered and blood was drawn prior to euthanasia. Whole blood samples were then collected by intracardiac puncture, centrifuged at 3,000 rpm for 5 minutes, and serum samples were stored at -20 °C in 1.5 mL Eppendorf tubes until analysis. A data file was created for each dog, including the day of blood sampling, sex, age (young, adult, or old), breed and ownership status.

The present study was approved by the Animal Ethics Committee (Protocol # 037/2008) of the Department of Agricultural Sciences at the Universidade Federal do Paraná.

3. Indirect immunofluorescence assay (IFA)

Indirect immunofluorescence assay (IFA) was performed following previous standard protocol (HORTA et al., 2004). In brief, the test consisted of serum samples reaction with VERO cells infected with *Rickettsia* spp. that were isolated from native Brazilian ticks. Each serum was individually screened at 1:64 dilution of antigens of *R. rickettsii* (strain Taiacu) and *R. parkeri* (strain AT24). Fluorescein isothiocyanate anti-dog immunoglobulin G (Antibodies, Zoonosis Control Center, São Paulo, Brazil) was applied for fixed antigen reaction and visualized by fluorescence microscope (Olympus BX60; Olympus, Tokyo, Japan). Positive and negative sera were used as controls for each slide reaction. Positive samples for at least one tested species were then submitted to serial dilutions until 1:1024 and tested against antigens of *R. rickettsii* (strain Taiacu), *R. parkeri* (strain AT24), *R. bellii*

(strain Mogi), *R. rhipicephali* (strain HJ5), *R. amblyommii* (strain Ac37), and *R. felis* (strain Pedreira). Samples with IgG titers $\geq 1:64$ were considered positive, as previously described for humans and dogs (SANGIONI et al., 2005). Samples with titers at least fourfold higher for a specific *Rickettsia* when compared to others were considered homologous or very closely related genotype (SAITO et al., 2008).

4. Statistics

Statistical analysis was conducted using the chi-square test (BioEstat 5.0®, National Council for Scientific and Technological Development [CNPq], Brazil). IFA results for *Rickettsia* spp. were also correlated by sex, age, breed and ownership status. Differences in the multivariate model were considered of statistical significance when $p < 0.05$.

Results

A total of 364 dogs were sampled and tested against *R. rickettsii* and *R. parkeri* antigens at 1:64 dilution, of which 16 (4.4%) reacted to at least one of the two species. Out of 16 initially positive samples, nine were positive against *R. parkeri*, seven against *R. rickettsii*, five against *R. felis*, five against *R. amblyommii*, four against *R. bellii*, and three against *R. rhipicephali* (Table 1). Among positive dogs, two had suggestive titers of *R. bellii* exposure, and one sample showed a homologous reaction to *R. felis*.

A total of 09/34 (26.5%) urban districts in São José dos Pinhais had at least one positive animal for *Rickettsia* spp. (Table 2 and Figure 2). Although one animal was from an unknown origin, no positive animal (0/31) was found from rural areas.

Table 1. Endpoint titers of indirect immunofluorescence assay (IFA) against antigens of six *Rickettsia* species in dog sera. City of São José dos Pinhais, State of Paraná, Southern Brazil, February 2006-2007.

Serum	Endpoint IgG titers						PAIHR
	<i>R. rickettsii</i>	<i>R. parkeri</i>	<i>R. bellii</i>	<i>R. felis</i>	<i>R. amblyommii</i>	<i>R. rhipicephali</i>	
8	64	-	-	64	-	-	
27	-	-	-	64	-	-	
86	-	128	-	-	-	64	
103	-	-	128	-	-	-	<i>R. bellii</i>
107	-	64	-	-	-	-	
138	256	128	-	-	128	128	
145	-	-	1024	-	-	-	<i>R. bellii</i>
162	64	128	-	-	-	-	
163	-	64	-	-	-	-	
183	64	64	64	128	-	-	
204	64	-	-	-	64	-	
244	-	-	-	-	64	-	
295	256	128	-	-	128	128	
344	-	-	-	128	-	-	<i>R. felis</i>
346	-	128	64	64	-	-	
354	128	256	-	-	64	-	

PAIHR: Possible antigen involved in a homologous reaction. Sera evidencing *Rickettsia* species titer at least fourfold higher than that observed for any other *Rickettsia* species were considered homologous to the first *Rickettsia* species. Therefore, dogs 8, 27, 86, 107, 138, 162, 163, 183, 204, 244, 295, 346, 354 were not considered PAIHR. -: Non reactive at IgG titer $\geq 1:64$; IFA: indirect immunofluorescence assay.

Out of the positive samples, 10 (62.5%) were males and six (37.5%) were females; 15 (93.7%) were adults and one (6.2%) was old. Eleven dogs (68.7%) were from mixed breed, two were Rottweiler, one German shepherd, one cocker spaniel, and one pit bull.

No differences were found when the 360 dogs were compared by age (young, adult, or old; $\chi^2 = 0.196$), sex (male, female; $\chi^2 = 0.047$), breed (pure breed, mixed breed; $\chi^2 = 0.223$) or ownership (stray and owned; $\chi^2 = 0.016$).

Table 2. Distribution of positive dogs (IgG titers ≥ 64 by indirect immunofluorescence assay) per district. City of São José dos Pinhais, State of Paraná. Southern Brazil, 2006-July 2007.

Neighborhood	Dogs (positive/total tested)		Total
	2006	2007	
Afonso Pena	1/16	0/15	1/31 (3.2%)
Borda do Campo	2/10	0/1	2/11 (18.2%)
Rio Grande	1/4	1/2	2/6 (33.3%)
Cruzeiro	2/3	0/6	2/9 (22.2%)
Independência	1/2	0/0	1/2 (50.0%)
Itália	2/10	0/2	2/12 (16.7%)
Centro	1/19	2/10	3/29 (10.3%)
Rio Pequeno	1/7	0/0	1/7 (14.3%)
Ipê	0/5	1/4	1/9 (11.1%)

Discussion and Conclusion

The present study found a relatively low prevalence of antibodies to *Rickettsia* spp. (4.4%) when compared to previous serological studies performed in dogs from endemic areas in the State of São Paulo that reported a seropositivity between 31.3 and 69.6% (LEMOS et al., 1996; HORTA et al., 2004, 2007; PINTER et al., 2008; MORAES-FILHO et al., 2008), from endemic areas of the State of Minas Gerais (13.7 and 81.3%) (LEMOS et al., 1994; VIANNA et al., 2008) and from endemic areas of the State of Espírito Santo (7.6%) (SPOLIDORIO et al., 2010). The seroprevalence found in the present study was also lower than that reported in dogs from non-endemic areas, 12.9% (LEMOS et al., 1996) and 20% (HORTA et al., 2007) in the State of São Paulo, and 11 to 23% in Northern Paraná (TAMEKUNI, 2009). These studies have shown the importance of dogs as sentinels for human infection and how wide may be the geographic distribution of BSF due to this interaction.

The relatively lower overall prevalence found corroborates a single previous report to date of a human case in the same municipality (FREITAS et al., 2010). However, when positive dogs were distributed by district, the seropositivity varied from 3.2 to 50.0%. The human case was reported in the district of Borda do Campo (18.2%) (Table 2). Thus, regular monitoring is required because, despite the low prevalence found in the present

Number of seropositive animals



*Occurrence area of the 1st human case of BSE in Paraná

Urban areas

1. Centro
2. São Domingos
3. Cidade Jardim
4. São Cristóvão
5. São Pedro
6. Bom Jesus
7. Pedro Moro
8. Aristocrata
9. Águas Betas
10. Boneca do Iguaçu
11. Afonso Pena
12. Aviação
13. Aeroporto
14. Quissisana
15. Cruzeiro
16. Ouro Fino
17. Itália
18. Santo Antônio
19. Rio Grande
20. Zacarias
21. Arujá
22. Barro Preto
23. Costeira
24. Costeirinha
25. Rio Pequeno
26. Iná
27. Independência
28. Ipê
29. Guatupê
30. Cristal
31. Academia
32. Roseira de São Sebastião
33. Borda do Campo
34. Dom Rodrigo
35. Del Rey
36. São Marcos
37. Campina do Taquaral
38. Passo do Campo
39. Campo Largo da Roseira
40. Contenda

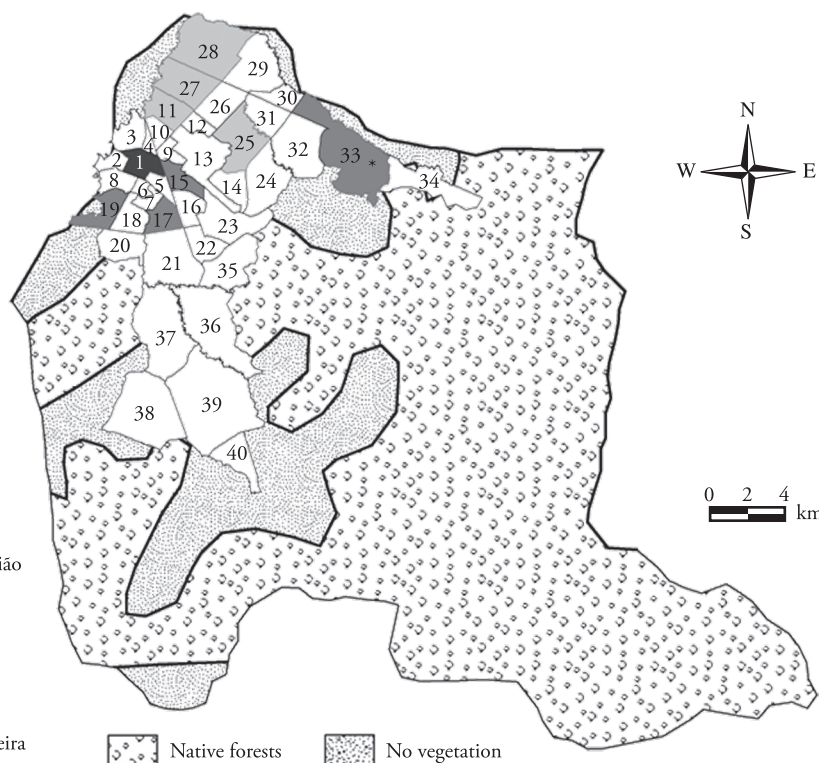


Figure 2. Positive dogs sera by indirect immunofluorescence assay (IFA) for *Rickettsia* spp., according to areas where at least one dog was found positive. City of São José dos Pinhais. State of Paraná, Southern Brazil, 2006-2007.

study, it may represent an important finding since BSF frequency may vary due to seasonal distribution of local vectors.

All positive samples came from the urban area of São José dos Pinhais, which contrast with that reported by Labruna et al. (2007a) who showed 11.6 and 3.9% prevalence of antibodies against *Rickettsia* spp. in rural and urban areas in Northern Brazil. Although forest areas are common in the region studied (dog origin) (Figure 2) and may favor tick proliferation (SAITO et al., 2008), no positive animal was found in the rural area. The fact that seropositive dogs were found only in urban areas may be explained by tick distribution associated to higher concentration of host species in these areas. Since it was out of the scope of the present study to determine tick species and their frequency, further studies are needed to fully establish this correlation.

Another serological survey conducted in 389 dogs of Southern Brazil have shown a high prevalence of positive animals (42.4%) for *Rickettsia* spp. by IFA, and *R. parkeri* was the causative agent in 100/389 (25.7%) dogs (SAITO et al., 2008). We hypothesize that low titers may be a result of a less recent or severe infection, probably due to low or non-pathogenic *Rickettsia* species.

The present data shows no infection evidence for either *R. rickettsii* or *R. parkeri*. Even in endemic areas of Southern Brazil, previous studies have proposed that BSF may be caused by *Rickettsia* species other than *R. rickettsii*. Moreover, regional particularities may also play a role such as host species, tick prevalence and risk of human exposure to potential infected vectors (PACHECO et al., 2007).

This is the first report on evidence of natural infection by *R. bellii* and *R. felis* in dogs from Southern Brazil. *Rickettsia bellii* has been reported in ticks of Southeastern Brazil (LABRUNA et al., 2004; HORTA et al., 2006) and *Amblyomma* ticks from rural areas of Brazil are mostly infected by *R. bellii* (PINTER et al., 2008). However, the first evidence in vertebrates has been only recently reported, and only in capybaras from the same region (PACHECO et al., 2007). Although strictly associated to ticks, *R. bellii* is not classified as belonging to the SFG group and its pathogenic effects in humans and dogs has never been demonstrated.

Serological evidence of infection by *R. bellii* in two dogs and *R. felis* in another animal may indicate that these two agents are probably circulating in the region. In addition, since *R. felis* is reportedly pathogenic for human in southeastern Brazil (PAROLA et al., 2005), these findings may alert of zoonotic risk in the area.

Although most reports on *R. felis* were associated to the flea *Ctenocephalides felis felis* collected in cats and/or dogs (LABRUNA et al., 2007b; NAVA et al., 2008; OLIVEIRA et al., 2008; GEHRKE et al., 2009), future studies should be conducted to fully establish flea rickettsial burden.

It was first evidenced dog exposure by *R. bellii* and *R. felis* in Southern Brazil, demonstrating *Rickettsia* circulation in the area. The low prevalence found in the study suggests that dogs are exposed to a small vector population infected by *Rickettsia* and the risk of infection for humans may be low too. However, active epidemiological surveillance should be periodically conducted as the area is considered vulnerable to BSF cases.

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