

Serological survey of *Rickettsia* sp. in horses and dogs in an non-endemic area in Brazil

Identificação sorológica de *Rickettsia* sp. em equinos e cães de área não endêmica no Brasil

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Abstract

Brazilian Spotted Fever (BSF) is a lethal rickettsiosis in humans caused by the bacteria *Rickettsia rickettsii*, and is endemic in some areas of Brazil. Horses and dogs are part of the disease's life cycle and they may also serve as sentinel animals in epidemiological studies. The first human BSF case in the State of Paraná was reported in 2005. The present study was conducted in the municipality of Almirante Tamandaré, where no previous case of BSF was reported. Serum samples were collected from 71 horses and 20 dogs from nine properties in the area. Ticks were also collected from these animals. All farmers completed a questionnaire about their knowledge of BSF and animal health management. Serum samples were analyzed by indirect immunofluorescent-antibody assay (IFA) using *R. rickettsii* and *R. parkeri* as antigens. Ticks were analyzed by PCR for *Rickettsia* sp., and all of them were PCR-negative. Six horses (8.45%) and 4 dogs (20%) were identified as seropositive. Farmers were not aware of the correlation between the presence of ticks and risk of BSF. Although a non-endemic area, Almirante Tamandaré is a vulnerable environment for BSF and effective tick control measures are required.

Keywords: Ticks, brazilian spotted fever, emerging zoonosis.

Resumo

A Febre Maculosa Brasileira (FMB) é uma riquetsiose letal para humanos, causada pela bactéria *Rickettsia rickettsii*, e é endêmica em algumas regiões brasileiras. Equinos e cães podem participar do ciclo da doença e podem também servir como sentinelas em estudos epidemiológicos. O primeiro caso humano relatado no Estado do Paraná ocorreu em 2005. O presente estudo foi realizado no município de Almirante Tamandaré, região onde não há relatos de casos de FMB. Foram coletadas amostras de sangue de 71 cavalos e 20 cães em nove propriedades rurais na região. Carrapatos também foram colhidos dos animais. Todos os proprietários responderam a um questionário sobre o manejo sanitário dos animais e o conhecimento a respeito da FMB. As amostras de soro foram processadas pela técnica de Reação de Imunofluorescência Indireta (RIFI), utilizando-se os antígenos de *R. rickettsii* e *R. parkeri*. Os carrapatos foram analisados por PCR para *Rickettsia* sp. e todos foram negativos. Seis cavalos (8,45%) e 4 cães (20%) foram identificados como soropositivos. Todos os proprietários desconheciam a relação de carrapatos com a FMB. Embora considerada uma área não endêmica, Almirante Tamandaré é um ambiente vulnerável à FMB e um controle eficiente de carrapatos deve ser implementado.

Palavras-chave: Carrapatos, febre maculosa brasileira, zoonose emergente.

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Introduction

Rickettsiae are gram-negative, obligate intracellular bacteria that inhabit salivary glands and ovaries of their arthropod hosts (BILLINGS et al., 1998). *Rickettsia rickettsii* is the primary etiological agent of Brazilian spotted fever (BSF) but *Rickettsia parkeri* and *Rickettsia felis* have also been described as causative agents. However, lethal human cases have been confirmed as caused only by *R. rickettsii* (SILVA, 2007). BSF is acquired through the bite of an infected tick that remains attached to the host for at least four to six hours (RICKETTS, 1906).

In humans these bacteria replicate in the vascular endothelium, causing vasculitis and leading to the activation of platelets and coagulation system, thrombosis and increased vascular permeability. Hemostatic disorders, including thrombocytopenia and prolonged clotting time, are attributed to cytopathic effects and cellular activity of *R. rickettsii* endotoxins (DAVIDSON et al., 1990). BSF is characterized by sudden onset of high fever, headache, myalgia, and prostration, followed by a maculopapular rash predominantly on palms and sole. Patients who do not receive early treatment can develop serious complications, and approximately 50% of these patients may die (WALKER, 2002).

Clinical cases have been described in dogs in the United States (McDADE; NEWHOUSE, 1986), but there have been no reported cases in Brazil (SANGIONI et al., 2005). In horses, there are no descriptions of clinical signs or symptoms, even when these animals have high titers ($\geq 1:1024$) against *R. rickettsii* (LEMONS et al., 1996).

The tick *Amblyomma cajennense* is the main vector of BSF. However, disease transmission is not exclusively associated to this tick species, as verified in a study by Pinter and Labruna (2006) in São Paulo that reported *A. aureolatum* as a vector; and Rozental et al. (2002) reported the tick *Rhipicephalus sanguineus* as a potential vector. Cunha et al. (2009) reported the first molecular detection by PCR of *R. rickettsii* on *R. sanguineus* naturally infected in Rio de Janeiro. These tick species are also responsible for maintenance of *R. rickettsii* in the environment through transovarial and transtadial transmission, and a single female can transmit the agent to all its progenies. Besides the tick, other amplified hosts of the disease include wild animals such as capybaras (SOUZA et al., 2008). Dogs and horses may help to maintain the cycle of disease as they are potential hosts for *R. sanguineus* (FREITAS, 2007) and *Amblyomma cajennense* ticks (TIRIBA, 1972; LABRUNA et al., 2002).

Cardoso et al. (2006) among others described that seropositive horses and dogs are often reported in endemic areas, serving as sentinel animals in epidemiological studies. Labruna (2009) remarked that horses and capybaras are also sentinel animals and that few populations of ticks are infected by pathogenic *Rickettsia*. Pinter et al. (2008) reported that dogs are important sentinels for the presence of the bacterium *R. rickettsii* in areas where the tick *A. aureolatum* is the main vector of BSF.

BSF can be considered a reemerging zoonosis in Brazil, and information about disease transmission is still incipient (SANGIONI et al., 2005). Cases have been underdiagnosed because of the lack of awareness of health providers about this disease and

difficult clinical and laboratorial diagnosis in humans and animals (PADDOCK et al., 1999). According to the Brazilian Ministry of Health (2005) BSF was first reported in Brazil in 1929. Between 1995 and 2005, 386 cases were reported in Southeastern States of São Paulo and Minas Gerais and in the Southern State of Santa Catarina. The first human case in the State of Paraná was reported in April, 2005, in a 45-year-old male owner of a rural property in the municipality of São José dos Pinhais (FREITAS, 2007). Freitas (2007) reported for the first time anti-*Rickettsia* antibodies of the spotted fever group in horses and dogs from that area.

The objective of the present study was to provide epidemiological input on BSF in the State of Paraná, Brazil, by determining the presence of anti-*R. rickettsii* and *R. parkeri* antibodies by IFA in sera of horses and dogs from the municipality of Almirante Tamandaré.

Material and Methods

1. Study area

The study was conducted in the municipality of Almirante Tamandaré, which is part of the metropolitan area of Curitiba, State of Paraná, Southern Brazil. The city has a population of 93,055 inhabitants and is located at latitude of 25° 19' 29" S and longitude 49° 18' 36" W. It has an altitude of 945 m above sea level. The climate is subtropical with temperatures varying from 18 to 22 °C.

2. Local knowledge of BSF

During field work, a questionnaire was applied to 17 residents including questions about their knowledge on the importance of ticks, the presence of ticks on animals and whether ticks could pose any risk to animals or transmit any disease.

3. Animals and techniques

A total of 71 horses and 20 dogs living in nine rural properties were sampled from August and September, 2008. A record was kept for each animal including information such as age and name. In addition, a questionnaire about husbandry practices was filled out for each property. Blood samples were collected from jugular vein using vacutainer needles and adapters (BD Vacutainer, England), centrifuged to separate the serum and frozen before analysis.

Each serum was tested by indirect immunofluorescent-antibody assay (IFA) using crude *R. rickettsii* and *R. parkeri* antigens as previously described (LABRUNA et al., 2007). Individual sera were initially screened at a dilution 1:64 against each of the rickettsial antigens. In case of a positive reaction, serum serial dilutions at two-fold increments were tested up to the endpoint titer.

Ticks found attached to horses and dogs were collected and preserved in isopropyl alcohol. They were individually identified to species and submitted to DNA extraction by guanidine thiocyanate as previously described by Sangioni et al. (2005). Ticks were processed in pools of up to three specimens, and tested by PCR

with primers CS-78 and CS-323, which target a 401-bp fragment of the *gltA* gene of *Rickettsia* spp. These procedures were performed as previously described by Labruna et al. (2004).

Results

Overall, 8.5% (6/71) of horses and 20% (4/20) of dogs showed positive anti-*Rickettsia* reactions (Table 1), with titers ranging from 1:64 to 1:1024 for both *R. rickettsii* or *R. parkeri* (Table 2). All sera reactive to *R. rickettsii* were also reactive to *R. parkeri*, usually with similar endpoint titers with at most 2-fold difference between them. One serum (horse 30) showed a 4-fold difference between anti-*R. rickettsii* and anti-*R. parkeri* endpoint titers.

Eleven horses (15.5%) were infested with *Dermacentor nitens*. Seven dogs (35%) were found infested, six with *Amblyomma aureolatum* and one with *Rhipicephalus microplus*. No rickettsial DNA was amplified by PCR from these ticks.

The questionnaire showed that 42.9% (6/14) farmers had no knowledge on BSF, 71.4% (10/14) did not know how the pathogen is transmitted, and none of them knew any clinical signs of BSF. Fifteen farmers (80%) reported having seen wild animals in the

area in the following order of frequency: hares (100%), capybaras (16.7%), and deers (8.3%).

Acaricide treatment was provided for 60 (7.1%), 90 (25%) or 180 days (21.4%) based on the presence of ticks reported by the owners (7.8%) and during summer months (3.6%). Only 21.5% of the owners did not use acaricides.

Regarding the origin of the horses studied, 60.7% (17/28) were from Almirante Tamandaré, and were used for different purposes (horseback riding, rodeos, and work). The average age of these animals was 9.5 years (range: 1 to 20 years). The dogs had an average age of 2.5 years (range: 0.3 to 9 years). All dogs were mostly reared unrestrained with free outdoor access.

Discussion

Almirante Tamandaré is in an area where rural tourism is of significant economic importance, with several horse stables and training centers. This is an area of rivers, abundant vegetation and reported wild animals such as capybaras, which can all favor the maintenance of many tick species and consequently of *Rickettsia*. The fact that antibodies against *Rickettsia* from the spotted fever

Table 1. Indirect immunofluorescence assay (IFA) using *Rickettsia rickettsii* and *Rickettsia parkeri* antigens in horses and dogs from nine rural properties. Municipality of Almirante Tamandaré, State of Paraná, Brazil.

Property No.	No. seroreactive animals / total number of animals tested (% seroreactivity)			
	Horses		Dogs	
	<i>R. rickettsii</i>	<i>R. parkeri</i>	<i>R. rickettsii</i>	<i>R. parkeri</i>
1	0/20 (0.00)	0/20 (0.00)	0/0 (0.00)	0/0 (0.00)
2	2/16 (0.13)*	0/16 (0.00)	1/4 (0.25)*	0/4 (0.00)
3	0/3 (0.00)	0/3 (0.00)	0/0 (0.00)	0/0 (0.00)
4	1/7 (0.14)*	0/7 (0.00)	0/1 (0.00)	0/1 (0.00)
5	0/9 (0.00)	0/9 (0.00)	0/1 (0.00)	0/1 (0.00)
6	1/6 (0.16)	1/6 (0.16)	0/0 (0.00)	0/0 (0.00)
7	1/2 (50.00)*	0/2 (0.00)	0/0 (0.00)	0/0 (0.00)
8	0/5 (0.00)	0/5 (0.00)	0/0 (0.00)	0/0 (0.00)
9	1/3 (0.33)	1/3 (0.33)	3/14 (0.21)	3/14 (0.21)
Total	6/71 (8.50)	2/71 (0.03)	4/20 (20.00)	3/20 (0.15)

*A homologous reaction was determined when an endpoint titer to a *Rickettsia* species was at least four-fold higher than that seen in other *Rickettsia* species. In this case, the *Rickettsia* species with the highest endpoint titer was considered the possible antigen involved in a homologous reaction (PAIHR).

Table 2. Antibody titers by indirect immunofluorescence assay (IFA) for two rickettsial antigens (*Rickettsia rickettsii* and *Rickettsia parkeri*) in horses and dogs from rural properties of Almirante Tamandaré municipality, State of Paraná, Brazil.

Property No.	Animal No.	Age	Sex	IFA titer		Presence of ticks
				<i>R. rickettsii</i>	<i>R. parkeri</i>	
2	horse-28	7 years	male	128	64	No
2	horse-30	4 years	male	256	64	No
2	dog-40	4 months	female	128	64	Yes*
4	horse-46	11 years	male	128	64	Yes**
6	horse-64	12 years	male	1024	1024	No
7	horse-72	12 years	male	256	128	No
9	dog-82	1.5 years	male	64	64	No
9	dog-84	3 years	female	512	512	No
9	dog-86	unknown	male	1024	1024	No
9	horse-69	4.5 years	male	64	64	No

Amblyomma aureolatum*; *Dermacentor nitens*.

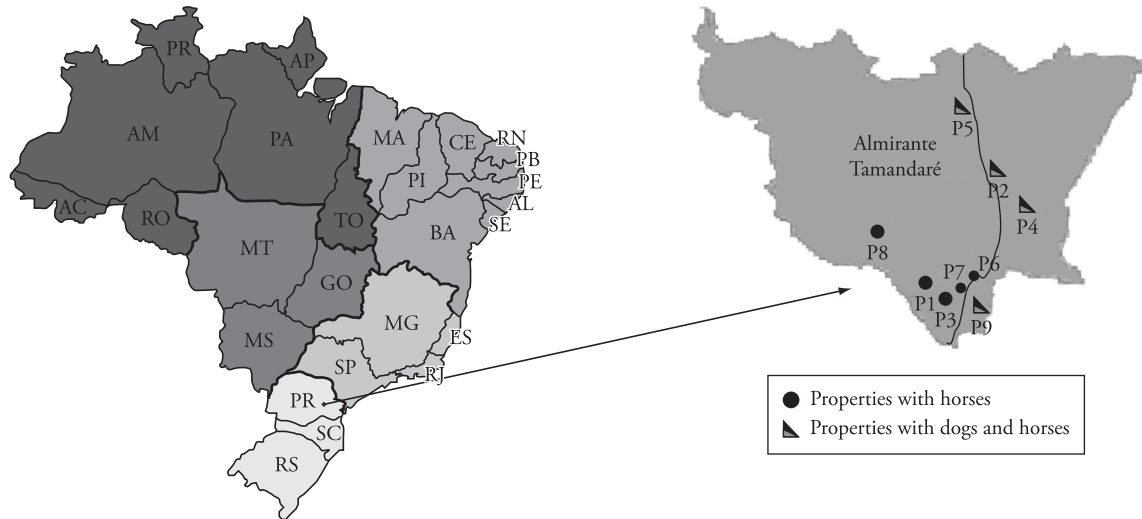


Figure 1. Map showing the sampled properties and the Belem River. Municipality of Almirante Tamandaré, State of Paraná, Brazil.

group have been found in animals in Almirante Tamandaré, a non-endemic area, reinforces the notion that BSF is an important reemerging zoonosis in Brazil.

Negative PCR tests for ticks could be explained by the small sample of ticks examined and also the varying refractory effect to the pathogen (LABRUNA et al., 2008), with lethal effect from *R. rickettsii* (NIEBYLSKI et al., 1999) that may alter the host-pathogen relationship.

In the present study, the seroprevalence of rickettsial antibodies in horses was 8.45% which is low compared to that reported (25 and 27.3%) in other studies in non-endemic areas of Brazil (LEMOS, 1996; HORTA et al., 2004; GALVÃO et al., 2005; CARDOSO et al., 2006; FREITAS, 2007; FREITAS et al., 2010). However Freitas (2007) investigated an area where there was an outbreak of BSF and a human case reported. Also, Vianna et al. (2008) reported that 100% of the sampled horses were positive to *R. rickettsii* in Minas Gerais. The prevalence of rickettsial antibodies in dogs in this study (20%) was higher than that found by Lemos et al. (1996) and Freitas (2007) of 12.9 and 12.5%, respectively.

In the present study all five properties that were crossed or delimited by rivers were considered BSF niches by IFA (Figure 1). Therefore, the city is within an area of biotic potential for the development of free-living ticks and hosts and the occurrence of BSF. The study showed positive animals living under environment conditions favorable to ticks where rivers and wild animals such as capybaras (MOLENTO, personal communication) may serve as reservoirs of *Rickettsia* sp. (LEMOS et al., 1996, 2001; MILAGRES, 2004; HORTA et al., 2004; SOUZA et al., 2009; FORTES et al., 2010).

Conclusion

There is serological evidence showing the presence of rickettsiae from the spotted fever group in the area of Almirante Tamandaré. Further studies are needed and although this is a non-endemic area, humans are vulnerable to BSF due to their close proximity to positive animals.

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