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# Serosurvey of antibodies against spotted fever group *Rickettsia* spp. in horse farms in Northern Paraná, Brazil

Soroprevalência de anticorpos contra *Rickettsia* spp. do grupo febre maculosa em equinos de haras no Norte do Paraná, Brasil

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## Abstract

Brazilian spotted fever (BSF) is an emerging disease most likely caused by *Rickettsia rickettsii*. The objective of the present study was to estimate the seroprevalence of BSF rickettsia infections in equines from six horse farms located in Londrina County, Paraná, Southern Brazil. Six owners of horse farms situated in Cambé, Santa Fé, Guaraci and Londrina municipalities participated in the study. All farms were located in areas where BSF has not been reported. A total of 273 horses were sampled and their sera were tested by indirect Immunofluorescence assay (IFA) using *R. rickettsii* and *R. parkeri* antigens. Titers equal to and greater than 64 were considered positive. Of 273 sera tested, 15 (5.5%) reacted to *R. rickettsii* and 5 (1.8%) to *R. parkeri*. Five out of the six farms studied revealed seropositive animals and seropositivity rate ranged from 0 to 13%. The titers ranged from 64 to 512, and four samples had a titer of 512. Nine animals reacted to *R. rickettsii* with titers four-fold higher than those for *R. parkeri*. These results suggest that horses in Northern Paraná may have been exposed to rickettsiae identical or closely related to *R. rickettsii*.

**Keywords:** Brazilian spotted fever, *Amblyomma* spp., *Rickettsia* spp., horse farms.

## Resumo

Febre Maculosa Brasileira (FMB) é uma doença emergente, sendo *Rickettsia rickettsii* o seu principal agente etiológico. O objetivo deste estudo foi determinar a soroprevalência de rickettsia do grupo da febre maculosa em equinos de seis haras localizados nos municípios de Cambé, Santa Fé, Guaraci e Londrina. As propriedades eram localizadas na região Norte do Paraná onde casos de FMB ainda não foram diagnosticados. Foram colhidas amostras de sangue de 273 equinos, e os soros foram testados pela RIFI, usando *R. rickettsii* e *R. parkeri* como antígenos, considerando-se como positivos títulos  $\geq 64$ . Entre 273 soros, 15 (5,5%) reagiram contra *R. rickettsii* e 5 (1,8%) para *R. parkeri*. Cinco de seis haras estudadas tinham animais reativos, e a taxa de sororreatividade variou de 0 a 13%. Os títulos variaram de 64 para 512, e três amostras apresentaram título de 512. Nove animais reagiram para *R. rickettsii* com títulos quatro vezes maiores que para *R. parkeri*. Esses resultados sugerem que equinos no Norte do Estado do Paraná, Brasil, podem ter sido expostos a uma rickettsia idêntica ou muito próxima a *R. rickettsii*.

**Palavras-chave:** Febre maculosa brasileira, *Amblyomma* spp., *Rickettsia* spp., equinos.

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Brazilian spotted fever (BSF) is an acute tick-borne disease caused by *R. rickettsii* and its clinical signs include fever, headache, myalgia, nausea and rash. The first case of BSF was reported in 1929 in the State of São Paulo (DIAS; MARTINS, 1939) and in recent years BSF has been reported in the State of Minas Gerais, Rio de Janeiro, Santa Catarina, Rio Grande do Sul and Paraná (FREITAS et al., 2010).

Indirect immunofluorescence assay (IFA) is currently the test of choice for the diagnosis of rickettsioses in humans and animals. However, cross-reactivity between *Rickettsia* species is seen in IFA. IFA does not allow to determining the etiologic *Rickettsia* species, but by comparing titers among different antigens, serological similarities and presumptive etiologic agents can be inferred (LASCOLA; RAOULT, 1997; HORTA et al., 2004; PACHECO et al., 2007).

*Rickettsia* spp. are transmitted by ticks of *Amblyomma* genus, and the most important species responsible for transmitting *R. rickettsii* that causes BSF is *A. cajennense*, which is often identified in humans with BSF (MOREIRA; MAGALHÃES, 1935). Horses are primary hosts for all parasitic stages of *A. cajennense*, and they have been described as excellent sentinels for BSF in many studies (SANGIONI et al., 2005; HORTA et al., 2007; PINTER et al., 2008).

The objective of the present study was to obtain seroepidemiological information about spotted fever group (SFG) rickettsia infections among horses on farms located in Northern Paraná, southern Brazil, through serological testing by IFA using *R. rickettsii* and *R. parkeri* antigens.

The study was carried out on six horse farms located in Londrina County (23° 18' 39" S and 51° 09' 53" W), Northern Paraná, a region with no reported BSF cases. A total of 273 horse blood samples were collected and sera were separated and stored at -20 °C before use. The number of samples collected at each farm was as follows: 50 in Cambé, 44 in Santa Fé, 25 in Guaraci, 35 in Londrina 1, 34 in Londrina 2, and 85 in Londrina 3. All of them are in a rural area, except for Londrina 1 farm that is located in an urban area. Londrina 1 farm is an equestrian school where horses are kept in stables with no access to pastures. In all other farms the animals were kept in pastures and had access to a forested area. Tick control was carried out by spraying with pyrethroids and oral ivermectin when ticks were found on the animals. The collection of animal blood was approved by the Universidade Estadual de Londrina (UEL) Ethics Committee on Animal Experiments (number 82/2006).

All horse sera were tested by IFA following Horta et al. (2004) protocol using two *Rickettsia* species: *R. rickettsii* strain Taiaçu (PINTER; LABRUNA, 2006) and *R. parkeri* strain São Paulo (SILVEIRA et al., 2007). Briefly, *Rickettsia* species were cultivated on Vero cells until 100% of cells were infected. Slides were then prepared by harvesting cells and air drying onto multiwell Teflon-coated glass slides, fixed in acetone, and stored at -20 °C before use. The slides were examined using an epifluorescence microscope (Olympus, Japan) and titers ≥64 were considered positive.

Table 1 summarizes the serological results of IFA for the six horse farms studied. Seropositive titers ranged from 64 to 512. Of 15 positive sera, 11 had *R. rickettsii* titers at least four-fold

higher than *R. parkeri* titers. These sera were considered reactive to *R. rickettsii* or very closely related species (Table 2).

Two different tick species identified as *A. cajennense* and *Anocentor nitens* were detected on the horses during sampling. The study variables ciliary forest ( $p = 0.029$ ) and wild animals (capybaras / *Hydrochoerus hydrochaeris* and New World opossums / *Didelphis* spp.) ( $p = 0.029$ ) showed significant statistical associations with *R. rickettsii* seropositivity. Only animals more than one year old were seropositive for *R. rickettsii*. There were no seropositivity differences between gender and breed of the animals. Ticks were found only on 2.9% of the horses studied.

The rate of seropositive horses found in this study is similar to that reported by Freitas et al. (2010) in São José dos Pinhais, Southern Paraná (9.33%), and lower than those found by HORTA et al. (2007) and LEMOS et al. (1996) (19 and 27.3%, respectively) in Southeast Brazil in BSF endemic areas. In contrast with our results, Toledo et al. (2010), while studying work horses in an urban area of Londrina, found a seroprevalence of 38.5%. This

**Table 1.** Seropositivity rates by indirect immunofluorescence assay (IFA) using two different *Rickettsia* antigens in horses from six farms in Northern Paraná, Brazil.

Farms studied	IFA positive reaction/Total collected (%)	
	Antigens	
	<i>R. rickettsii</i>	<i>R. parkeri</i>
Cambé	6/50 (12)	0/50 (0)
Santa Fé	1/44 (2.3)	0/44 (0)
Guaraci	1/25 (4)	0/25 (0)
Londrina 1	0/35 (0)	0/35 (0)
Londrina 2	4/34 (11.8)	2/34 (5.9)
Londrina 3	3/85 (3.5)	3/85 (3.5)
Total	15/273 (5.5)	5/273 (1.8)

**Table 2.** Antibody titers by indirect immunofluorescence assay (IFA) using two different *Rickettsia* spp. antigens in horses from six farms in Northern Paraná, Brazil.

Sera	Area	IFA titers		Probable causative species
		<i>R. rickettsii</i>	<i>R. parkeri</i>	
H2	Cambé	518	NR	<i>R. rickettsii</i>
H7	Cambé	64	NR	<i>R. rickettsii</i>
H16	Cambé	518	NR	<i>R. rickettsii</i>
H25	Cambé	256	NR	<i>R. rickettsii</i>
H37	Cambé	256	NR	<i>R. rickettsii</i>
H42	Cambé	256	NR	<i>R. rickettsii</i>
H109	Guaraci	64	NR	<i>R. rickettsii</i>
H112	Londrina 2	128	64	ND
H117	Londrina 2	128	NR	<i>R. rickettsii</i>
H138	Londrina 2	512	256	ND
H144	Londrina 2	128	NR	<i>R. rickettsii</i>
H191	Londrina 3	64	64	ND
H207	Londrina 3	64	64	ND
H226	Londrina 3	256	64	<i>R. rickettsii</i>
H184	Santa Fé	128	NR	<i>R. rickettsii</i>

NR = Nonreactive. ND = Not determined.

discrepancy could be explained by environmental and animal tick infestations, which were higher in urban Londrina than in horse farms.

Our study found only few *A. cajennense* ticks on horses and low seropositivity rate in these animals. Thus, rickettsia infection in humans transmitted by *A. cajennense* in these areas is unlikely because of very limited exposure of humans to ticks. And, in fact, there are no human BSF cases reported in this area.

Sangioni et al. (2005) recommended surveys of horse sera as useful methods for BSF surveillance in areas where humans are exposed to *A. cajennense* ticks. However, due to low seropositivity rates and under the conditions present in our study, horses cannot be used as sentinel animals because of low exposure to *Amblyomma* spp. ticks and consequent lower risk to humans and other animals living in these areas.

The titers against *Rickettsia* spp. found in this study ranged from 64 to 512 with three samples reaching 512. Nine animals reacted to *R. rickettsii* showing titers that were four-fold higher than those seen for *R. parkeri* suggesting the circulation of *Rickettsia* spp. homologous to *R. rickettsii* in the population studied.

IFA is the gold standard test for rickettsioses in humans and animals (LASCOLA; RAOULT, 1997). However, cross-reactivity between *Rickettsia* species is common and therefore identification of *Rickettsia* species causing infection in BSF is difficult (HORTA et al., 2004; LABRUNA et al., 2004; PACHECO et al., 2007).

The present study provides important evidence that horses in farms in Northern Paraná may have been exposed to rickettsiae identical or closely related to *R. rickettsii*. Monitoring rickettsial infections in horses is a valuable action for preventing BSF in humans exposed to ticks. Further epidemiological studies examining other potential domestic and wild reservoirs for BSF are needed in this area and other areas in the State of Paraná.

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