

# Rickettsiae of the Spotted Fever group in dogs, horses and ticks: an epidemiological study in an endemic region of the State of Rio de Janeiro, Brazil\*

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**ABSTRACT.** Cunha N.C., Lemos E.R.S., Rozental T., Teixeira R.C., Cordeiro M.D., Lisboa R.S., Favacho A.R., Barreira J.D., Rezende J. & Fonseca A.H. **Rickettsiae of the Spotted Fever group in dogs, horses and ticks: an epidemiological study in an endemic region of the State of Rio de Janeiro, Brazil.** [Rickettsias do grupo da febre maculosa em cães, equinos e carrapatos: um estudo epidemiológico em região endêmica do estado do Rio de Janeiro, Brasil.] *Revista Brasileira de Medicina Veterinária*, 36(3):294-300, 2014. Departamento de Epidemiologia e Saúde Pública, Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro, BR 465, Km 7, Seropédica, RJ 23890-000, Brasil. E-mail: adivaldo@ufrj.br

Spotted fever is a disease of which *Rickettsia rickettsii* is the most pathogenic agent. Its transmission is by tick bites and the infected ticks can act as vectors, reservoirs or amplifiers. The purpose of this paper is to assess the potential of dogs and horses as sentinels for brazilian spotted fever (BSF) emergence and become acquainted with the tick species in a municipal region of Resende, Rio de Janeiro State, Brazil, where five BSF cases in man were registered. Dog and horse blood samples were collected from rural and periurban properties to assess IgG anti-*Rickettsia rickettsii*, using the indirect immunofluorescence assay (IFA). First, an analysis was conducted to detect association between IFA results and answers obtained from a questionnaire. Afterwards, a multivariate investigation was undertaken that presented significant statistical differences. Ticks were collected directly from dogs and horses for taxonomic identification. Out of the 107 canine serum samples, 30 (28.0%) were reactive, with titers varying from 1:64 to 1:4096, and 77 (72.0%) were not reactive. Of 96 animals in

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the serum analysis of horses, 9 (9.4%) were reactive, all with titers of 1:64, and 87 (90.6%) were non-reactive. The tick species collected from dogs were *Rhipicephalus sanguineus*, *Amblyomma sculptum*, *Amblyomma aureolatum*, *Amblyomma ovale*, *Rhipicephalus microplus* and nymphs of *Amblyomma* sp., *R. sanguineus* and *R. microplus*. Adult ticks gathered from horses were *A. cajennense*, *R. microplus* and *Dermacentor nitens*, in addition to nymphs of *Amblyomma* sp., *R. microplus* and *D. nitens*. The results suggest that: (i) the habit of dogs entering forests and living in rural environments positively influenced the presence of anti-rickettsiae of the spotted fever group serum antibodies, (ii) horses were not good sentinels for this study area and (iii) *R. sanguineus* as well as *A. cajennense* ticks were the most prevalent ixodidae fauna of the region.

KEY WORDS. Brazilian Spotted Fever, *Rickettsia rickettsii*, ticks, sentinel, epidemiology.

**RESUMO.** A febre maculosa é uma doença que tem a bactéria *Rickettsia rickettsii* como a mais patogênica. Sua transmissão ocorre pela picada de carrapatos infectados, os quais atuam como vetores, reservatórios ou amplificadores. O objetivo deste trabalho foi avaliar o potencial de cães e equinos como sentinelas para febre maculosa brasileira (FMB) e conhecer a fauna de ixodídeos em uma região do município de Resende, estado do Rio de Janeiro, Brasil, localidade onde ocorreram cinco casos de FMB em humanos. Amostras sanguíneas de caninos e equinos foram coletadas de áreas rurais e periurbanas para a detecção de IgG anti-*Rickettsia rickettsii*, por meio da técnica de reação de imunofluorescência indireta (RIFI). Em um primeiro momento, uma análise foi realizada para identificar associação entre os resultados da RIFI e as respostas obtidas no questionário. Posteriormente, uma análise multivariada foi utilizada para avaliar os resultados que apresentaram diferença estatística. Os carrapatos foram coletados diretamente dos cães e equinos para a identificação taxonômica. Observou-se que das 107 amostras de cães, 30 (28,0%) foram reativas, com títulos variando de 1:64 a 1:4096, e 77 (72,0%) foram não reativas. Dos 96 equinos estudados, 9 (9,4%) foram reativos, todos com titulação de 1:64 e 87 (90,6%) foram não reativos. As espécies de carrapatos coletadas de cães foram *Rhipicephalus sanguineus*, *Amblyomma sculptum*, *Amblyomma aureolatum*, *Amblyomma ovale*, *Rhipicephalus microplus* e ninfas de *Amblyomma* sp., *R. sanguineus* e *R. microplus*. Os ixodídeos adultos coletados de equinos foram *A. cajennense*, *R. microplus* e *Dermacentor nitens*, além de ninfas de *Amblyomma* sp., *R. microplus* e *D. nitens*. Os resultados encontrados sugerem que: i) o hábito de caninos frequentarem matas e viverem em ambiente rural influenciou positivamente na presença de anticorpos séricos anti-rickettsia do grupo da febre maculosa; ii) os equinos não foram bons sentinelas para estudos epidemiológicos de FMB na região es-

tudada e (iii) que os carrapatos *R. sanguineus* e *A. cajennense* foram os mais prevalentes na fauna de ixodídeos da região.

PALAVRAS-CHAVE. Febre Maculosa Brasileira, *Rickettsia rickettsii*, carrapatos, sentinelas, epidemiologia.

## INTRODUCTION

The genus *Rickettsia* is composed of different species of Gram-negative and intracellular bacteria, with low viability outside their reservoirs and vectors. Spotted fever (SF) is a disease of which *Rickettsia rickettsii* is the most pathogenic agent, spread throughout the Americas, with cases reported in Canada, USA, Mexico, Costa Rica, Panama, Colombia, Brazil and Argentina (Dumler et al. 2005, Paddock et al. 2008).

In Brazil, Rock Mountain Spotted Fever (RMSF) is known as Brazilian spotted fever (BSF) and the notification of occurrences has been mandatory since 2001. The disease has received growing attention from public health authorities and resulting in increased reports of cases in different locations areas previously recognized as endemic, as well as the high mortality rate. Spotted fever (SF) transmission is by tick bites, the infected ticks can act as vectors, reservoirs or amplifiers of spotted fever group rickettsiae (SFGR) (Parola et al. 2005). Domestic and wild animals are involved in the disease ecology, and dogs as well as horses are used as sentinels in epidemiological studies to detect and/or confirm endemic sites (Lemos et al. 1996, Horta et al. 2004, Sangioni et al. 2005, Pinter et al. 2008).

Identifying tick species in areas considered endemic to BSF has been the subject of many studies to better understand the ecology of the disease. The presence of *A. sculptum* on different hosts, including man, and its abundance in endemic areas together with the SFGR diagnosis in this tick species, has determined it as the main BSF vector (Moreira & Magalhães 1935, Dias & Martins 1939, Lemos et

al. 1997, Guedes et al. 2005). To a lesser extent, other tick species are also involved in BSF epidemiology, such as *A. aureolatum* (Pinter & Labruna 2006) and *A. ovale* (Sabatini et al. 2010). *Rhipicephalus sanguineus* tick was found infected with *R. rickettsii* in the same area of our study (Cunha et al. 2009, Rozental et al. 2009) as well as in an endemic region of the state of São Paulo (Moraes-Filho et al. 2008), suggesting that this tick species may also be involved in BSF epidemiology.

The purpose of this paper was to assess the potential of dogs and horses as sentinels for BSF and become acquainted with the tick species in a municipal region of Resende, Rio de Janeiro State, Brazil.

## MATERIALS AND METHODS

### Characterization of the study area

The study was conducted in the district of Engenheiro Passos (22°30'9.46''S; 44°42'44.29''O), municipality of Resende, State of Rio de Janeiro, where in 2006, five cases of BSF took place in a single family, resulting in two deaths. The municipality of Resende is located in the mezzo-region, known as Sul Fluminense, and has a climate classified as tropical. The district of Engenheiro Passos is characterized as a rural area, crossed by an important river, Paraíba do Sul, which flows through the states of Rio de Janeiro, São Paulo and Minas Gerais. Traditionally, this used to be primarily a ranching region with secondary Atlantic Rainforest. However, in the last ten years, the pastures have been reforested with *Eucalyptus* and *Pine*, thereby altering the ecosystem of the region.

### Blood sample collections

The study was designed as cross-sectional, in the period from June 2006 to July 2007, with blood samples collected from 107 dogs and 96 horses from rural and periurban properties, no further than six kilometers from the rural area where the cases of BSF took place. Blood samples were collected by cephalic venipuncture of dogs and jugular vein of horses after signature of a consent form by the owners. Upon collection, an interview was conducted through a standardized questionnaire filled out by the owners to obtain information on the animals and management. Most of the rural dogs were mongrel, untethered and/or maintained in places close to the owners' homes, as pets, guardians and/or for hunting. The dogs in the periurban properties were raised loose or kept in kennels, for pets and watch dogs. The horses were for farm management, sale, shows or recreation.

### Indirect immunofluorescence Assay

The detection of IgG anti-*R. rickettsii* was performed through an indirect immunofluorescence assay (IFA), according to a cut-off point established by the manufacturer PANBIO/MEDVAX®, with a titer cut-off value of 1:64. Animal serum samples that were reactive were diluted until they reached the maximum titer.

### Data processing

Initially, the study consisted of a univariate analysis, adopting the chi-square with 95% significance level and was conducted to detect any association between IFA results and the answers obtained from a questionnaire involving sex and age for dogs and horses; urban or peri-urban habitat and habit entering the forest for dogs; and horses raised rurally or stud farm bred. A multivariate analysis was applied to variables that revealed statistically significant differences, and an *odds ratio* was also calculated. Data were analyzed by SAS software, version 9.2, for Windows.

### Ixodidae fauna study

In the period from June 2006 to July 2007, ticks were collected monthly from dogs and horses with ophthalmic tweezers or by manual torsion and conditioned in plastic flasks. Animals that had the ticks collected were those from which blood samples were taken. However, some of these animals were inspected more than once during the field work, and thus at the end of the study, there were 200 dog and 200 horse evaluations.

The species identification was performed based on the dichotomy key of Barros-Battesti et al. (2006). The ticks in the ingurgitated nymph stage were maintained at 28°C with a relative humidity of 85% until molting to their adult stage, when identification at a specific level was performed.

## RESULTS

### Seroepidemiological study

**Dogs:** Of the 107 canine serum samples analyzed, 30 (28.0%) were reactive and 77 (72.0%) not reactive. Of the reactive dogs, titers were 1:64 (76.9%), 1:128 (6.6%), 1:256 (3.3%), 1:512 (6.6%) and 1:4096 (6.6%). Analyses were carried out associating results obtained from serology considering sex and age, divided into two categories (up to 5 years old and over 5 years old), with no significant difference observed in these parameters. When analyzing habitat type (periurban or rural) and dogs customarily entering forested areas, there was a significant difference in relation to the animals' reactivity. This study observed a high chance of evidence of serologically reactive dogs regarding the type of environment in which they lived emphasizing forest access (Tables 1 and 2).

**Horses:** Nine of the 96 equines (9.4%) were reactive, all with a 1:64 titer, and 87 (90.6%) were non-reactive. There was no statistical difference between serological results in relation to sex, age (up to 10 and over 10 years old) and the site where the animals lived (stud farm or rural property). According to the *odds ratio* analysis, it was not possible to establish an occurrence probability of reactive animals with the parameters analyzed (Table 3).

Table 1. Results of indirect immunofluorescence assay (IFA) for detection of IgG anti *Rickettsia rickettsii* in dogs according to type of habitat (Periurban or Rural), habit of entering forests, age and sex. Serum samples from dogs at the district of Engenheiro Passos, municipality of Resende in the State of Rio de Janeiro, collected in the period from June 2006 to July 2007.

Variable	IFA (reactive)	IFA (not reactive)	P	Chi-squared	Odds ratio	Confidence interval (95%)
Periurban	5	31	0.0203*	5.3827	0.2968	0.1063-0.8287
Rural	25	46				
Forests Yes	27	51	0.0130*	6.1720	4.5882	1.3575-15.5078
No	3	26				
Up to 5 years	24	67	0.3609	0.8349	0.5970	0.2041-1.7463
Above 5 years	6	10				
Male	14	43	0.3927	0.7305	0.6919	0.3016-1.5873
Female	16	34				

\*Significant.

Table 2. Multivariate analysis of variables with significant statistical differences in reaction results of indirect immunofluorescence assay (IFA) for detection of IgG anti *Rickettsia rickettsii* in dogs at the district of Engenheiro Passos, municipality of Resende in the State of Rio de Janeiro, collected in the period from June 2006 to July 2007.

Variable	IFA (reactive)	IFA (not reactive)	Odds ratio	Confidence interval (95%)
Periurban	5	31	2.275	0.672-7.702
Rural	25	46		
Forests Yes	27	54	2.804	0.612-12.847
No	3	23		

### Ixodidae fauna

**Dogs:** 44% of the 200 dog inspections revealed ticks. A total of 470 specimens were collected: adult species of *R. sanguineus*, *A. cajennense*, *A. aureolatum*, *A. ovale*, *R. microplus* and nymphs of *Amblyomma* sp., *R. sanguineus* and *R. microplus*, in addition to 33 larvae (Table 4). Out of the ingurgitated nymphs collected, 29 developed to the adult stage, of which 25 were identified as species *A. cajennense* and four *R. sanguineus*. *Rhipicephalus sanguineus* ticks were found throughout the year, with no peak in relation to season. *A. aureolatum* was encountered in the months from April to November, the *Amblyomma* spp. nymphs from April to December, and no seasonal aspects apparently influenced the remaining species due to the small quantity of ixodidae.

**Horses:** 71% of the 200 horse examinations rendered ticks throughout all the months (all twelve months) of field work. Of the 975 ticks gathered, adults were identified as species *A. cajennense*, *R. microplus* and *D. nitens*. Additionally, nymphs of *Amblyomma* sp., *R. microplus* and *D. nitens* were collected together with 15 larvae (Table 5). All 38 nymphs that evolved to the adult stage were identified as *A. cajennense*. We noted that adult ixodidae of the *A. cajennense* species were present throughout the entire year, with peaks in the warmer, humid months. *Amblyomma* spp. nymphs were witnessed

from June to November, the remaining species evidencing no seasonal distribution.

## DISCUSSION

### Seroepidemiological study

In the present study, we observed that 28.0% of the dogs presented anti-*R. rickettsii* antibodies according to IFA, results similar to those in other studies in endemic areas with frequencies of 36.4%, 31.25% and 31.3% (Lemos et al. 1996, Horta et al. 2004, Sangioni et al. 2005) and where the primary vector was identified as *A. cajennense* tick. In studies where the primary vector of BSF was the *A. aureolatum* tick, frequencies of reactive dogs were 64% and 69.6%, with a greater percentage of reactive serum animals in relation to our study (Moraes-Filho et al. 2008, Pinter et al. 2008).

We determined that dogs with habits of frequenting forested areas demonstrated a 2.8 fold higher chance of producing anti-RGSF antibodies, which agrees with Saito et al. (2008), who noted that contact with pastures or forests was statistically associated to anti SFGR reactive serum dogs. Results of this study can be explained, because dogs that roam freely in rural areas are more likely to be parasitized by the SF potential vectors, such as *A. aureolatum* and *A. cajennense*, due to the host/vector proximity (Labruna et al. 2002, Guglielmone et al. 2003, Pinter et al. 2004). Moreover, replacement of pastures by *Eucalyptus* and *Pine* forests in the study site may have influenced the approximation of wild animals and their parasites to peridomestic environments. Pet dogs in rural areas generally roam freely in pastures and forests. Thus, the presence of these animals with such habits is a risk factor for BSF in man, due to the migration of the ticks from the sylvatic to the peridomestic environment.

Studies in Brazil confirm the serological evidence of other SFGR, determined by *R. rickettsii* in en-

Table 3. Results of indirect immunofluorescence assay (IFA) for detection of IgG anti *Rickettsia rickettsii* in horses according to type of habitat (Rural property or Stud farm), age and sex. Serum samples from horses at the district of Engenheiro Passos, Municipality of Resende in the State of Rio de Janeiro, collected in the period from June 2006 to July 2007.

Variable	IFA (reactive)	IFA (not reactive)	P	Chi-squared	Odds ratio	Confidence interval (95%)
Rural property	6	50	0.5943	0.2837	1.4800	0.3808-5.7515
Stud farm	3	37				
Up to 10 years	5	69	0.1065	2.6054	0.3261	0.0860-1.2358
Above 10 years	4	18				
Male	2	28	0.5394	0.3767	0.6020	0.1338-2.7096
Female	7	59				

demetic areas (Horta et al. 2004, Sangioni et al. 2005, Horta et al. 2007, Labruna et al. 2007, Pinter et al. 2008). Therefore, the majority of the results in this study with a 1:64 titer may be the result of a cross relation with other species of *Rickettsia* which possibly circulate in the region.

We observed that, despite the insignificant difference among age groups, there was a tendency for older animals to be reactive, which may be explained by either the prolonged exposure of older animals over time to tick parasites infected with *R. rickettsii* (Pinter et al. 2008) or lower immunity.

The frequency of anti-*R. rickettsii* antibodies in horses was 9.4%, which is different from findings in other studies in BSF endemic areas where frequencies of nearly 70% were reported (Lemos et al. 1996, Horta et al. 2004, Horta et al. 2007). In these studies, the authors indicated evidence that the primary vector of BSF was the *A. cajennense* tick.

Guedes et al. (2005) found, in an SF endemic area in Minas Gerais (MG), 1.28% of *A. cajennense* free-living ticks infected by *R. rickettsii*, and Pacheco et al. (2011) reported that, in a area neighboring in MG, 13.1% of the *R. sanguineus* ticks and none of the *A. cajennense* were found to be infected with *R. rickettsii*; but, at least 41% of these horses were seropositive for *R. rickettsii* with some indication of recent infection, as evidenced by high endpoint titres. However, the results of this study are similar for non-endemic areas, as Batista et al. (2010) reported a prevalence of 8.5%, and Sangioni et al. (2005) stated that in a non-endemic area, no sample from horses reacted positively. Thus, it is suggested that the *A. cajennense* tick is not involved in disease transmission in the study area, although it is prolific in the area. Thereby, it is recommended that in epidemiological studies for active search of sentinel animals such as dogs and horses, the researcher should be aware of what is involved in tick transmission of rickettsiae in the study area, because IFA reactive animal percentages can change considerably.

In our study, however, we noted a new profile for the BSF epidemiological characterization, where serological evidence of SFGR in horses was much lower than expected, reinforcing the possible function of other tick species in cases in the studied region. Reports in literature (Lemos et al. 1996, Horta et al. 2004, Sangioni et al. 2005, Pinter et al. 2008) have indicated both horses and dogs as efficient sentinels for BSF, yet in this study, horses were not good references for the circulation of the agent in the region.

#### **Ixodidae fauna study**

During this study, 1,445 ticks were collected from dogs and horses in larval, nymph and adult stages. We noted that they were all similar to tick species presented in other studies in BSF endemic areas (Lemos et al. 1997, Rozental et al. 2002, Sangioni et al. 2005, Horta et al. 2007). Seven hundred forty of the specimens were *A. cajennense*, throughout the period of field work. There was a tendency of for adults in the more humid months and nymphs from June to November (Brazilian winter and early spring), but we cannot confirm any suspicion of a pattern for this species (Lemos et al. 1997, Labruna et al. 2002), because the research was performed in only one single year. The *R. sanguineus* tick was apparent during all months of collections, attaining the status of most prevalent tick species among dogs both in rural and periurban habitats. In urban environments, this species is very common for dogs according to Ribeiro et al. (1997), as it is in rural environments possessing similar characteristics to urban (Labruna et al. 2001).

The report by Labruna et al. (2008) of the high *R. sanguineus* nymph susceptibility to *R. rickettsii* when infected in the larval phase, together with cases of attacking man (Dantas-Torres et al. 2006) and a description of its infection with the *R. rickettsii* bacteria in the same area of our study (Cunha et al. 2009) and other endemic areas (Demma et al. 2005, Wikswo et al. 2007, Moraes-Filho et al. 2008,

Table 4. Ticks collected from dogs from 19 properties at the district of Engenheiro Passos, Municipality of Resende in the State of Rio de Janeiro in the period from June 2006 to July 2007.

Species of ticks	Total ticks captured (%)	Occurrence of dogs with parasites	Intensity of ticks per dog*	No. of properties with infestation(n/N)
<b>Adults</b>				
<i>Rhipicephalus sanguineus</i>	197 (41.9)	39	5.1±6.0 (1-26)	10/19
<i>Amblyomma cajennense</i>	8 (1.7)	2	4.0±4.2 (1-7)	1/19
<i>Amblyomma aureolatum</i>	27 (5.7)	13	2.1±2.1 (1-8)	6/19
<i>Amblyomma ovale</i>	5 (1.1)	3	1.7±0.9 (1-3)	2/19
<i>Rhipicephalus microplus</i>	12 (2.6)	5	2.4±2.2 (1-6)	3/19
<b>Nymphs</b>				
<i>Rhipicephalus sanguineus</i>	19 (4.0)	9	2.1±1.3 (1-4)	5/19
<i>Amblyomma</i> sp.	167 (35.5)	51	3.3±2.5 (1-9)	11/19
<i>Rhipicephalus microplus</i>	2 (0.4)	2	1 (1)	2/19
Larvae	33 (7.0)	6	4.7±8.1 (1-23)	5/19

\*average± standard deviation and amplitude.

n= Number of properties with dogs with parasites.

N= Total of properties visited.

Table 5. Ticks collected from horses from 12 properties at the District of Engenheiro Passos, Municipality of Resende in the State of Rio de Janeiro in the period from June 2006 to July 2007.

Species of ticks	Total ticks captured (%)	Occurrence of horses with parasites	Intensity of ticks per horse*	Properties with infestation (n/N)
<b>Adults</b>				
<i>Amblyomma cajennense</i>	740 (75.9)	113	6.5±4.5(1-18)	10/12
<i>Dermacentor nitens</i>	56 (5.7)	14	3.9±2.8(1-10)	5/12
<i>Rhipicephalus microplus</i>	58 (5.9)	15	3.9±5.8(1-24)	6/12
<b>Nymphs</b>				
<i>Amblyomma</i> sp.	85 (8.7)	32	2.7±2.1(1-10)	9/12
<i>Dermacentor nitens</i>	5 (0.5)	1	5 (1)	1/12
<i>Rhipicephalus microplus</i>	15 (1.5)	7	2.1±1.8(1-6)	4/12
Larvae	16 (1.6)	9	1.8±1.2(1-4)	5/12

\*average± standard deviation and amplitude.

n= Number of properties with horses with parasites.

N= Total of properties visited.

Rozental et al. 2009) leads us to believe that this tick is associated with BSF epidemiology.

The area studied offers the necessary conditions for *A. aureolatum* species survival and proliferation (Rodrigues et al. 2002, Guglielmone et al. 2003, Pinter et al. 2004), with the presence of domestic dogs, eucalyptus vegetation and proximity to a natural reserve (Itatiaia National Park) containing wild animals. In Brazil, there are areas where this tick species is the vector responsible for BSF transmission (Pinter & Labruna 2006). However, our study did not test these ticks for rickettsial infection, although, due to the ecology of the site, we cannot discard the possibility that *A. aureolatum* is also involved in BSF epidemiology.

## CONCLUSIONS

Based on the results in the study region, we may confirm the role of dogs as important sentinels for BSF. Additionally, we established that: (i) the habit of dogs roaming forests while living in rural en-

vironments positively influenced the presence of anti-RGSF serum antibodies, (ii) horses were not good sentinels and (iii) *R. sanguineus* and *A. cajennense* ticks were the most prevalent ixodidae of the region.

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