

# Serological evidence of exposure to tick-borne agents in opossums (*Didelphis* spp.) in the state of São Paulo, Brazil

Evidência sorológica de exposição a agentes transmitidos por carrapatos em gambás (*Didelphis* spp.) no estado de São Paulo, Brasil

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## Abstract

This work involved a serological investigation of tick-borne pathogens in opossums in eight municipalities of the state of São Paulo, Brazil. Serum samples from 109 opossums (91 *Didelphis aurita* and 18 *Didelphis albiventris*) were tested to detect antibodies to *Rickettsia rickettsii* (Taiacu strain, 1:64 cut-off) and *Ehrlichia canis* (São Paulo strain, 1:40 cut-off), by indirect immunofluorescence assay (IFA); and against *Borrelia burgdorferi* (strain G39/40) by enzyme-linked immunosorbent assay (ELISA). The presence of antibodies to anti-*R. rickettsii*, anti-*E. canis* and anti-*B. burgdorferi* was detected in 32 (29.35%), 16 (14.67%) and 30 (27.52%) opossums, respectively. Opossum endpoint titers ranged from 64 to 1,024 for *R. rickettsii*, from 40 to 160 for *E. canis*, and from 400 to >51,200 for *B. burgdorferi*. These serological results suggest that opossums have been exposed to *Rickettsia* spp., *Ehrlichia* spp., and *B. burgdorferi*-related agents in the state of São Paulo. Our study underscores the need for further research about these agents in this study area, in view of the occurrence of Spotted Fever and Baggio-Yoshinari Syndrome disease in humans in the state of São Paulo, Brazil.

**Keywords:** *Didelphis albiventris*, *Didelphis aurita*, *Rickettsia*, *Ehrlichia*, *Borrelia*, ticks.

## Resumo

O presente estudo investigou evidência sorológica de agentes transmitidos por carrapatos em gambás em oito municípios do Estado de São Paulo, Brasil. Amostras de soro de 109 gambás (91 *Didelphis aurita* e 18 *Didelphis albiventris*) foram testadas para detecção de anticorpos contra *Rickettsia rickettsii* (cepa Taiacu, ponto de corte 1:64) *Ehrlichia canis* (cepa São Paulo, ponto de corte 1:40), pela reação de imunofluorescência indireta (RIFI); e contra *Borrelia burgdorferi* (cepa G39/40) pelo teste imunoenzimático (ELISA). A presença de anticorpos anti-*R. rickettsii*, anti-*E. canis* e anti-*B. burgdorferi* foi detectada em 32 (29,35%), 16 (14,67%) e 30 (27,52%) gambás, respectivamente. Os títulos finais variaram de 64 a 1.024 para *R. rickettsii*, de 40 a 160 para *E. canis*, e de 400 a >51.200 para *B. burgdorferi*. Esses resultados sugerem que os gambás foram expostos a agentes relacionados à *Rickettsia* spp., *Ehrlichia* spp., e *B. burgdorferi* no Estado de São Paulo. Neste estudo salienta a necessidade de novas pesquisas sobre esses agentes nessas áreas de trabalho, devido à ocorrência da Febre Maculosa e da Síndrome Baggio-Yoshinari em humanos no Estado de São Paulo, Brasil.

**Palavras-chave:** *Didelphis albiventris*, *Didelphis aurita*, *Rickettsia*, *Ehrlichia*, *Borrelia*, carrapatos.

## Introduction

Worldwide tick-borne diseases have been highlighted in both human and veterinary medicine. Various tick species act as vectors of several pathogens, and many wild animals act as reservoirs for these agents. Therefore, rickettsiosis, ehrlichiosis, and borreliosis

have been reported around the world to cause illness in animals and humans (CASTELLAW et al., 2011).

Ticks are considered vectors of a large number of infectious agents (HOOGSTRAAL, 1967). Only a few of the approximately 825 species of ticks described worldwide are directly relevant to public health, because of the possibility of these ticks parasitizing humans (OLIVER, 1989). However several other species that have never been described in humans may play an important, albeit

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indirect role in public health because they contribute to the enzootic maintenance of infectious agents in nature (HOOGSTRAAL, 1967). In this context, the genera *Rickettsia*, *Ehrlichia* and *Borrelia* are important tick borne agents that cause disease in humans and animals (DUMLER et al., 2001; KRUPKA et al., 2007; PAROLA et al., 2013).

In Brazil, opossums are infested with different species of ticks and can often act as amplifier hosts of some pathogens, such as *R. rickettsii* (HORTA et al., 2009), which is the world's most pathogenic species of *Rickettsia* and is responsible for causing Brazilian Spotted Fever (BSF) (PAROLA et al., 2013). These animals are abundant in all areas endemic for BSF, where they present high antibody titers to *R. rickettsii* (HORTA et al., 2007) and are usually infested with ticks of the *A. cajennense* complex, the most important vector in South America (LABRUNA, 2009).

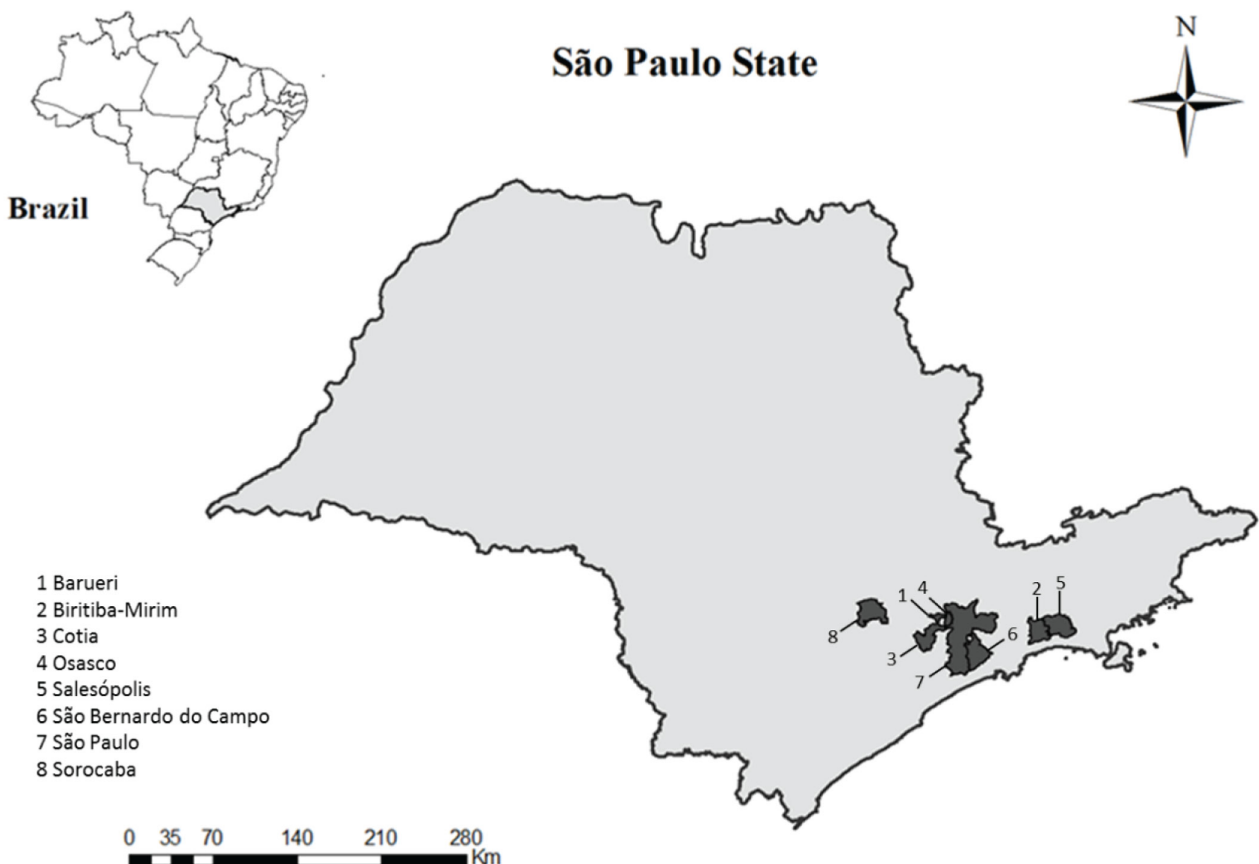
As for the genus *Ehrlichia*, there are few studies about the presence of antibodies in opossums. Animals seropositive to *Ehrlichia chaffeensis* have been reported in the United States (CASTELLAW et al., 2011). On the other hand, Lyme disease, a common zoonosis in the northern hemisphere (United States, Europe and Asia), is considered an infectious disease caused by spirochetes of the *Borrelia burgdorferi* sensu lato complex which is transmitted by ticks of the *Ixodes ricinus* complex (STEERE, 2001). In the case of borreliosis, this disease can be caused by a number of species of worldwide distribution, such as *Borrelia*

*burgdorferi* (United States and Europe); *Borrelia garinii* and *Borrelia afzelii* (Europe), and *Borrelia japonica* (Asia) (STEERE, 2001; FELSENFELD, 1965; BARANTON et al., 1992). These species were isolated from *Ixodes* sp. ticks and another spirochete, called *Borrelia lonestari*, found in the United States, was isolated from *Amblyomma americanum* (BARBOUR et al., 1996). In Brazil, the disease is known as Baggio-Yoshinari Syndrome (BYS) or Lyme-like disease, and the first cases similar to this disease in the country were described in 1992 in siblings, who developed erythema migrans, flu-like symptoms and arthritis after being bitten by ticks. However, *B. burgdorferi* has so far not been isolated in this country. Specimens of the genera *Ixodes* and *Amblyomma* naturally infected with unidentified spirochetes have been found in areas where Lyme-like disease has been reported (YOSHINARI et al., 2010).

This work involved a serological evaluation of tick-borne agents (*Ehrlichia* spp., *Rickettsia* spp. and *Borrelia* spp.) in blood collected from opossums (*Didelphis* spp.) in the state of São Paulo, Brazil.

## Materials and Methods

This study was conducted in eight municipalities (Barueri, Biritiba Mirim, Cotia, Osasco, Salesópolis, São Bernardo do Campo, São Paulo, and Sorocaba) in the state of São Paulo, Brazil (Figure 1). Free-living opossums were captured between 2003 and 2008. The opossums were anesthetized with 15-30 mg/kg



**Figure 1.** Geographic locations of the 8 municipalities in which the opossums were caught. State of São Paulo, Brazil.

of ketamine prior to drawing blood samples. The serum was prepared individually by centrifugation (3.000 g for 15 minutes), and stored at  $-20^{\circ}\text{C}$  for the immunofluorescence assay (IFA) and enzyme-linked immunosorbent (ELISA) assays.

This study was approved by the Ethics Committee on Animal Use of the University of São Paulo (under Protocol FMVZ 301/2003, ICB 495/CEP/2003), and by the Brazilian Institute of Environment and Renewable Natural Resources – IBAMA (under Protocol No. 02027.023912/2002)

The IFA was used to detect antibodies to *Ehrlichia* spp. and *Rickettsia* spp. Anti-*Ehrlichia* spp. antibodies were evaluated using the São Paulo strain of *E. canis* with a cut-off point at an initial dilution of 1:40 (AGUIAR et al., 2007). Anti-*Rickettsia* spp. antibodies were evaluated using the Taiaçu strain of *R. rickettsii*, with an initial dilution of 1:64 as previously described (PINTER & LABRUNA, 2006). Each slide contained serum previously shown to be non-reactive (negative control) and serum known to be reactive (positive control, obtained by experimentally infection of the agents in opossums). Anti-*Borrelia* antibodies were evaluated by ELISA using the North American strain G39/40 of *B. burgdorferi* as antigen (JOPPERT et al., 2001).

## Results

Sera samples were obtained from 109 young and adult opossums, i.e., 91 *Didelphis aurita* and 18 *Didelphis albiventris*. The presence of anti-*Rickettsia* sp., anti-*Ehrlichia* sp., and anti-*B. burgdorferi* antibodies was detected in 32 (29.35%), 16 (14.67%) and 30 (27.52%) opossums, respectively (Table 1). Opossum endpoint titers ranged from 64 to 1,024 for *R. rickettsii*, from 40 to 160 for *E. canis*, and from 400 to >51,200 for *B. burgdorferi*.

## Discussion

This study detected the presence of antibodies against *Rickettsia* spp., *Ehrlichia* spp. and *B. burgdorferi* in free-living opossums in the state of São Paulo. In Brazil, serological studies in opossums infected experimentally with *R. rickettsii*, *R. parkeri*, *R. bellii*, and *R. felis* have shown that these animals have enough seroconversion to be positive by IFA (HORTA et al., 2009, 2010). Studies on

free-living opossums in the states of São Paulo and Minas Gerais detected reactivity rates of 59.5% (56/94) and 42.1% (16/38), respectively, against *Rickettsia*-specific antigen (HORTA et al., 2007; MILAGRES et al., 2010). More recently Silveira et al. (2015) found free living opossums seroreactive to *Rickettsia* spp. (*R. parkeri*, *R. rickettsii*, *R. amblyommii* and *R. rhipicephali*) from Paulicéia, state of São Paulo. Although our results indicated lower percentages than those found in another studies, we found evidence of rickettsial infection circulating among opossums in Brazil, an important synanthropic animal commonly present in areas endemic for BSE. Horta et al. (2009) had already demonstrated that *R. rickettsii* is able to infect opossums without clinical signs and to develop rickettsemia that can cause infection in guinea pigs and ticks. These results consistently demonstrate that opossums can serve as amplifier hosts for the horizontal transmission of this pathogen to *A. cajennense* ticks. Thus, it can be inferred that the participation of opossums in the epidemiology of the disease is very important for the maintenance of the agent in nature, not only in ticks but also in vertebrates hosts, including humans.

This study reports the presence of anti-*Ehrlichia* spp. antibodies in opossums. Evidence of the exposure of opossums to *E. chaffeensis* has been reported in the USA (CASTELLAW et al., 2011). Although research into *Ehrlichia* spp. infections in opossums are scanty, our results indicate the possibility that they occur due to the presence of vector ticks. The existence of cross-reactivity observed in the IFA among the species of the genus *Ehrlichia* (HARRUS & WANER, 2011) implies the possibility that an unrecognized ehrlichial agent is circulating among these animals. To date, only *E. canis* and an ehrlichial agent related to cattle and *Rhipicephalus* (*Boophilus*) *microplus* ticks have been isolated in Brazil (CABEZAS-CRUZ et al., 2012; AGUIAR et al., 2013, 2014).

Our results support the evidence of *B. burgdorferi* infection in opossums (27.52%). Similar results were observed in the state of Minas Gerais, where the presence of antibodies against *B. burgdorferi* was detected in 7.69% opossums (MONTANDON et al., 2014). Other studies in Brazil have reported humans and animals seropositive for *B. burgdorferi* (YOSHINARI et al., 2010; CORRADI et al., 2006; MADUREIRA et al., 2007; MONTANDON et al., 2014), demonstrating different prevalence rates in dispersed areas and showing that both domestic and wild animals are becoming

**Table 1.** Presence of anti-*R. rickettsii*, anti-*E. canis*, and anti-*B. burgdorferi* antibodies in opossums (*D. albiventris* and *Didelphis aurita*) from the state of São Paulo, Brazil.

Municipality	<i>Rickettsia rickettsii</i>		<i>Ehrlichia canis</i>		<i>Borrelia burgdorferi</i>	
	<i>D. albiventris</i>	<i>D. aurita</i>	<i>D. albiventris</i>	<i>D. aurita</i>	<i>D. albiventris</i>	<i>D. aurita</i>
Barueri	-	0% (0/1)	-	0% (0/1)	-	0% (0/1)
Biritiba-Mirim	100% (1/1)	25% (3/12)	0% (0/1)	33.33% (4/12)	100% (1/1)	50% (6/12)
Cotia	-	100% (1/1)	-	0% (0/1)	-	100% (1/1)
Osasco	-	50% (1/2)	-	0% (0/2)	-	50% (1/2)
Salesópolis	-	60% (3/5)	-	20% (1/5)	-	40% (2/5)
São Bernardo do Campo	50% (1/2)	20% (1/5)	50% (1/2)	20% (1/5)	50% (1/2)	40% (2/5)
São Paulo	-	34% (17/50)	-	12% (6/50)	-	26% (13/50)
Sorocaba	20% (3/15)	6.67% (1/15)	13.33% (2/15)	6.67% (1/15)	20% (3/15)	0% (0/15)
Total	29.35% (32/109)		14.67% (16/109)		27.52% (30/109)	

infected with a closely related *Borrelia* spp., although the agents have never been isolated in Brazil.

Our findings reinforce the probable presence of these agents in opossums in eight municipalities in the state of São Paulo and emphasize the need for further research into these pathogens in this study area, in view of the occurrence of BSF and BYS in humans in the state of São Paulo.

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