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

Andréia Lima Tomé Melo¹; Daniel Moura de Aguiar¹; Mariana Granziare Spolidorio²; Natalino Hajime Yoshinari¹; Eliana Reiko Matushima³; Marcelo Bahia Labruna³; Mauricio Claudio Horta⁴⁵

¹ Universidade Federal do Mato Grosso – UFMT, Cuiabá, MT, Brasil
² Universidade de São Paulo – USP, São Paulo, SP, Brasil
³ Universidade Federal do Vale do São Francisco – Univasf, Petrolina, PE, 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 (Taiaçu 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 Borrelia-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 Taiaçu, 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 Borrelia-related agents 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
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
of ketamine prior to drawing blood samples. The serum was prepared individually by centrifugation (3.000 g for 15 minutes), and stored at −20 °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

<table>
<thead>
<tr>
<th>Municipality</th>
<th><em>Rickettsia rickettsii</em></th>
<th><em>Ehrlichia canis</em></th>
<th><em>Borrelia burgdorferi</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D. albiventris</td>
<td>D. aurita</td>
<td>D. albiventris</td>
</tr>
<tr>
<td>Barueri</td>
<td>0% (0/1)</td>
<td>-</td>
<td>0% (0/1)</td>
</tr>
<tr>
<td>Birita-Mirim</td>
<td>100% (1/1)</td>
<td>25% (3/12)</td>
<td>0% (0/1)</td>
</tr>
<tr>
<td>Cotia</td>
<td>-</td>
<td>100% (1/1)</td>
<td>-</td>
</tr>
<tr>
<td>Osasco</td>
<td>-</td>
<td>50% (1/2)</td>
<td>-</td>
</tr>
<tr>
<td>Salesópolis</td>
<td>-</td>
<td>60% (3/5)</td>
<td>-</td>
</tr>
<tr>
<td>São Bernardo do Campo</td>
<td>50% (1/2)</td>
<td>20% (1/5)</td>
<td>50% (1/2)</td>
</tr>
<tr>
<td>São Paulo</td>
<td>-</td>
<td>34% (17/50)</td>
<td>-</td>
</tr>
<tr>
<td>Sorocaba</td>
<td>20% (3/15)</td>
<td>6.67% (1/15)</td>
<td>13.33% (2/15)</td>
</tr>
<tr>
<td>Total</td>
<td>29.35% (32/109)</td>
<td>14.67% (16/109)</td>
<td>27.52% (30/109)</td>
</tr>
</tbody>
</table>
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 BSY and BYS in humans in the state of São Paulo.

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**References**


Tick-borne agents in opossums


