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Survey of Ticks (Acari: Ixodidae) and Their *Rickettsia* in an Atlantic Rain Forest Reserve in the State of São Paulo, Brazil

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Survey of Ticks (Acari: Ixodidae) and Their *Rickettsia* in an Atlantic Rain Forest Reserve in the State of São Paulo, Brazil

GUILHERME S. SABATINI, ADRIANO PINTER, FERNANDA A. NIERI-BASTOS, ARLEI MARCILI, AND MARCELO B. LABRUNA


**ABSTRACT** The current study investigated the occurrence of ticks and their rickettsiae in the Serra do Mar State Park, which encompasses one of the largest Atlantic rain forest reserves of Brazil. From July 2008 to June 2009, a total of 2,439 ticks (2,196 free living and 243 collected on hosts) was collected, encompassing the following 13 species: *Amblyomma aureolatum* (Pallas), *Amblyomma brasilienise* Aragão, *Amblyomma dubitatum* Neumann, *Amblyomma fuscum* Neumann, *Amblyomma incisum* Neumann, *Amblyomma longirostre* (Koch), *Amblyomma naponense* (Packard), *Amblyomma nodosum* Neumann, *Amblyomma ovale* Koch, *Haemaphysalis juxtakochi* Cooley, *Ixodes aragaoi* Fonseca, *Ixodes loricatus* Neumann, and *Rhipicephalus sanguineus* (Latreille). Ticks were submitted to polymerase chain reaction assays targeting portions of the rickettsial genes gltA and ompA. Polymerase chain reaction products were DNA sequenced and compared with corresponding sequences available in GenBank. *Rickettsia bellii*, a rickettsia of unknown pathogenicity, was detected in one *A. aureolatum* and three *A. incisum* specimens. At least 8.8% (3/34) of the free-living *A. ovale* ticks, 13.6% (8/59) of the *A. ovale* ticks collected from dogs, and 1.9% (1/54) of the *R. sanguineus* (Latreille) ticks were found to be infected by *Rickettsia* sp strain Atlantic rain forest, a novel strain that has been shown to cause an eschar-associated spotted fever in the state of São Paulo. Our results suggest that *A. ovale* is the vector of *Rickettsia* sp strain Atlantic rain forest in the state of São Paulo.

**KEY WORDS** ticks, *Amblyomma*, *Haemaphysalis*, *Ixodes*, *Rickettsia*

The tick fauna of Brazil is currently composed of 61 tick species, divided into the families Ixodidae and Argasidae. The former family is the largest, composed of the genera *Amblyomma* (30 species), *Ixodes* (8), *Haemaphysalis* (3), *Rhipicephalus* (2), and *Dermacentor* (1) (Dantas-Torres et al. 2009). Ticks of the genus *Amblyomma* are of greater medical importance in South America, where the vast majority of human infestations refer to *Amblyomma* species (Guglielmonne et al. 2006), and because several rickettsial agents pathogenic for humans are transmitted by *Amblyomma* ticks (Labruna 2009).

Bacteria within the genus *Rickettsia* are obligate intracellular short rods, 0.3–0.5 × 0.8–2.0 μm. Gram negative, primarily associated with invertebrate hosts. Pathogenic *Rickettsia* species have been classically classified into the spotted fever group (SFG) and the typhus group (Fournier and Raoult 2007). More recently, part of the SFG species was split into a new group, designated as the transitional group (Gillespie et al. 2007). Currently, all SFG species are primarily associated with ticks (Weinert et al. 2009). Whereas most of the SFG species are pathogenic for humans, to whom they are transmitted via tick bites, other SFG species are still considered of unknown pathogenicity (Fournier and Raoult 2007, Weinert et al. 2009).

The Brazilian Atlantic rain forest is characterized by species diversity higher than most of the Amazonian forests (Morellato and Haddad 2000). One of the greatest remnants of this forest is the Serra do Mar State Park, a nature reserve of 315,000 ha along the Serra do Mar mountain chain, just east to the largest metropolitan area of South America, which is composed of São Paulo city and various adjacent cities. The current study investigated the occurrence of ticks within the Serra do Mar State Park. In addition, we investigated rickettsial infection in part of the collected ticks.

**Materials and Methods**

This study was conducted in the Itutinga-Pilões branch of the Serra do Mar State Park, state of São Paulo, Brazil. For this purpose, six sites of the park (trails 1–6) were visited during consecutive 6–12 mo, from July 2008 to June 2009. On each visit in each site, the vegetation on both sides of a trail was sampled for 60 min, always by four investigators, by using flagging.
stages of most impossible to identify, by morphology, the immature counted. While conducting the current study, it was ally available for examination and collection of ticks. These ticks were also taken alive to the laboratory and were always found as large groups, from dozens to thousands of individuals per cluster. For convenience, were not counted individually because they larvae were brought alive to the laboratory, where adults species identiﬁcation of the former immature ticks, obtained from the engorged nymphs were used for previded by feeding them on tick-bite naive rabbits, as conducted by visual search of questing ticks on the vegetation, as previously reported (Szabo et al. 2004). In each set of reactions, negative control tubes containing water were included, and also a positive control tube containing DNA of the strain NOD of Rickettsia parkeri. Samples that yielded visible ampliﬁcations of the expected size by the gltA-PCR were further tested by a second PCR assay targeting a 532-bp fragment of the rickettsial gene ompA, as previously described (Regnery et al. 1991). All ompA-PCR ampliﬁcations of the expected size were submitted to direct DNA sequencing in an automated ABI Prism 310 genetic analyzer (Applied Biosystems, Foster City, CA), as well as the gltA-PCR ampliﬁcations from ticks that were negative by the ompA-PCR. The BLAST program (National Center for Biotechnology Information, Bethesda, MD) was used to compare appropriate similarities of the rickettsial partial sequences generated in the current study.

Results

A total of 2,439 ticks (2,196 free living and 243 collected on hosts) encompassing 13 different species was collected during the current study, as shown in Table 2. Two Rickettsia species were found infecting ticks (Table 3). Rickettsia bellii was detected in one Amblyomma aureolatum (Pallas), one Amblyomma

Table 1. Details of the six trails used for tick sampling in the Serra do Mar State Park, state of São Paulo, Brazil

<table>
<thead>
<tr>
<th>Trails</th>
<th>Coordinates</th>
<th>Altitude (m above sea level)</th>
<th>Distance from trail 1 (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>23°49’29.4”S 46°30’47.4”W</td>
<td>759</td>
<td>1 —</td>
</tr>
<tr>
<td>2</td>
<td>23°51’28.5”S 46°30’43.1”W</td>
<td>746</td>
<td>3,930</td>
</tr>
<tr>
<td>3</td>
<td>23°53’36.2”S 46°34’22.6”W</td>
<td>764</td>
<td>10,600</td>
</tr>
<tr>
<td>4</td>
<td>23°55’47.6”S 46°31’07.5”W</td>
<td>69</td>
<td>11,700</td>
</tr>
<tr>
<td>5</td>
<td>23°47’35.4”S 46°18’33.3”W</td>
<td>993</td>
<td>20,600</td>
</tr>
<tr>
<td>6</td>
<td>23°54’17.7”S 46°29’28.3”W</td>
<td>36</td>
<td>9,300</td>
</tr>
</tbody>
</table>

Table 2. Ticks found in the Serra do Mar State Park, state of São Paulo, Brazil, 2008–2009

<table>
<thead>
<tr>
<th>Tick species</th>
<th>Trailsa</th>
<th>No. ticks collected on vegetation</th>
<th>Animals (no. parasitized)—no. ticks collected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adults</td>
<td>Nymphs</td>
<td>Larval clusters</td>
</tr>
<tr>
<td>Amblyomma aureolatum</td>
<td>1,2,5</td>
<td>7</td>
<td>—</td>
</tr>
<tr>
<td>Amblyomma brasiliense</td>
<td>3,4</td>
<td>36</td>
<td>—</td>
</tr>
<tr>
<td>Amblyomma dubitatum</td>
<td>3</td>
<td>—</td>
<td>11</td>
</tr>
<tr>
<td>Amblyomma fuscum</td>
<td>4</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Amblyomma incisum</td>
<td>1,2,3,4,6</td>
<td>101</td>
<td>482</td>
</tr>
<tr>
<td>Amblyomma longicervix</td>
<td>1</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>Amblyomma naponense</td>
<td>4</td>
<td>2</td>
<td>—</td>
</tr>
<tr>
<td>Amblyomma nodosum</td>
<td>4</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Amblyomma ovale</td>
<td>4,5,6</td>
<td>34</td>
<td>—</td>
</tr>
<tr>
<td>Haemaphysalis juxtakochi</td>
<td>1,2,3,4</td>
<td>34</td>
<td>146</td>
</tr>
<tr>
<td>Iodes aragai</td>
<td>5</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>Iodes lorettus</td>
<td>4</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Rhipicephalus sanguineus</td>
<td>4</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Amblyomma sppb</td>
<td>1,2,3,4,5,6</td>
<td>1,295</td>
<td>26</td>
</tr>
<tr>
<td>Total</td>
<td>216</td>
<td>1,934</td>
<td>46</td>
</tr>
</tbody>
</table>

a See trail geographic information in Table 1.

b Subadult ticks that died before reaching the adult stage in the laboratory, precluding their identiﬁcation to species.
Rhipicephalus sanguineus 1.9% (1/54) of the (8/59) of the 8.8% (3/34) of the free-living Bank (CP000087, DQ865204, EU567181). However, in addition, no distribution of between these trials could have provided different microbiota and vertebrate fauna, the different altitude between these trials could have provided different microclimatic conditions, resulting in this marked distribution of A. aureolatum and A. ovale in the Serra do Mar State Park.

Discussion

The 13 tick species found in the current study have been previously reported in Atlantic rain forest areas of the state of São Paulo (Aragão and Fonseca 1961, Barros-Battesti and Knysak 1999, Barros-Battesti et al. 2005, Szabó et al. 2009). Adults of A. aureolatum and A. ovale were collected in relatively large amounts, both on the vegetation and on dogs during the current study. Dogs have been reported to be one of the main hosts for the adult stage of these two tick species, whereas immature stages seem to feed primarily on birds and small rodents (Guglielmone et al. 2006). Because the SFG agent Rickettsia sp strain Atlantic rain forest, were found infecting ticks in the current study (Table 3). R. bellii is of unknown pathogenicity, and has been previously reported infecting A. aureolatum, A. incisum, and A. ovale ticks from other Atlantic rain forest areas in the state of São Paulo (Pinter and Labruna 2006, Pacheco et al. 2008). However, strain Atlantic rain forest is the etiological agent of a novel SFG rickettsiosis recently reported in a human patient in the state of São Paulo, Brazil (Spolidorio et al. 2010), and is the subject of much speculation as to its taxonomic status (see Walker and Ismail 2008 and Goddard 2009 for further discussion). According to the original case-report description, infection by the strain Atlantic rain forest was acquired through the bite of an infected tick in Barra do Una, an Atlantic rain forest area of low altitude (<100 m above sea level), ≈80 km southwest of trails 1–6 of the current study. In fact, this Barra do Una locality belongs to the Serra do Mar State Park along the Atlantic coast in the state of São Paulo, where A. ovale is found throughout low altitude areas (unpublished data from the Coleção Nacional de Carrapatos). Unfortunately, the tick specimen that transmitted rickettsia to the patient in Barra do Una was not saved for taxonomic identification. A. ovale is an important human-biting tick in Brazil (Labruna et al. 2005, Guglielmone et al. 2006). Thus, our results suggest that A. ovale is the main vector of Rickettsia sp strain Atlantic rain forest in the state of São Paulo, especially at low altitude areas among the Serra do Mar State Park, where thousands of tourists visit annually.

We also found strain Atlantic rain forest in the tick R. sanguineus, which is highly specific to dogs, and has been seldom reported biting humans in South America (Guglielmone et al. 2006). Because the R. sanguineus specimens of the current study were collected on dogs together with A. ovale specimens in trail 4 (data not shown), it is possible that the rickettsial infection found in this single R. sanguineus specimen was acquired through cofeeding with an infected A. ovale tick on a dog. Cofeeding transmission might also be the reason for the higher infection rate of A. ovale collected from dogs than from vegetation (Table 3).

Finally, intense serologic cross-reactions were shown between strain Atlantic rain forest and Rickettsia sp refers to strain Atlantic rain forest reported by Spolidorio et al. (2010).

Two different rickettsiae, R. bellii and the SFG agent Rickettsia sp strain Atlantic rain forest, were tested for infecting ticks in the current study (Table 3). R. bellii is of unknown pathogenicity, and has been previously reported infecting A. aureolatum, A. incisum, and A. ovale ticks from other Atlantic rain forest areas in the state of São Paulo (Pinter and Labruna 2006, Pacheco et al. 2008). However, strain Atlantic rain forest is the etiological agent of a novel SFG rickettsiosis recently reported in a human patient in the state of São Paulo, Brazil (Spolidorio et al. 2010), and is the subject of much speculation as to its taxonomic status (see Walker and Ismail 2008 and Goddard 2009 for further discussion). According to the original case-report description, infection by the strain Atlantic rain forest was acquired through the bite of an infected tick in Barra do Una, an Atlantic rain forest area of low altitude (<100 m above sea level), ≈80 km southwest of trails 1–6 of the current study. In fact, this Barra do Una locality belongs to the Serra do Mar State Park along the Atlantic coast in the state of São Paulo, where A. ovale is found throughout low altitude areas (unpublished data from the Coleção Nacional de Carrapatos). Unfortunately, the tick specimen that transmitted rickettsia to the patient in Barra do Una was not saved for taxonomic identification. A. ovale is an important human-biting tick in Brazil (Labruna et al. 2005, Guglielmone et al. 2006). Thus, our results suggest that A. ovale is the main vector of Rickettsia sp strain Atlantic rain forest in the state of São Paulo, especially at low altitude areas among the Serra do Mar State Park, where thousands of tourists visit annually.

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ettsia rickettsii (Spolidorio et al. 2010). This later agent is the etiological agent of Brazilian spotted fever (BSF), an acute tick-borne spotted fever endemic in southeastern Brazil, including the state of São Paulo. Because *R. rickettsii* comprises the sole antigen employed for serological diagnosis of BSF in Brazil (Labruna 2009), it is likely that more cases of clinical infection by *Rickettsia* sp strain Atlantic rain forest have been misdiagnosed with BSF, because relatively high infection rates by strain Atlantic rain forest were found in the human-biting tick *A. ovale*.

**Acknowledgments**

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