Environmental infestation and rickettsial infection in ticks in an area endemic for Brazilian spotted fever

Infestação ambiental e infecção por rickéttisias em carrapatos de área endêmica para Febre Maculosa Brasileira

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Abstract

Brazilian spotted fever (BSF), caused by Rickettsia rickettsii, is endemic in the municipality of Americana, southeastern Brazil, where the disease is transmitted by the tick Amblyomma cajennense. This study evaluated the tick fauna and rickettsial infection in free-living ticks that were captured monthly using dry ice traps in areas endemic for BSF in Americana, from July 2009 to June 2010. Two tick species were captured: A. cajennense (6,122 larvae; 4,265 ninfas; 2,355 adults) and Amblyomma dubitatum (7,814 larvae; 3,364 ninfas; 1,193 adults). The immature stages of A. cajennense and A. dubitatum had similar distribution through the 12-month period, with larvae of both species collected in highest numbers between April and July, and nymphs between June and October. The highest numbers of A. cajennense adults were collected between October and December, whereas A. dubitatum adults were collected in relatively similar numbers throughout the 12-month period. Rickettsial infection was evaluated by means of PCR in 1,157 A. cajennense and 1,040 A. dubitatum ticks; only 41 (3.9%) A. dubitatum ticks were found to be infected by Rickettsia bellii. The present study showed that the areas of Americana that are endemic for BSF are characterized by high environmental burdens of A. cajennense and A. dubitatum.

Keywords: Amblyomma cajennense, Amblyomma dubitatum, Rickettsia bellii, Brazil.

Introduction

The bacterium Rickettsia rickettsii is the etiological agent for the deadliest form of rickettsiosis in the world, namely Brazilian spotted fever (BSF) (LABRUNA, 2009). This disease is endemic in southeastern Brazil, especially in the state of São Paulo, where 555 laboratory-confirmed cases occurred from 1985 to 2012, with a 40% case-fatality rate (official data from the São Paulo State Health Office available at http://www.cve.saude.sp.gov.br/). BSF is transmitted by ticks. In Brazil, Amblyomma cajennense is the most important vector, since it is incriminated in transmitting R. rickettsii to humans in most of the endemic areas, including...
Materials and Methods

From July 2009 to June 2010, free-living ticks were collected by means of dry ice traps, as previously described (WILSON et al., 1972; OLIVEIRA et al., 2000), in the following areas within the municipality of Americana, state of São Paulo, Brazil: 1- Sobrado Velho (22°41’16”S and 47°15’09”W, 516 m); 2- Carioba (22°41’75”S and 47°19’30”W, 496 m); 3- Bosque das Nascentes (22°41’94”S and 47°17’93”W, 534 m); 4- Fazenda Palméiras (22°45’01”S and 47°16’82”W, 558 m); 5- Museu Histórico (22°41’60”S and 47°17’42”W, 508 m); and 6- Ribeirão Quilombo (22°43’39”S and 47°19’66”W, 528 m). All of these six areas (1 to 6) were inhabited by capybaras, whereas horses were present only in areas 1, 2, and 4. Human parasitism by ticks had been reported in these risk areas, and to investigate rickettsial infection in these ticks.

Results

Over the 12-month period, a total of 3,548 adult ticks (1,649 males and 1,899 females) and 21,565 immature ticks (7,629 nymphs and 13,936 larvae) were collected in the 830 dry ice traps mounted in the six sampled areas. Two tick species, A. cajennense and A. dubitatum, were collected in all sampled areas (Table 1). Adults of A. cajennense and A. dubitatum were collected in similar numbers, except in areas 2 and 4, where significantly more A. cajennense were collected. Taxonomic differentiation of unfed larvae of A. cajennense and A. dubitatum under a stereo microscope relied basically on the idiosome size, which was visually larger in A. dubitatum (Figure 1). The numbers of ticks collected in the six areas were pooled and are presented in Figure 2. Overall, the immature stages of A. cajennense and A. dubitatum had similar distribution throughout the 12-month period, with larvae of both species collected in highest numbers during the autumn and early winter (April to July), and nymphs during late autumn, winter and early spring (June to October). On the other hand, the highest numbers of A. cajennense adult ticks were collected during the spring and early summer months (October to December), whereas A. dubitatum adult ticks were collected in relatively similar numbers throughout the 12-month period (Figure 2).

The salivary glands of 2,197 adult ticks were subjected to DNA extraction and PCR. Among the 1,157 A. cajennense ticks, none was positive according to the initial gIgA-PCR protocol. Among...
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According to the initial gltA-PCR, 41 (3.9%) were positive, negative according to the ompA-PCR and positive according to the R. bellii-specific PCR protocol. These PCR positive samples were found in 5 of the 6 sampled areas, with infection rates varying from 1.3 to 8.5% (Table 2). PCR products were randomly selected from 10 of these ticks, and were subjected to DNA sequencing. The 10 ticks generated sequences that were 100% identical to the corresponding sequence of R. bellii in GenBank (accession number CP000087).

Discussion

This study was conducted in six areas of the municipality of Americana, which has been considered to be an area endemic for BSF since 2004 (PINTER et al., 2011). It was found that all the active stages of A. cajennense and A. dubitatum were abundant in the six sampled areas, which were also all inhabited by free-ranging capybaras. Indeed, the environmental tick burdens found in the present study are directly related to capybaras, which are primary hosts for all the parasitic stages of both A. cajennense and A. dubitatum (PEREZ et al., 2008; NAVA et al., 2010; LABRUNA, 2013). Horses, another primary host species for all parasitic stages of A. cajennense (LABRUNA et al., 2002), were also present in areas 1, 2 and 4. Interestingly, areas 2 and 4 were the only ones where significantly greater numbers of A. cajennense than of A. dubitatum were collected. It is possible that this higher A. cajennense burden was related to higher host availability, namely horses and capybaras. Unfortunately, we could not quantify the populations of capybaras or horses in the present study, thus

<table>
<thead>
<tr>
<th>Area</th>
<th>Amblyomma cajennense</th>
<th>Amblyomma dubitatum</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Larvae</td>
<td>Nymphs</td>
</tr>
<tr>
<td>1</td>
<td>31</td>
<td>262</td>
</tr>
<tr>
<td>2</td>
<td>388</td>
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<td>5</td>
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<tr>
<td>6</td>
<td>3,007</td>
<td>1,151</td>
</tr>
<tr>
<td>Total</td>
<td>6,122</td>
<td>4,265</td>
</tr>
<tr>
<td>%*</td>
<td>43.9</td>
<td>55.9</td>
</tr>
</tbody>
</table>

* different superscript italic letters in the same line mean significantly different proportions of A. cajennense and A. dubitatum adult ticks in the area. † refers to the proportion (%) of each tick species according to the total number of larvae, nymphs or adults.
precluding any comparative analysis of host density and tick burdens. Interestingly, the six sampled areas were interconnected by water courses, in which capybaras were present and to which they had free access (data not shown). Therefore, it is possible that constant gene (and pathogen) exchange exists between ticks in the six sampled areas. Nonetheless, the present study corroborates a number of previous studies that have reported the presence of capybaras associated with the ticks *A. cajennense* and *A. dubitatum* in southeastern Brazil, in both BSF-endemic and non-endemic areas (GUEDES et al., 2005; PEREZ et al., 2008; TOLEDO et al., 2008; PACHECO et al., 2009; QUEIROGAS et al., 2012).

The seasonal dynamics of *A. cajennense* has been well studied in southeastern Brazil, where this tick completes one generation per year, with larvae predominating in autumn, nymphs in winter and adults during spring and summer (OLIVEIRA et al., 2000, 2003; LABRUNA et al., 2002). This seasonal pattern is determined by the behavioral diapause of unfed larvae, as regulated by photoperiod and ground temperature (LABRUNA et al., 2003; CABRERA; LABRUNA, 2009). In the present study, albeit encompassing only a 12 month period, the highest peaks of larvae, nymphs and adults peaking during spring and summer; immature stages were not identified to species level. On the other hand, we would have missed any *Rickettsia* species that did not infect the salivary glands and hemolymph, as is the case of infection by *Rickettsia peacockii* in Dermacentor andersoni ticks in the United States (NIEBYLSKI et al., 1997). *R. bellii* is known to infect *A. dubitatum* hemolymph (LABRUNA et al., 2004), but it is not known whether it infects the salivary glands. If it does not infect the salivary glands, then our findings of PCR-positive ticks would have resulted from hemolymph residues that were inevitably collected with the salivary glands. Thus, our low *R. bellii*-infection rate may have been related to lower tropism of *R. bellii* to tick salivary glands, which has yet to be demonstrated.

In two recent studies in an area endemic for BSF in the state of Minas Gerais, Guedes et al. (2005, 2011) found that the proportions of *A. cajennense* ticks infected with *R. rickettsii* were 1.28% (1/78) and 0.5% (2/400), respectively. Even though Americana is an area endemic for BSF where *R. rickettsii* is presumably transmitted by *A. cajennense* (PINTER et al., 2011), we failed to encounter any *R. rickettsii*-infected ticks. However, this finding is not totally unexpected, since the prevalence of this pathogen among *A. cajennense* ticks can be very low due to the low efficiency of transovarial and transstadial transmission of *R. rickettsii* in *A. cajennense* ticks (SOARES et al., 2012). Similarly to the present study, Sangioni et al. (2005) was unsuccessful in finding any infected ticks among 810 *A. cajennense* adult ticks collected from areas endemic for BSF in the state of São Paulo.

In conclusion, the present study showed that the areas at risk of BSF in Americana are characterized by high environmental burdens of *A. cajennense* and *A. dubitatum*, which are primarily sustained by capybaras. It was shown for the first time that larvae and nymphs of *A. dubitatum* are active during the same periods as the corresponding stage of *A. cajennense*. While *R. bellii* was found infecting *A. dubitatum* ticks, infection by *R. rickettsii* among *A. cajennense* ticks was not found, thus indicating that it probably has a very low infection rate, as also seen in other areas endemic for BSF in the interior of the state of São Paulo.

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References

Brites-Neto J. Diagnóstico epidemiológico de infectividade para Rickettsia rickettsii em Amblyomma sp. no Município de Americana, SP [Dissertação]. Nova Odessa: Instituto de Zootecnia; 2011.


