

Rickettsia* spp., *Ehrlichia* spp. e *Anaplasma* spp. em carrapatos de vida livre nas mesorregiões Sul Fluminense e Metropolitana do Rio de Janeiro, RJ

***Rickettsia* spp., *Ehrlichia* spp. and *Anaplasma* spp. in free-living ticks of mesoregions South Fluminense and Metropolitan area of Rio de Janeiro, RJ**

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Resumo

Os carrapatos são ectoparasitas obrigatórios e vetores de agentes patogênicos como bactérias, parasitos e vírus. O presente estudo teve como objetivo identificar a presença de bactérias dos gêneros *Rickettsia*, *Ehrlichia* e *Anaplasma* em carrapatos de vida livre coletados em cinco áreas, localizadas nas mesorregiões Sul Fluminense e Metropolitana do Rio de Janeiro. Foram coletados 9.353 carrapatos distribuídos em 372 pools. A análise dos dados pelo método PCR-RFLP indicou que as bandas verificadas na análise eram de *Rickettsia bellii* em 0,25% dos carrapatos coletados de duas áreas. A pesquisa molecular para os gêneros *Ehrlichia* e *Anaplasma* revelou resultados negativos para ambos os gêneros. A presença de genoma de *R. bellii* em carrapatos sugere a importância da execução de novas pesquisas com o intuito de entender a epidemiologia dos agentes estudados na região.

Palavras-chave: ixodídeos, *Rickettsia bellii*, Rickettsiales.

Abstract

Ticks are obligate ectoparasites and vectors of several bacterial, parasitic and viral pathogens. This study aimed to identify bacteria of the *Rickettsia*, *Ehrlichia* and *Anaplasma* genera in free-living ticks, collected in five areas located in both South Fluminense and Metropolitan mesoregions of Rio de Janeiro state. We collected a total of 9,353 ticks which were distributed in 372 pools. Data analysis using the PCR-RFLP method showed that the bands observed in the analysis are of the type *Rickettsia bellii*. The positivity level to *R. bellii* found in this study was 0.25%. The molecular search for *Ehrlichia* and *Anaplasma* genera revealed negative results for both genera. The presence of *Rickettsiae* DNA in the Rocky Mountain Spotted Fever group in ticks suggests the importance of undertaking new research in order to understand the epidemiology of the agents.

Keywords: ixodids, *Rickettsia bellii*, Rickettsiales.

Introduction

Ticks are important vectors of pathogens, including bacteria, viruses, and protozoa (Dantas-Torres et al., 2012). Ticks of the genera *Argas*, *Amblyomma*, *Dermacentor*, *Haemaphysalis*, *Hyalomma*, *Ixodes* and *Rhipicephalus* were described parasitizing human beings (Otranto et al., 2014). When searching for the presence of blood parasites, these authors detected pathogens of the genera *Rickettsia*, *Anaplasma*, *Borrelia* and *Babesia*, thus demanding the importance of ticks as vectors of important zoonosis. Some pathogens can influence a tick's gene expression, demonstrating a molecular interaction between tick's species and the pathogens transmitted by them (Liu & Bonnet, 2014).

The Rocky Mountain Spotted Fever Group rickettsiae are transmitted by ticks and may cause severe human infectious diseases and transmit agents of human tick-borne rickettsiosis in Brazil, originated from wildlife hosts (Szabó et al., 2013).

Whatever the origin of pathogenic *Rickettsia*, the human activities can be responsible for the amplification of both wildlife hosts and tick infections, and may be also associated to factors ranging from natural environment to human dwellings. Nonetheless, acknowledgment of the ecological background of each rickettsiosis is a major step to provide diagnosis, treatment and preventive measures (Szabó et al., 2013). According to Parola et al. (2013), microbial isolation and animal transmission studies of rickettsiae are important tools to better understand the role of ticks as potential reservoirs and vectors for both pathogenic and species of unknown pathogenicity.

Canine monocytic ehrlichiosis has a worldwide distribution and is a highly prevalent disease in Brazil, where *Ehrlichia canis* revealed substantial genetic diversity (Aguilar et al., 2013). Canine granulocytic ehrlichiosis is caused by either *Ehrlichia ewingii* or *Anaplasma phagocytophilum*. *Anaplasma phagocytophilum* has been found throughout the world in large quantities of vertebrate hosts (e.g., dogs, ruminants, humans and rodents) and is

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vectored largely by the Ixodes species (Yabsley et al., 2008). In addition, Santos et al. (2013) demonstrated the presence of *A. phagocytophilum* in 6.03% of 398 blood samples from dogs in the state of Rio de Janeiro, as well as its presence in *Amblyomma sculptum* (*A. cajennense* complex) and *Rhipicephalus sanguineus* naturally parasitized.

This study aimed to identify the presence of bacteria belonging to the genera *Rickettsia*, *Ehrlichia* and *Anaplasma* in free-living ticks collected in five areas located in both South Fluminense and Metropolitan mesoregions of Rio de Janeiro state, Brazil.

Material and methods

The ticks assessed in this study were collected in the State of Rio de Janeiro from October 2009 to August 2011, as described by Silveira and Fonseca (2013). The collections in the Itatiaia National Park - PNI and National Forest Mario Xavier - FLONA were authorized by SISBIO 16622-1. The activities in military areas, like the Navy of Brazil and the Brazilian Army, were authorized by their respective commands. The ADN samples extracted were submitted to PCR and agarose gel electrophoresis at the Parasitic Diseases Laboratory and the Molecular Biology Multi-User Laboratory of the Federal Rural University of Rio de Janeiro.

Ticks were collected using three sampling techniques: **1)** CO₂ chemical trap, **2)** flannel drag nets and **3)** from the clothing of the researchers who participated in the research (Silveira & Fonseca, 2011). For the specific identification of tick's dichotomous keys were used, according to Aragão & Fonseca (1961) and Battesti et al. (2006). Adults were identified as species, and immature stages as gender. The ticks were pooled according to the capture areas, relocated in polypropylene tubes and frozen at -20°C until extraction of their nucleic acids.

ADN extraction was performed with protocol phenol/phenol-chloroform, according Costa Santolin et al. (2013). To research the genus *Rickettsia* a PCR was carried out in order to amplify the portion of 549 bp of the *htrA* gene (17kDa), using the 17K-5 and 17K-3 primers and the portion of 834 bp of the *gltA* gene (Citrate synthase), using CS-239 and CS-1069 primers (Labruna et al., 2004a).

Positive samples in the two reactions were subjected to a new PCR with primers that identified *Rickettsia* of the Rocky Mountain Spotted Fever group. For amplification of the 532 bp portion of the *ompA*, Rr190,70p and Rr190,602n genes, primers were used (Labruna et al., 2004a). "Amplicons" derived from positive tick's samples in conventional PCR assays for *Rickettsia* were digested using the restriction enzymes *HindIII*, *MspI* and *RsaI* (New England Biolabs), selected based on the analysis of digestion *in silico* (Santolin et al., 2013).

To study the *Ehrlichia* genus, we used the *dsb330* and *dsb720* primers for the first amplification, which had a product with 401 bp; and *dsb380* and *dsb720* primers were used for the second amplification, as well as the amplified product size 349 bp, according to Aguiar et al. (2014). In the molecular research of the genus *Anaplasma*, primers ge3a and ge10r primary PCR were used, whose product is 932 bp (Massung et al., 1998). For the secondary PCR, ge9f and ge2 primers were used, whose target is the 16S rRNA gene with 546 bp. We analyzed all these products by electrophoresis on agarose gel at 1.5% to confirm the size amplicon reached by comparison with a ADN-based molecular weight marker (GeneRuler 100 bp ADN Ladder, produto # SM024, Thermo Scientific). The bands were visualized with the aid of transillumination, and the photographic record was carried out with a specific electronic device.

Results

Nine thousand three hundred and fifty-three specimens were analyzed, distributed in 372 samples according to their taxonomic classification, evolutionary stage, area and year of collection. Each pool contained 50 larvae, 20 nymphs and one adult. This total consisted of 7,273 larvae of *Amblyomma*, one larva of *R. (B.) microplus*, 1,952 nymphs of *Amblyomma*, 120 adult *A. sculptum*, four adults and three adult *Amblyomma brasiliense* and *Amblyomma dubitatum* (Table 1).

Table 1: Ticks analyzed by molecular biology, separated by evolutionary stage, genus, species and sex, collected between October 2009 and August 2011 in five institutional areas in the state of Rio de Janeiro, Brazil

Genus/Specie /Gender	PNI	Marambaia	FLONA	DCMun	UFRRJ	Total
<i>Amblyomma</i> spp. - larvas	0	544	538	4463	1728	7273
<i>Amblyomma</i> spp. - ninfas	6	445	528	295	678	1952
<i>Amblyomma sculptum</i> - ♂	0	14	11	16	19	60
<i>Amblyomma sculptum</i> - ♀	0	12	11	31	6	60
<i>Amblyomma brasiliense</i> - ♂	2	0	0	0	0	2
<i>Amblyomma brasiliense</i> - ♀	2	0	0	0	0	2
<i>Amblyomma dubitatum</i> - ♂	0	0	0	0	0	0
<i>Amblyomma dubitatum</i> - ♀	0	0	2	1	0	3
<i>Rhipicephalus microplus</i> - larvas	0	0	0	0	1	1
Total	10	1015	1090	4806	2432	9353

PNI: Itatiaia National Park; Marambaia: Marambaia Sandbank; FLONA: National Forest Mário Xavier; DCMun: Central Ammunition Depot; UFRRJ: Federal Rural University of Rio de Janeiro.

Two samples were positives: one comes from three larvae of *Amblyomma* from FLONA and one from a pool of 20 nymphs of *Amblyomma* sp. from Marambaia. When the samples were subjected to PCR reaction using primers specific to rickettsiae of the Rocky Mountain Spotted Fever group (GFM) (*ompA*), was showed that they were negative for this group of *Rickettsia*. Data analysis by Restriction Fragment Length Polymorphism – Polymerase Chain Reaction (RFLP-PCR) method showed that the bands observed in the analysis were similar to the pattern expected for *Rickettsia bellii*, whose positivity was 0.25%.

Discussion

Rickettsia bellii has already been identified in several species of ticks in Brazil (Estrada et al., 2006; Horta et al., 2007; Labruna et al., 2004a, 2004b; McIntosh et al., 2015). In a Brazilian native people's reservation in the state of Mato Grosso, the circulation of *R. bellii* in *Amblyomma* sp. larvae and *A. sculptum* adult was recorded by Moura-Martiniano et al. (2014). In areas of occurrence of Brazilian Spotted Fever (BSF) in the state of Mato Grosso, Lopes et al. (2014) confirmed the presence of *R. bellii* in a specimen of *A. dubitatum*, suggesting the importance of these species of rickettsiae as a potential threat for the native local population and for differential diagnostic of BSF, nevertheless of unknown pathogenicity for humans.

Competition for ecological niche between rickettsia species within the tick has been previously reported, resulting in the fact that once infected by a rickettsia, a tick is refractory to a second species (Burgdorfer et al., 1980). It was experimentally observed that in an artificially infected tick, a second species of *Rickettsia* had its transovarial transmission inhibited (Macaluso et al., 2002).

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Thus, the presence of *Rickettsia Bellii* can inhibit, for example, an infection by other rickettsiae of known pathogenicity, such as *Rickettsia rickettsii* and *Rickettsia parkeri* in ticks.

In this study, in both areas where the circulation of *R. bellii* was found, there was the presence of capybaras, whose are very important in the epidemiology of the Brazilian Spotted Fever. This mammal acts as the primary host and amplifier and vectors tick such as *A. sculptum* and *A. dubitatum* (Souza et al., 2009). *R. bellii* has the potential to induce immune response in mammals, either by natural or experimental infection (Horta et al., 2010), which is exciting for a thorough investigation into its possible pathogenicity to vertebrate hosts.

Circulation of organisms belonging the genus *Rickettsia* in the areas studied was found, but *Ehrlichia* and *Anaplasma* were not diagnosed. The presence of the genome of *R. bellii* in free-living ticks reinforces the need to elucidate the importance in their epidemiology, and suggests the importance of undertaking new research in order to understand the epidemiology of this specie of rickettsia.

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