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Ticks infesting wild small rodents in three areas of the state of São Paulo, Brazil

Carrapatos infestando pequenos roedores silvestres em três municípios do estado de São Paulo, Brasil

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ABSTRACT

From May to September 2011, a total of 138 wild rodents of the Cricetidae family were collected in the cities of Anhembi, Bofete and Torre de Pedra, in São Paulo State. All animals were examined for the presence of ticks, which were collected and identified at species level in the laboratory by morphological examination (for adults, nymphs and larvae) and molecular biology, by sequencing of a fragment of the 16S mitochondrial rDNA (for larvae). A total of 47 ticks (21 larvae and 26 nymphs) were collected from rodents, identified as 21 larvae and 23 nymphs of *Amblyomma ovale* (collected on *Oligoryzomys nigripes*, *Oligoryzomys flavescens*, *Sooretamys agouya* and *Nectomys squamipes*), two nymphs of *Ixodes schulzei* (*O. nigripes* and *O. flavescens*) and an *Amblyomma sculptum* nymph in *S. agouya*. The overall prevalence of parasitism by *A. ovale* was 4.3% for larvae, and 10.1% for nymphs. The mean intensity of parasitism was 3.5 larvae/parasitized animal, and 1.6 nymphs/parasitized animal. One *O. nigripes* was found parasitized by both larva and nymph of *A. ovale*, and another *O. nigripes* was parasitized simultaneously by an *I. schulzei* nymph and five *A. ovale* nymphs. This research reports the following unpublished records: *A. ovale* on *O. flavescens*, *O. nigripes* and *S. agouya*; *A. sculptum* on *S. agouya*; and *I. schulzei* on *O. flavescens* and *O. nigripes*.

Key words: ticks, wild rodents, *Amblyomma ovale*, *Amblyomma sculptum*, *Ixodes schulzei*, Brazil.

RESUMO

De maio a setembro de 2011, um total de 138 roedores silvestres da família Cricetidae foram coletados nos municípios de Anhembi, Bofete e Torre de Pedra, no estado de São Paulo. Todos os animais foram examinados quanto à presença de

carrapatos, os quais foram coletados e identificados ao nível de espécie em laboratório, através de análises morfológicas (para adultos, ninfas e larvas) e por biologia molecular para confirmar estas análises, através do sequenciamento de um fragmento do gene 16S rDNA mitocondrial (para uma parte das larvas). Um total de 47 carrapatos (21 larvas e 26 ninfas) foi coletado dos roedores, sendo identificados como 21 larvas e 23 ninfas de *Amblyomma ovale* (coletados sobre *Oligoryzomys nigripes*, *Oligoryzomys flavescens*, *Sooretamys agouya* e *Nectomys squamipes*), duas ninfas de *Ixodes schulzei* (em *O. nigripes* e *O. flavescens*) e uma ninfa de *Amblyomma sculptum* em *S. agouya*. A prevalência geral de parasitismo por *A. ovale* foi de 4,3% e de 10,1% para larvas e ninfas, respectivamente. As intensidades médias de parasitismo foram de 3,5 larvas/animal parasitado e de 1,6 ninfas/animal parasitado. Um *O. nigripes* foi encontrado parasitado simultaneamente por larva e ninfa de *A. ovale*, e outro *O. nigripes* estava parasitado simultaneamente por uma ninfa de *I. schulzei* e cinco ninfas de *A. ovale*. O presente trabalho reporta os seguintes registros inéditos: *A. ovale* em *O. flavescens*, *O. nigripes* e *S. agouya*; *A. sculptum* em *S. agouya*; e *I. schulzei* em *O. flavescens* e *O. nigripes*.

Palavras-chave: carrapatos, roedores silvestres, *Amblyomma ovale*, *Amblyomma sculptum*, *Ixodes schulzei*, Brasil.

INTRODUCTION

Ticks are arthropod ectoparasites of the class Arachnida, with worldwide distribution, which parasitize terrestrial vertebrates, amphibians, reptiles, birds and mammals (BARROS-BATTESTI et al.,

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2006). Given the particularities of their feeding habits, ticks are considered the first group of importance as vectors of infectious diseases to animals, and the second for humans (JONGEJAN & UILENBERG, 2004). Tick-borne microorganisms include viruses, bacteria, protozoa and helminths (BARROS-BATTESTI et al., 2006). In Brazil, bacteria of the genus *Rickettsia* are considered the most important tick-borne agents, which are spotted fever diseases in different regions of the country (LABRUNA et al., 2011). The species of ticks which infest domestic animals are the most studied; however, the species that parasitize wildlife are still poorly known, especially in relation to their taxonomy, biology, ecology, geographical distribution, usual host, and vector capacity of bioagents (BARROS-BATTESTI et al., 2006). Recent studies have shown that small rodents are important hosts for several species of *Ixodidae* ticks, especially for immature stages (GUGLIELMONE & NAVA 2010, 2011). In this context, the aim of this study was to report the occurrence of ticks infesting wild rodents in three areas of the state of São Paulo, southeastern Brazil.

MATERIALS AND METHODS

Ticks were collected from wild rodents from May to September 2011 in three municipalities located in the State of São Paulo: Anhembi (22°47'09.11"S, 48°07'30.90"W), Bofete (23°05'54.51"S, 48°11'26.61"W) and Torre de Pedra (23°14'58.76"S, 48°11'39.49"W). Rodents were captured as part of a study on the wild cycle of the Vaccinia viruses, in which rodents were evaluated as potential reservoirs of the virus (PERES et al., 2013). Rodent captures were performed with Sherman and pitfall intercepts traps, as previously described (PERES et al., 2013). Rodents were identified to species according to BONVICINO et al. (2008), REIS et al. (2006) and WILSON & REEDER (2005). Attached ticks were removed using tweezers and stored in 70% alcohol until its taxonomic identification in the laboratory, following taxonomic keys for tick genera (BARROS-BATTESTI et al., 2006) and for nymph of the genus *Amblyomma* (MARTINS et al., 2010), and morphological description previously reported for the nymph of *Ixodes schulzei* (BARROS-BATTESTI et al., 2007). In the case of *Amblyomma* spp. larvae, collected samples were compared morphologically with *Amblyomma ovale* larvae originated from a laboratory colony, as previously described (BARBIERI et al., 2008; SZABÓ et al., 2013.). The morphological identification of these

larvae was confirmed by molecular analysis of a few specimens, which had the DNA extracted by the guanidine isothiocyanate method (SANGIONI et al., 2005) and tested by polymerase chain reaction (PCR) targeting a fragment of 460 nucleotides of the tick mitochondrial 16S rDNA gene, according to MANGOLD et al. (1998). Amplicons of the expected size were generated to DNA sequencing in an automatic sequencer (Model ABI 3500 Genetic Analyzer; Applied Biosystems/Thermo Fisher Scientific, Foster City, CA, USA) according to manufacturer's instructions. For each host species, prevalence and mean intensity of infestation by certain tick species was calculated following BUSH et al. (1997). In this case, prevalence = number of infested individuals / number of examined individuals X 100; mean intensity = total number of ticks/number of infested individuals.

RESULTS

A total of 138 rodents of the *Cricetidae* family was captured and examined for the presence of ticks. Animals taken belonged to eight different species, of which 75 *Oligoryzomys nigripes*, 31 *Oligoryzomys flavescens*, 14 *Sooretamys agouya*, eight *Akodon montensis*, four *Nectomys squamipes*, four *Calomys tener*, one *Juliomys pictipes*, and one *Necomys lasiurus*. A total of 47 ticks (21 larvae and 26 nymphs) were collected from rodents and identified as 21 larvae and 23 nymphs of *A. ovale* (collected on *O. nigripes*, *O. flavescens*, *S. agouya* and *N. squamipes* in at least one of the three sampled municipalities), two nymphs of *I. schulzei* (one on *O. nigripes* from Anhembi, one on *O. flavescens* from Bofete) and a *Amblyomma sculptum* nymph on *S. agouya* from Anhembi.

Once the tick *A. ovale* accounted for 44 (93.7%) of the 47 collected ticks, prevalence and mean intensity of parasitism values were calculated only for this tick species, as detailed in table 1. The overall prevalence of parasitism by *A. ovale* was 4.3% for larvae, and 10.1% for nymphs. The mean intensity of parasitism was 3.5 larvae/infested animal, and 1.6 nymphs/infested animal. Considering the four species of rodents in which larvae or nymphs of *A. ovale* were collected, prevalence values ranged from 5.3 to 6.5% for larvae, and from 3.2 to 25.0% for nymphs. The mean intensity of larvae ranged from 2.5 to 4.0 larvae per infested animal, whereas the mean intensity of nymphs ranged from 1.0 to 1.8 nymphs per infested animal. Only one individual rodent (*O. nigripes* from Bofete) was simultaneously infested by larva and

Table 1 - *Amblyomma ovale* ticks collected from wild rodents (*Oligoryzomys nigripes*, *Oligoryzomys flavescens*, *Sooretamys agouya*, *Nectomys squamipes*, *Akodon montensis*, *Calomys tener*, *Juliomys pictipes*, *Necomys lasiurus*) that were captured in three municipalities (Anhembi, Bofete, and Torre de Pedra) of the state of São Paulo, Brazil, from May to September 2011.

	-----Number of infested animals / Number of examined animals (Prevalence); Mean intensity*-----						
Host	Anhembi	-----Bofete-----		-----Torre de Pedra-----		-----Total-----	
	Nymph	Larva	Nymph	Larva	Nymph	Larva	Nymph
<i>O. nigripes</i>	7/48 (14.5); 2.3	2/18 (11.1); 1.0	1/18 (5.6); 1.0	2/9 (22.2); 7.0	3/9 (33.3); 1.0	4/75 (5.3); 4.0	11/75 (14.6); 1.8
<i>O. flavescens</i>	0/14 (0); 0	1/13 (7.7); 1.0	1/13 (7.7); 1.0	1/4 (25.0); 4.0	0/4 (0); 0	2/31 (6.5); 2.5	1/31 (3.2); 1.0
<i>S. agouya</i>	1/13 (7.6); 1.0	0/1 (0); 0	0/1 (0); 0	-	-	0/14 (0); 0	1/14 (7.1); 1.0
<i>N. squamipes</i>	0/3 (0); 0	0/1 (0); 0	1/1 (100); 1.0	-	-	0/4 (0); 0	1/4 (25.0); 1.0
<i>A. montensis</i>	-	0/8 (0); 0	0/8 (0); 0	-	-	0/8 (0); 0	0/8 (0); 0
<i>C. tener</i>	0/2 (0); 0	0/2 (0); 0	0/2 (0); 0	-	-	0/4 (0); 0	0/4 (0); 0
<i>J. pictipes</i>	-	-	-	0/1 (0); 0	0/1 (0); 0	0/1 (0); 0	0/1 (0); 0
<i>N. lasiurus</i>	-	0/1 (0); 0	0/1 (0); 0	-	-	0/1 (0); 0	0/1 (0); 0
Total	8/80 (10.0); 2.1	3/44 (6.8); 1.0	3/44 (6.8); 1.0	3/14 (2.1); 6.0	3/14 (2.1); 1.0	6/138 (4.3); 3.5	14/138 (10.1); 1.6

* : Mean intensity = total number of ticks / number of infested animals.

- : No animal of this species was collected in this locality.

nymph of *A. ovale*. The *O. nigripes* specimen that was parasitized by a *I. schulzei* nymph also contained five nymphs of *A. ovale*.

Of the 21 larvae that were morphologically identified as *A. ovale* through comparison with larvae derived from a laboratory colony, four larvae (one from *O. nigripes* and one from *O. flavescens* from Torre de Pedra, two from *O. nigripes* from Bofete) were subjected to PCR for the mitochondrial 16S rDNA gene, resulting in 409-412-bp DNA sequences that differed to each other by only 1 (0.2%) to at most 16 nucleotides (3.9%). These four sequences were shown to be 96 to 99% identical to corresponding sequences of *A. ovale* in GenBank (AF541255, KF179347, JN573304, FJ424409). After *A. ovale*, the sequences from GenBank with larger similarity values to these larvae were *Amblyomma aureolatum* (KF179343), *Amblyomma multipunctum* (KC677673), *Amblyomma naponense* (KC677674) and *Amblyomma dubitatum* (DQ858954), with similarity values always lower than 88%. The four sequences of *A. ovale* larvae generated in this study were deposited in GenBank under accession number KR605466-KR605469. Voucher tick specimens collected in this study were deposited in the tick collection "Coleção Nacional de Carrapatos" (CNC) of the Faculty of Veterinary Medicine of the University of São Paulo, under the accession numbers CNC-2457 to CNC-2462.

DISCUSSION

The tick *A. ovale* was the major species found in small rodents in this study, the most prevalent and abundant (Table 1). Previous studies indicated that immature *A. ovale* parasitize mainly rodents of the family *Cricetidae* (SZABÓ et al., 2013; SPONCHIADO et al., 2015) and *Echimyidae* (SARAIVA et al., 2012), whereas the adult stage parasitizes chiefly wild carnivores and domestic dogs (GUGLIELMONE et al., 2003). In this study, immature *A. ovale* were reported on four species of rodents, which includes the first records for *O. flavescens*, *O. nigripes* and *S. agouya*. Generally, the values of prevalence and mean intensity of *A. ovale* reported in this study were similar or within the range reported for other species of small rodents in the state of São Paulo (SZABÓ et al., 2013) and Mato Grosso do Sul (SPONCHIADO et al., 2015).

In the present study, we found an *A. sculptum* nymph parasitizing *S. agouya* for the first time. Although *A. sculptum* (formerly known as *Amblyomma cajennense*) is widely distributed in southeastern Brazil (NAVA et al., 2014), this tick species has a preference for medium to large-sized mammals (BARROS-BATTESTI et al., 2006) and is rarely found on *Cricetidae* rodents, such as *Cerradomys subflavus* and *O. nigripes* in Minas

Gerais (SARAIVA et al., 2012) and *Calomys callosus* and *Rhipidomys macrurus* in Mato Grosso do Sul (SPONCHIADO et al., 2015).

The present reports of *I. schulzei* on two species of *Oligoryzomys* are unprecedented for this tick. Although all parasitic stages of *I. schulzei* are associated with *Cricetidae* rodents, the vast majority of records have been on a single species, *N. squamipes* (LABRUNA et al., 2003; SARAIVA et al., 2012). There are occasional records on *A. montensis* and *Euryoryzomys russatus* in the state of Paraná (ONOFRIO et al., 2013).

In this study, *A. ovale* was the most frequent tick on small rodents in three areas of the state of São Paulo. Findings of larvae and nymphs parasitizing small rodents indicated that *A. ovale* is established in the studied area, where wild carnivores and/or domestic dogs should be serving as hosts for the adult stage of the tick. In fact, during the present fieldwork, two adult males of *A. ovale* were collected from a free-ranging coati (*Nasua nasua*) in Anhembi (CNC-2464). Human parasitism by the adult stage of *A. ovale* is relatively common in areas of occurrence of this tick species (LABRUNA et al., 2005; SZABÓ et al., 2006). Once *A. ovale* is vector of *Hepatozoon canis* for domestic dogs (FORLANO et al., 2005) and a causative agent of spotted fever (*Rickettsia* sp. strain Atlantic Forest) for humans in the state of São Paulo (SZABÓ et al., 2013), studies such as the present one are essential for a better understanding of the natural history of *A. ovale* and its associated pathogens.

BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

Protocol no. 112/201-CEUA of the Universidade Estadual Paulista Júlio de Mesquita Filho (UNESP-Botucatu).

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