

Diversity of ticks in the wildlife screening center of São Paulo city, Brazil

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ABSTRACT: *The Wildlife Screening Center (CETAS) of the Tietê Ecological Park (PET), situated at the municipality of São Paulo, receives, treats and rehabilitates wild animals that have been dislodged from their natural environment due to different reasons. This study analyzed the ixodid fauna, and the rickettsial infection in these ticks, collected on wild animals received at the PET's CETAS. During the period from March 2003 to November 2016, 936 ticks were collected from 96 wild animals (16 bird and 18 mammal species) that were sent to CETAS. The following 12 ixodid species were identified: Amblyomma aureolatum, Amblyomma brasiliense, Amblyomma calcaratum, Amblyomma dubitatum, Amblyomma longirostre, Amblyomma ovale, Amblyomma parkeri, Amblyomma sculptum, Amblyomma varium, Haemaphysalis juxtakochi, Ixodes loricatus and Rhipicephalus microplus. From 67 tick specimens tested by the Real Time PCR for rickettsiae, none were positive. The present research records for the first time in Brazil the following association between the tick stages and hosts that have never been reported before: Amblyomma sculptum nymphs on Caprimulgus parvulus, Asio clamator, Buteo brachyurus, Coragyps atratus, Amazona aestiva and Aramus guarauna, Amblyomma dubitatum nymphs on Alouatta guariba and Sphiggurus villosus, Amblyomma aureolatum adults on Bradypus variegatus, Amblyomma longirostre larvae and nymphs on A. clamator, and nymphs on Megascops choliba and Pyroderus scutatus, besides Amblyomma parkeri nymphs on Penelope obscura and Callicebus nigrifrons, and adult on Nasua nasua.*

Key words: *Amblyomma, Ixodes, Haemaphysalis, Rhipicephalus.*

Diversidade de carrapatos no centro de triagem de animais silvestres na cidade de São Paulo, Brasil

RESUMO: *O Centro de Triagem de Animais Silvestres (CETAS) do Parque Ecológico do Tietê (PET), localizado no município de São Paulo, recebe, atende e reabilita animais silvestres que vem sendo retirados do seu ambiente natural por diversas razões. Este estudo relata a diversidade de carrapatos coletados em animais silvestres recebidos no PET, assim como a pesquisa de riquetsias nestes ectoparasitas. Durante os meses de março de 2003 a novembro de 2016, foram coletados e identificados 936 carrapatos de 96 animais silvestres (16 espécies de aves e 18 de mamíferos) atendidos no CETAS. Foram identificadas 12 espécies de ixodídeos: Amblyomma aureolatum, Amblyomma brasiliense, Amblyomma calcaratum, Amblyomma dubitatum, Amblyomma longirostre, Amblyomma ovale, Amblyomma parkeri, Amblyomma sculptum, Amblyomma varium, Haemaphysalis juxtakochi, Ixodes loricatus e Rhipicephalus microplus. De 67 carrapatos testados por PCR em tempo real para riquetsias, nenhum foi positivo. O presente trabalho registra pela primeira vez no Brasil as seguintes associações dos estágios de carrapatos e hospedeiros que nunca foram relatadas anteriormente: ninfas de Amblyomma sculptum em Caprimulgus parvulus, Asio clamator, Buteo brachyurus, Coragyps atratus, Amazona aestiva e Aramus guarauna, ninfas de Amblyomma dubitatum em Alouatta guariba e Sphiggurus villosus, adultos de Amblyomma aureolatum em Bradypus variegatus, larvas e ninfa de Amblyomma longirostre em A. clamator e ninfas em Megascops choliba e Pyroderus scutatus, além de ninfas de Amblyomma parkeri em Penelope obscura e Callicebus nigrifrons e adulto em Nasua nasua.*

Palavras-chave: *Amblyomma, Ixodes, Haemaphysalis, Rhipicephalus.*

INTRODUCTION

Ticks are arthropods of the Ixodida order and Acarina subclass, comprising ectoparasites that are highly specialized in hematophagy. Different species of mammals, birds, reptiles, and amphibians serve as hosts feeding stages of ticks, namely larvae, nymphs, and adults. Ticks are considered the

second largest group of vectors of human diseases, behind only mosquitoes, and the most important vectors of pathogens to wild and domestic animals (MEDIANNIKOV & FENOLLAR, 2014).

The Wildlife Recovery Center (CRAS) of the Tietê Ecological Park (PET) of the Water and Electric Power Department (DAEE) is a Wildlife Screening Center (CETAS) class "A", according to

IBAMA's 169th normative instruction from February 20th, 2008. The CETAS receives wild animals from illegal wildlife trade, from apprehensions made by the Environmental Military Police, Brazilian Institute of the Environment and Renewable Natural Resources (IBAMA), Civilian, Municipal and Federal Police inspections; those rescued by the City Hall, Fire Departments, Urban Parks, Private Companies or even those brought by common citizens. Since CETAS's opening, CRAS has received more than 72 thousand wild animals, on average birds represent 84% of the animals received; mammals 5% and reptiles 11%, adding up to 520 species (MILANELO & FITORRA, 2012).

Due to the importance of knowledge of ticks fauna and the parasite-host relation in wild animals and the pathogens transmitted by ticks that parasitize the Brazilian wild fauna, the aim of the present study was to analyze the ixodid fauna biodiversity and rickettsial infection in these same ticks coming from the animals received and treated at PET's CETAS.

MATERIALS AND METHODS

A total of 96 wild animals were received by the PET during the period from March 2003 to November 2016. Animals were rescued in 28 different municipalities, including São Paulo city and nearby municipalities (Table 1 and 2). Sampled animals belonged to different age groups and genders, and were identified by the PET's veterinarians and biologists. Animals comprised 21 bird and 75 mammals specimens (Table 1 and 2).

During the treatment at the PET's Veterinarian Hospital of those animals received at the CETAS, in every inspection the ticks reported on the animals were collected and forwarded alive in 70% alcohol to the Parasitic Disease Laboratory of the Faculty of Veterinary Medicine and Animal Science of University of São Paulo (FMVZ-USP). Some engorged immature ticks were placed in B.O.D incubators for ecdysis, as detailed elsewhere (LABRUNA et al., 2002). Ticks were taxonomically identified according to current taxonomic literature (BARROS-BATTESTI et al., 2006; MARTINS et al., 2010, 2016). Voucher specimens were deposited in the tick collection "Coleção Nacional de Carrapatos Danilo Gonçalves Saraiva" (CNC) of the FMVZ-USP (Table 1 and 2).

Some tick specimens (n=67, Table 2) had their DNA extracted by the guanidine isothiocyanate method (SANGIONI et al., 2005) and tested for

rickettsiae by a Taqman Real-Time PCR (qPCR) targeting the citrate synthase gene (*gltA*) of the genus *Rickettsia*, according to LABRUNA et al. (2004b). This protocol uses a pair of primers (CS5 e CS6) that amplify a 147 nucleotide fragment of the rickettsial *gltA* gene, combined with an internal fluorogenic probe (5' 6-FAM - BHQ-1 3') of 23 nucleotides. This protocol has shown to be sensitive to the detection of one single gene copy of spotted fever group *Rickettsia* spp. (LABRUNA et al., 2004b). To ensure the viability of the extracted DNA, all samples that were analyzed by the qPCR for molecular detection of rickettsiae were also tested by a conventional PCR targeting a 460-bp fragment of the tick 16S mitochondrial rDNA gene (BLACK & PIESMAN, 1994).

RESULTS AND DISCUSSION

A total of 936 tick specimens (58 larvae, 303 nymphs, 319 males and 256 females) were collected from 96 sampled wild animals, comprising 16 bird and 18 mammal species. Overall, 936 tick specimens were collected and identified to 12 distinct ixodid species (Table 1 and 2).

No rickettsial DNA was detected in any of the 67 tick specimens that were tested by qPCR targeting the *gltA* gene (Table 2). These same ticks yielded visible amplicons by the PCR protocol targeting the mitochondrial 16S rDNA, validating our DNA extraction protocol.

While the majority of tick-host species associations reported in this study has been previously described in the literature (BARROS & BAGGIO, 1992; PEREIRA et al., 2000; ARZUA et al., 2005; LABRUNA et al., 2005, 2009; MARTINS et al., 2006; MARTINS et al., 2011, 2013, 2015, 2016; SARAIVA et al., 2012; LAVINA et al., 2015; TEIXEIRA et al., 2017), the present study records for the first time in Brazil, the parasitism of *Amblyomma longirostre* larva and nymph on *Asio clamator*, and nymphs on *Megascops choliba* and *Pyroderus scutatus*, in addition to a nymphal record of *Amblyomma sculptum* on *Caprimulgus parvulus*, *A. clamator*, *Buteo brachyurus*, *Coragyps atratus*, *Amazona aestiva*, and *Aramus guarauna* in the country.

We reported a nymph of *A. sculptum* (molted to a male in the laboratory) in *Tyto furcata* in the municipality of Guarulhos. While there is one previous report of *A. sculptum* (published as *Amblyomma cajennense*) parasitizing *T. furcata* (published as *Tyto alba*) at the Belo Horizonte CETAS, Minas Gerais State (ANDERY et al.,

Table 1 - Ticks collected from wild birds and mammals received and treated by the Wildlife Screening Center in São Paulo city, Brazil, from March 2003 to November 2016.

Hosts ⁺	Municipality	Dates	Ticks	Accession number
-----Birds-----				
<i>Tyto furcata</i>	Guarulhos	III-2003	1N [‡] <i>Amblyomma sculptum</i>	CNC-797
<i>Saltator similis</i>	São Paulo	VIII-2010	1L <i>Amblyomma</i> sp.	CNC-2419
<i>S. similis</i>	Diadema	X-2015	2N <i>Amblyomma longirostre</i>	CNC-3421
<i>S. similis</i>	São Paulo	VI-2015	1N <i>A. longirostre</i>	CNC-3422
<i>Celeus flavescens</i>	Mogi das Cruzes	XI-2012	1N <i>A. longirostre</i>	CNC-2420
<i>Caprimulgus parvulus</i>	São Paulo	IX-2012	1N <i>A. sculptum</i>	CNC-2485
<i>Megascops choliba</i>	São Paulo	II-2014	1N <i>A. longirostre</i>	CNC-2736
<i>Penelope obscura</i>	São Paulo	X-2013	1N <i>Amblyomma parkeri</i>	CNC-2737
<i>Asio clamator</i>	São Paulo	VII-2014	11L [‡] <i>A. longirostre</i> ; 9L <i>Amblyomma</i> sp.	CNC-2822
<i>A. clamator</i>	Guarulhos	VIII-2016	1N <i>A. longirostre</i> ; 3N <i>A.</i> <i>sculptum</i>	CNC-3401
<i>A. clamator</i> (2)	São Paulo	XI-2016	13N <i>A. sculptum</i>	CNC-3423
<i>Buteo brachyurus</i>	Rio Grande da Serra	IX-2016	5N <i>A. sculptum</i>	CNC-3402
<i>Cariama cristata</i>	Caçapava	IX-2010	35N <i>A. sculptum</i> ; 5L <i>Amblyomma</i> sp.	CNC-3424
<i>Crotophaga ani</i>	Guarulhos	VII-2016	2L <i>Amblyomma</i> sp.	CNC-3425
<i>Carcara plancus</i>	Guararema	IX-2014	5N <i>A. sculptum</i> ; 1L <i>Amblyomma</i> sp.	CNC-3426
<i>Tachyphonus coronatus</i>	Suzano	V-2015	3L <i>Amblyomma</i> sp.	CNC-3427
<i>Pyroderus scutatus</i>	Osasco	XII-2015	2N <i>A. longirostre</i>	CNC-3428
<i>Aramus guaranauna</i>	São Paulo	XI-2016	2N <i>A. sculptum</i>	CNC-3429
<i>Coragyps atratus</i>	São Paulo	VI-2013	1N <i>A. sculptum</i>	CNC-3430
<i>Amazona aestiva</i>	São Paulo	X-2016	2N <i>A. sculptum</i>	CNC-3431
-----Mammals-----				
<i>Didelphis aurita</i>	Guarulhos	IX-2005	1F <i>Ixodes loricatus</i>	CNC-921
<i>D. aurita</i> (3)	São Paulo	IX-2005; XI-2013	1M, 5F <i>I. loricatus</i>	CNC-922, 923, 2734
<i>D. aurita</i>	Embu das Artes	XI-2013	1F <i>I. loricatus</i>	CNC-2433
<i>D. aurita</i>	Francisco Morato	XI-2016	1F <i>I. loricatus</i>	CNC-3432
<i>Alouatta guariba</i>	São Paulo	I-2014	1F <i>Amblyomma aureolatum</i> ; 1N <i>A. sculptum</i>	CNC-2738
<i>A. guariba</i>	Mairiporã	I-2014	2N <i>A. parkeri</i>	CNC-2739
<i>A. guariba</i> (2)	Itapecerica da Serra	IX-2013	1M, 3F <i>A. aureolatum</i> ; 1N <i>A.</i> <i>sculptum</i>	CNC-3433
<i>A. guariba</i> (2)	São Paulo	X, XII-2015	1N <i>Amblyomma dubitatum</i> ; 1M, 24N <i>A. sculptum</i> ; 1L <i>Amblyomma</i> sp.	CNC-3434
<i>Callicebus nigrifrons</i>	Nazaré Paulista	VII-2013	3N <i>A. parkeri</i>	CNC-3435
<i>Bradypus variegatus</i>	Bertioga	XII-2010	1F <i>Amblyomma varium</i>	CNC-1777
<i>B. variegatus</i> (2)	Mogi das Cruzes	XII-2010	1F <i>A. aureolatum</i> ; 1M, 2F <i>A.</i> <i>varium</i>	CNC-2437
<i>B. variegatus</i>	Itapecerica da Serra	IV-2016	2M <i>A. varium</i>	CNC-3436
<i>B. variegatus</i>	Atibaia	XI-2016	2M <i>A. varium</i>	CNC-3437
<i>B. variegatus</i>	Mogi das Cruzes	VIII-2015	6M, 2F <i>A. varium</i>	CNC-3438
<i>Tamandua tetradactyla</i>	Registro	VII-2016	1F <i>Amblyomma calcaratum</i>	CNC-3439
<i>T. tetradactyla</i>	Pariquera-Açu	IV-2015	6F <i>A. calcaratum</i>	CNC-3440
<i>Dasybus novemcinctus</i>	São Paulo	I-2013	1F <i>A. sculptum</i>	CNC-2424

M: male; F: female; L: larva; N: nymph.

⁺ One specimen of each animal species, except when stated in parenthesis.

[‡] This tick was collected as an engorged nymph, which molted to adult in the laboratory.

[‡] These ticks were collected as engorged larvae, which molted to nymphs in the laboratory.

CNC: Tick collection "Coleção Nacional de Carrapatos Danilo Gonçalves Saraiva" of the Faculty of Veterinary Medicine and Animal Science of the University of São Paulo.

Table 2 - Ticks collected from wild mammals received and treated by the Wildlife Screening Center in São Paulo city, Brazil, from March 2003 to November 2016.

Hosts [†]	Municipality	Dates	Ticks	Accession number
-----Mammals-----				
<i>Leopardus pardalis</i>	Salesópolis	IX-2012	2F+2F* <i>Amblyomma aureolatum</i>	CNC-2421
<i>Leopardus guttulus</i>	Biritiba Mirim	III-2013	1F <i>A. aureolatum</i>	CNC-2422
<i>Eira barbara</i>	São Bernardo do Campo	IV-2007	1M+2M*, 1F+3F* <i>Amblyomma ovale</i>	CNC-2425
<i>Cercocyon thous</i>	Mogi das Cruzes	X-2012	2M+7M*, 2F <i>A. aureolatum</i> ; 2N <i>Amblyomma sculptum</i>	CNC-2426
<i>C. thous</i>	São Paulo	V-2013	1F <i>A. aureolatum</i>	CNC-2486
<i>Nasua nasua</i> (4)	São Paulo	IX-2012; VI, X-2013	5N <i>Amblyomma brasiliense</i> ; 1M <i>A. ovale</i> ; 1F <i>Amblyomma parkeri</i> ; 1F, 3N <i>A. sculptum</i> ; 1L <i>Amblyomma</i> sp.	CNC-2431, 2484, 2735
<i>N. nasua</i> (8)	São Paulo	V, VIII, X-2013; X-2015; VII-2016	1F, 25N <i>A. sculptum</i> ; 14L <i>Amblyomma</i> sp.	CNC-3441
<i>Sphiggurus villosus</i> (3)	São Paulo	III-2003; IX-2012; I-2014	4N <i>Amblyomma dubitatum</i> ; 4M, 2F <i>Amblyomma longirostre</i> ; 1L <i>Amblyomma</i> sp.	CNC-761, 2434, 2741
<i>S. villosus</i>	Itaquaquecetuba	VIII-2010	1M <i>A. longirostre</i>	CNC-2428
<i>S. villosus</i>	Poá	II-2011	1F <i>A. longirostre</i>	CNC-2429
<i>S. villosus</i>	Caieiras	VIII-2006	1M <i>A. longirostre</i>	CNC-2430
<i>S. villosus</i> (3)	Mogi das Cruzes	IX-2012; I-2014	4M, 2F+1F*, 1N <i>A. longirostre</i> ; 1M <i>A. parkeri</i>	CNC-2432, 2740
<i>S. villosus</i>	São Paulo	X-2013	5M, 1F <i>A. longirostre</i>	CNC-3442
<i>Guerlinguetus ingrami</i>	São Paulo	IV-2015	12L <i>Amblyomma</i> sp.	CNC-3443
<i>Tapirus terrestris</i> (2)	São Paulo	II-2010; VI-2012	2M, 7F, 1N <i>A. sculptum</i> ; 8M, 8F <i>Haemaphysalis juxtakochi</i>	CNC-2423, 2742
<i>Pecari tajacu</i>	Sete Barras	II-2013	118M+15M*, 33F+10F* <i>A. sculptum</i>	CNC-2427
<i>P. tajacu</i> (2)	São Paulo	I, II-2015	15M, 34F <i>A. sculptum</i>	CNC-3444
<i>Mazama gouazoubira</i> (3)	Mogi das Cruzes	V-2012; II, IX-2013	3N <i>A. sculptum</i> ; 31M+15M*, 15F+12F*, 10N <i>H. juxtakochi</i>	CNC-2436, 2745
<i>M. gouazoubira</i>	São Paulo	XII-2013	2F <i>H. juxtakochi</i>	CNC-2743
<i>M. gouazoubira</i>	Osasco	I-2014	2N <i>A. sculptum</i> ; 1M, 1F <i>H. juxtakochi</i> ; 2F <i>Rhipicephalus microplus</i>	CNC-2744
<i>M. gouazoubira</i>	Juquiá	V-2015	1N <i>A. brasiliense</i>	CNC-3445
<i>Myocastor coypus</i>	Mogi das Cruzes	VIII-2016	73N <i>A. dubitatum</i> ; 7L <i>Amblyomma</i> sp.	CNC-3446
<i>Hydrochoerus hydrochaeris</i> (6)	São Paulo	VII-2005; XII-2012; VI, XI-2013	31M, 23F, 2N <i>A. dubitatum</i> ; 9M, 10F <i>A. sculptum</i> ; 1L <i>Amblyomma</i> sp.	CNC-2435, 2487, 2748
<i>H. hydrochaeris</i>	Itaquaquecetuba	I-2014	14M, 11F <i>A. dubitatum</i> ; 1F, 1N <i>A. sculptum</i>	CNC-2746
<i>H. hydrochaeris</i> (2)	Guarulhos	VII, X-2013	6M, 5F, 1N <i>A. dubitatum</i> ; 1F, 5N <i>A. sculptum</i>	CNC-2747
<i>H. hydrochaeris</i>	São Paulo	IX-2013	5F, 36N <i>A. dubitatum</i>	-
<i>H. hydrochaeris</i> (2)	Cubatão	IX-2013	7M, 21F, 1N <i>A. dubitatum</i>	-
<i>H. hydrochaeris</i>	Mogi das Cruzes	XI-2016	2M, 5F, 6N <i>A. dubitatum</i> ; 1M, 2F, 2N <i>A. sculptum</i>	CNC-3447

M: male; F: female; L: larva; N: nymph.

[†] One specimen of each animal species, except when stated in parenthesis.

* Ticks tested by the PCR.

CNC: Tick collection "Coleção Nacional de Carrapatos Danilo Gonçalves Saraiva" of the Faculty of Veterinary Medicine and Animal Science of the University of São Paulo.

2013), these authors did not specify the tick stage that was collected. Noteworthy, *A. sculptum* is the main vector of *Rickettsia rickettsii* in Brazil (MARTINS et al., 2016).

Even though *Amblyomma parkeri* has been stated parasitizing various species of birds and mammals in the Brazilian territory (LABRUNA et al., 2009; MARTINS et al., 2013), we demonstrated in this research for the first time the occurrence of *A. parkeri* nymphs on *Penelope obscura*, and *Callicebus nigrifrons*, and adult on *Nasua nasua*.

Still unprecedentedly, we reported nymphs of *Amblyomma dubitatum* on *Sphiggurus villosus* in the Brazilian territory, corroborating with previous reports that rodents are the main hosts for all stages of this tick species (LABRUNA et al., 2004a; NAVA et al., 2010). Additionally, the present study reported for the first time a nymph of *A. dubitatum* on *Alouatta guariba*.

While LABRUNA et al. (2005) reported *Amblyomma aureolatum* as one of the most common tick species of wild carnivores in Brazil, we reported an *A. aureolatum* female on *Bradypus variegatus* in the municipality of Mogi das Cruzes. Such unusual record has been previously reported by GUGLIELMONE et al. (2003), who recorded two *A. aureolatum* females on *Bradypus* spp. sloths.

This research does not reported the presence of rickettsiae in the analyzed tick specimens, it is unequivocally accepted that there has been an increase of reports of rickettsiae in ticks from wild animals during recent years in Brazil (SPOLIDORIO et al., 2012; SOARES et al., 2015; ACOSTA et al., 2016; WITTER et al., 2016). The proportion of undiagnosed diseases, especially inland, may suggest that there are even more rickettsiae associated to ticks in the national territory.

CONCLUSION

The present study contributed with important data to the ixodid fauna of wild animals in Brazil as well as on the parasite-host interface, bringing various unpublished records of ectoparasitism of ticks on wild animals in the country. These data may contribute to a more comprehensive knowledge in the biology and behavior of these arthropods in relation to their hosts in the Brazilian territory.

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