



## Short Communication

# Ticks (Acari: Ixodidae, Argasidae) from humans, domestic and wild animals in the state of Espírito Santo, Brazil, with notes on rickettsial infection



Igor C.L. Acosta<sup>a</sup>, Thiago F. Martins<sup>a</sup>, Arlei Marcili<sup>a,b</sup>, Herbert S. Soares<sup>a</sup>, Felipe S. Krawczak<sup>a</sup>, Fernanda T. Vieira<sup>c</sup>, Marcelo B. Labruna<sup>a,\*</sup>

<sup>a</sup> Departamento de Medicina Veterinária Preventiva e Saúde Animal, Universidade de São Paulo, Faculdade de Medicina Veterinária e Zootecnia, São Paulo, SP, Brazil

<sup>b</sup> Mestrado em Medicina Veterinária e Bem Estar Animal, Universidade de Santo Amaro, São Paulo, SP, Brazil

<sup>c</sup> Departamento de Patologia Animal, Universidade Vila Velha, Faculdade de Medicina Veterinária, Vila Velha, ES, Brazil

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## ABSTRACT

Among the 69 tick species currently recognized in Brazil, only 13 have been reported in the state of Espírito Santo, southeastern Brazil. This study reports new tick records from Espírito Santo, and investigated the infection by rickettsial bacteria in these ticks. During 2012–2016, ticks were occasionally collected from different sources in Espírito Santo. In addition, we revised tick unpublished records from Espírito Santo in a tick collection. A total of 1263 tick specimens comprising 19 tick species (17 Ixodidae, 2 Argasidae) were collected. The following seven tick species are reported for the first time in Espírito Santo: *Amblyomma aureolatum*, *Amblyomma coelebs*, *Amblyomma ovale*, *Amblyomma rotundatum*, *Amblyomma varium*, *Haemaphysalis juxtakochi*, and *Ornithodoros hasei*. The only tick species previously reported for Espírito Santo, but not found in the present study, is *A. longirostre*. A total of 194 ticks of 7 species were tested for rickettsial infection. Three *Rickettsia* species were detected: *Rickettsia bellii* in *A. aureolatum*, *Rickettsia rhipicephali* in *H. juxtakochi*, and *Rickettsia amblyommii* in *A. humerale*. The present study increases the tick fauna of Espírito Santo to 20 species (29% of the Brazilian tick fauna). Additionally, 3 *Rickettsia* species are reported for the first time in this region of Brazil.

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## 1. Introduction

The tick fauna of Brazil is currently composed by 69 species divided into two families, Ixodidae (46 species) and Argasidae (23 species) (Barros-Battesti et al., 2015; Krawczak et al., 2015; Wolf et al., 2016). The state of Espírito Santo is located in the southeastern coast of Brazil, occupying an area of approximately 46,000 km<sup>2</sup>, within the Atlantic forest biome. Despite being located within the most populated region of Brazil (the Southeastern geopolitical region), very few studies on ticks and tick-borne diseases have been conducted in the state of Espírito Santo. Only the following 13 tick species (18.8% of the Brazilian tick fauna) have been reported to occur in the state of Espírito Santo: Ixodidae: *Amblyomma brasiliense* Aragão, *Amblyomma dubitatum* Neumann, *Amblyomma sculptum* Berlese [reported as *Amblyomma cajennense* (Fabricius)], *Amblyomma humerale* Koch, *Amblyomma incisum* Neumann, *Amblyomma longirostre* (Koch), *Amblyomma naponense* (Packard), *Amblyomma nodosum* Neumann, *Amblyomma oblongoguttatum* Koch, *Dermacentor*

*nitens* Neumann, *Rhipicephalus microplus* (Canestrini), *Rhipicephalus sanguineus* sensu lato (Latreille); and Argasidae: *Argas persicus* (Oken) (Aragão, 1936; Labruna et al., 2005a; Ogrzewalska et al., 2007; Oliveira et al., 2008; Silveira et al., 2008; Mazioli et al., 2012; Martins et al., 2016). Moreover, while at least eight *Rickettsia* species have been reported to infect different ticks in Brazil (Oliveira et al., 2008; Labruna et al., 2011; Almeida et al., 2011), studies in the state of Espírito Santo have been restricted to a single report of *Rickettsia felis* infecting *R. sanguineus* sensu lato ticks (Oliveira et al., 2008). In addition, several clinical (including fatal) cases of human infection by spotted fever group rickettsiae, presumably *Rickettsia rickettsii*, have been reported in Espírito Santo (Sexton et al., 1993; Barros e Silva et al., 2014). In this study, we report a list of new tick records from Espírito Santo, with some species reported for the first time in the state. In addition, we report different rickettsiae infecting these ticks.

## 2. Materials and methods

During 2012–2016, ticks were occasionally collected from different wild and domestic animals, humans, and from the environment in different areas of the state of Espírito Santo. In addition, we revised all unpublished records of ticks from Espírito Santo that have been

\* Corresponding author at: Universidade de São Paulo, Faculdade de Medicina Veterinária e Zootecnia, Departamento de Medicina Veterinária Preventiva e Saúde Animal, Av. Prof. Orlando Marques de Paiva 87, São Paulo, SP 05508-270, Brazil.

E-mail address: [labruna@usp.br](mailto:labruna@usp.br) (M.B. Labruna).

**Table 1**  
Localities in the state of Espírito Santo where ticks were collected for the present study.

Locality no.	Municipality	Latitude, longitude	Altitude (m)
1	Alegre	-20.76, -41.53	300
2	Cachoeiro de Itapemirim	-20.84, -41.11	110
3	Cariacica	-20.26; -40.42	50
4	Guarapari	-20.65, -40.51	10
5	Linhares	-19.39, -40.07	30
6	Marechal Floriano	-20.41, -40.68	750
7	Nova Venécia	-18.71, -40.40	140
8	Pinheiros	-18.38, -40.12	130
9	Santa Maria de Jetibá	-20.04, -40.74	780
10	Santa Teresa	-19.93, -40.60	720
11	São Mateus	-18.71, -39.85	30
12	Serra	-20.12, -40.30	40
13	Sooretama	-19.19, -40.09	65
14	Vila Valério	-18.99, -40.38	130
15	Vila Velha	-20.33, -40.29	7

deposited at the tick collection “Coleção Nacional de Carrapatos” (CNC) of the University of São Paulo, Brazil. Ticks were taxonomically identified following Kohls et al. (1970); Barros-Battesti et al. (2006); Martins et al. (2010), and Martins et al. (2016). Some of the recently collected ticks were selected to be tested for rickettsial infection. For this

purpose, adult ticks were processed individually, while nymphs were tested individually or in pools of up to 10 ticks by DNA extraction using the guanidine isothiocyanate phenol technique (Sangioni et al., 2005), and tested by polymerase chain reaction (PCR) protocol using primers CS-78 and CS-323, targeting a 401-bp fragment of the citrate synthase gene (*gltA*) of the genus *Rickettsia* (Labruna et al., 2004). Samples yielding a visible PCR product by this PCR were further tested with primers Rr190.70p and Rr190.701n, targeting a 631-bp fragment of the rickettsial 190-kDa outer membrane protein gene (*ompA*) (Eremeeva et al., 2006). Negative control tubes containing water and a positive control tube containing DNA of *Rickettsia* sp. strain NOD were included in each PCR run. In addition, DNA sample from an individual larva of the genus *Ornithodoros* was tested by a PCR assay targeting a portion of the tick mitochondrial 16S rRNA gene, as previously described (Mangold et al., 1998). Amplicons were purified with ExoSap (USB, Cleveland, Ohio, USA) and DNA-sequenced in an ABI automated sequencer (Applied Biosystems/Thermo Fisher Scientific, model ABI 3500 Genetic Analyzer, Foster City, California, USA) with the same primers used for PCR. The sequences obtained were submitted to BLAST analyses ([www.ncbi.nlm.nih.gov/blast](http://www.ncbi.nlm.nih.gov/blast)) to infer the closest similarities available in GenBank.

**Table 2**  
Ticks collected in the state of Espírito Santo, Brazil, according to hosts and localities.

Hosts (no. specimens with ticks)	Locality no.*	Year	Tick species: no. per stage #
Amphibia			
<i>Rhinella</i> sp. (1)	14	2007	<i>Amblyomma rotundatum</i> : 2 N
Reptilia			
<i>Chelonoidis denticulata</i> (1)	8	2012	<i>Amblyomma humerale</i> : 15 M
<i>Boa constrictor</i> (1)	15	2012	<i>A. rotundatum</i> : 2 N
Aves			
<i>Spheniscus magellanicus</i>	3	2012	<i>Amblyomma sculptum</i> : 1F
Mammals			
<i>Didelphis aurita</i> (1)	8	2012	<i>Amblyomma coelebs</i> : 1 N
<i>D. aurita</i> (2)	10	2015	<i>Amblyomma dubitatum</i> : 2 N; <i>Amblyomma</i> sp.: 10 L
<i>Marmosops incanus</i>	10	2015	<i>Amblyomma</i> sp.: 3 L
<i>Myrmecophaga tridactyla</i> (1)	4	2004	<i>Amblyomma nodosum</i> : 4 M, 1F
<i>Bradypus torquatus</i> (1)	4	2005	<i>Amblyomma varium</i> : 2 M, 2F
<i>Dasyprocta agouti</i> (2)	8	2012	<i>Amblyomma brasiliense</i> : 3 N; <i>Amblyomma naponense</i> : 1 N
<i>Tayassu pecari</i> (1)	8	2012	<i>A. brasiliense</i> : 1F, <i>A. naponense</i> : 4 M, 6F, 1 N; <i>Amblyomma oblongoguttatum</i> : 5 M, 11F, 4 N
<i>Tapirus terrestris</i> (1)	5	2012	<i>A. brasiliense</i> : 1 M, 2F; <i>Amblyomma incisum</i> : 1 M, 5F, 3 N; <i>A. naponense</i> : 1F, 1 N
<i>T. terrestris</i> (1)	8	2012	<i>A. brasiliense</i> : 3 M, 4F, 3 N; <i>A. incisum</i> : 1 M, 3F, 1 N; <i>A. naponense</i> : 1F, 2 N; <i>A. oblongoguttatum</i> : 7 M, 3F; <i>Amblyomma</i> sp.: 1 L
<i>T. terrestris</i> (1)	2	2011	<i>A. sculptum</i> : 10 M, 13 N
<i>T. terrestris</i> (1)	6	2012	<i>A. sculptum</i> : 4 M, 12F, 21 N; <i>Amblyomma</i> sp.: 10 L
<i>Nasua nasua</i> (1)	8	2012	<i>A. brasiliense</i> : 6 N; <i>A. oblongoguttatum</i> : 3 N; <i>Amblyomma</i> sp.: 1 L
<i>Equus caballus</i> (5)	8	2011	<i>A. incisum</i> : 1 M; <i>Dermacentor nitens</i> : 2 M, 12F, 2 N, 1 L; <i>Rhipicephalus sanguineus</i> s.l.: 1F; <i>Rhipicephalus microplus</i> : 2 M, 4F
<i>E. caballus</i> (1)	1	2013	<i>D. nitens</i> : 3F
<i>E. caballus</i> (1)	3	2013	<i>D. nitens</i> : 4F
<i>E. caballus</i> (1)	15	2013	<i>A. sculptum</i> : 3 N; <i>R. microplus</i> : 1F
<i>E. caballus</i> (1)	12	2013	<i>D. nitens</i> : 3 M, 3F
<i>E. caballus</i> (6)	7	2007	<i>D. nitens</i> : 31 M, 25F, 64 N, 2 L; <i>R. microplus</i> : 10 M, 32F, 7 N, 2 L
<i>Canis lupus familiaris</i> (6)	9	2013, 2015	<i>Amblyomma aureolatum</i> : 8 M, 9F; <i>Amblyomma ovale</i> : 3F
<i>C. lupus familiaris</i> (1)	10	2012	<i>A. brasiliense</i> : 1F, 2 N; <i>Amblyomma</i> sp.: 2 L
<i>C. lupus familiaris</i> (72)	8	2011, 2012	<i>A. ovale</i> : 1F
<i>C. lupus familiaris</i> (1)	5	2011	<i>A. naponense</i> : 2F, 3 N; <i>A. oblongoguttatum</i> : 5 M, 2F, 4 N; <i>A. sculptum</i> : 1 M, 1F, 3 N; <i>D. nitens</i> : 11F, 2 N; <i>R. sanguineus</i> s.l.: 76 M, 129F, 3 N; <i>R. microplus</i> : 1 M, 10F; <i>Amblyomma</i> sp.: 1 L
<i>C. lupus familiaris</i> (1)	8	2012	<i>R. sanguineus</i> s.l.: 1F
<i>Artibeus lituratus</i> (1)	8	2012	<i>Ornithodoros hasei</i> : 17 L
<i>Homo sapiens</i> (3)	10	2012	<i>A. brasiliense</i> : 2 N; <i>A. sculptum</i> : 1 N
Environment			
Hen house	11	2007	<i>Argas miniatus</i> : 6 M, 7F, 7 N
Vegetation	10	2016	<i>A. brasiliense</i> : 14 N
Vegetation	13	2015	<i>A. incisum</i> : 1F
Vegetation	5	2011	<i>A. brasiliense</i> : 2 M, 1F, 27 N; <i>A. oblongoguttatum</i> : 6 N; <i>Amblyomma</i> sp.: 30 L
Vegetation	8	2012	<i>A. brasiliense</i> : 8 M, 12F, 80 N; <i>A. incisum</i> : 7 M, 3F, 64 N; <i>A. naponense</i> : 4 M, 2F, 43 N; <i>A. oblongoguttatum</i> : 17 M, 20F, 144 N; <i>A. sculptum</i> : 3 M, 1F, 10 N; <i>Haemaphysalis juxtakochi</i> : 9 N; <i>Amblyomma</i> sp.: 15 L

\* See Table 1; # L: larvae; N: nymph; M: male; F: female.

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