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Evaluation of rickettsial infection in free-range capybaras (*Hydrochoerus hydrochaeris* Linnaeus, 1766) (Rodentia: Caviidae) and ticks (Acari: Ixodidae) in the Western Amazon, Brazil

Karla Dayane Gruhn[a,*], Maria Ogrzewalska[b], Tatiana Rozental[b], Itacir Olivio Farikoski[a], Carolina Blanco[b], Lucas de Souza Freitas[a], Elba Regina Sampaio de Lemos[b], Vânia Maria França Ribeiro[a]

[a] Centro de Ciências Biológicas e da Natureza, Universidade Federal do Acre, Rio Branco, AC, Brazil
[b] Laboratório de Hantaviroses e Rickettsioses, Fundação Oswaldo Cruz, Rio de Janeiro, RJ, Brazil

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ABSTRACT

Capybaras (*Hydrochoerus hydrochaeris* Linnaeus, 1766) (Rodentia: Caviidae) are important hosts of *Amblyomma* ticks (Acari: Ixodidae), which in turn can transmit rickettsiae to humans and animals. However, there is a scarcity of studies about the tick fauna and rickettsial infection in the Amazon region. The present study evaluated rickettsial infection in capybaras and ticks in different areas of the municipality of Rio Branco, state of Acre, in the Western Brazilian Amazon, where rickettsiosis has never been reported. Blood sera from 43 capybaras from four localities in Rio Branco were tested by indirect immunofluorescence assay using *Rickettsia rickettsii* antigens. Ticks were collected from capybaras and from vegetation as well. Ticks were taxonomically identified to the species level and some of them were tested by PCR, targeting a fragment of the rickettsial *gltA* gene. Additionally, ticks were tested for bacteria from the genus *Borrelia* and family Anaplasmataceae. All capybaras submitted to the serological examination were considered non-reactive to *R. rickettsii*. A total of 410 ticks were collected directly from the capybaras. *Amblyomma dubitatum* Neumann, 1899 was the most abundant species (82.4%), followed by *Amblyomma naponense* (Packard, 1869) (14.3%), *Amblyomma humerale* Koch, 1844 (0.7%), *Amblyomma pacae* Aragão, 1911 (0.4%), *Amblyomma rotundatum* Koch 1844 (0.2%) and *Amblyomma* sp. (1.7%). From the environment 262 ticks were collected: *Rhipicephalus microplus* (Canestrini, 1888) (88.9%), *Dermacentor nitens* Neumann, 1897 (9.9%), *Amblyomma varium* Koch, 1844 (0.7%) and *A. rotundatum* (0.3%). With the exception of *A. humerale, A. rotundatum* and *R. microplus*, all other species are reported here for the first time in the state. Some of the ticks sampled (N = 317) were tested by molecular methods for infection by *Rickettsia* spp. *Rickettsia bellii* was identified infecting *A. dubitatum* and *A. rotundatum*, while *Rickettsia amblyommatis* only was found infecting *A. humerale* and *Rickettsia* sp. strain Tapirapé was found in *A. naponense*. This is the first detection of *R. bellii* and *Rickettsia* sp. strain Tapirapé in Acre. No *Borrelia* or Anaplasmataceae were found in the tested ticks. These results add relevant knowledge about the *Rickettsia* spp. and the acarological fauna in the region of the Western Amazon, and are essential for the maintenance of vigilance about possible pathogens that occur in the state and determination of the risks that they pose to humans and animals that inhabit the region.

1. Introduction

The capybara (*Hydrochoerus* spp.) (Rodentia: Caviidae) is considered the largest rodent present in Central and South America (Reis et al., 2011; Reid, 2016). Studies have shown that it plays an important role in the epidemiological cycle of *Rickettsia rickettsii* (Rickettsiales, Rickettsiaceae), the etiological agent of Brazilian spotted fever, transmitted by ticks (Souza et al., 2009; Krawczak et al., 2014).

Ticks are obligate ectoparasites of vertebrates, belonging to the class Arachnida and suborder Ixodida (Barros-Battesti et al., 2006; Guglielmone et al., 2014). Of the 73 species endemic or already established in Brazil, 47 belong to the Ixodidae family (Labruna et al.,

* Corresponding author at: Departamento de Ciências da Natureza, Universidade Federal do Acre, Campus Universitário, BR 364, Km 04, Distrito Industrial, CEP: 69915-900, Rio Branco, AC, Brazil.
E-mail address: karla_gruhn@hotmail.com (K.D. Gruhn).

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2016; Dall'Agnola et al., 2017; Muñoz-Leal et al., 2018), which comprises the ticks known as "hard ticks", of which the genus *Amblyomma* is the most numerous (Barros-Battesti et al., 2006; Dantas-Torres et al., 2009). These ectoparasites are well known for transmitting various pathogens to animals and humans around the world (Barros-Battesti et al., 2006).

The capybara has a large body area and serves as host for several species of ticks, including *Amblyomma sculptum* Berlesse, 1888 (*A. cajennense* complex), a vector of Brazilian spotted fever in southern Brazil, and *Amblyomma dubitatum* Neumann, 1899 (Szabó et al., 2013; Pajuaba-Neto et al., 2018). Moreover, capybaras were experimentally proven to be amplifier hosts of *R. rickettsii* for *A. sculptum* ticks and can be used as sentinels to detect the circulation of rickettsiae (Souza et al., 2009; Brites-Neto et al., 2015).

The state of Acre, located in the North of Brazil and inserted in the Western Amazon, has an area of 164,123.737 km^2 (IBGE, 2018). It is located in one of the world's richest regions in terms of biodiversity, hosting a large number of species of fauna and flora (Myers, 1988).

However, there is a scarcity of studies on tick fauna in this region and no studies aimed at investigating tick-borne pathogens and the carriers in capybaras in the state. In particular, data are scanty regarding Acre's acarological fauna. Rohr (1909); Aragão (1936); Guimarães et al. (2001) reported the species *Amblyomma calcaratum* Neumann, 1899, *Amblyomma coelebs* Neumann, 1899, *Amblyomma dissimile* Koch, 1844, *Amblyomma incisum* Neumann, 1906, *Amblyomma longirostre* (Koch, 1844), *Amblyomma oblongoguttatum* Koch, 1844, *Amblyomma ovale* Koch, 1844 and *Rhipicephalus microplus* (Canestrini, 1888) in the state. Souza et al. (2016) made the first report of the species *Amblyomma geayi* Neumann, 1899 in the region, collected from a free-living sloth, and Lima et al. (2018) first reported the species Amblyomma humerale Koch, 1844 and Amblyomma nodosum Neumann, 1899 on birds, Amblyomma scalpturatum Neumann, 1906 from vegetation, Amblyomma rotundatum Koch, 1844 on a toad Rhinella sp., and Ixodes luciae Sénevet, 1940 on the opossum Didelphis marsupialis.

In state of Acre, Lima et al. (2018) reported *Rickettsia parkeri* strain NOD in *A. nodosum* and *Rickettsia amblyommatis* in *A. longirostre* and *A. geayi*, collected from birds.

The present study aimed to investigate the exposure to spotted fever group rickettsiae (*R. rickettsii* antigen) in free-living capybaras and *Rickettsia* spp., *Borrelia* spp. and Anaplasmataceae in ticks collected from capybaras and vegetation in the municipality of Rio Branco, Acre, Western Amazon, Brazil.

2. Material and methods

2.1. Study area

The study was conducted in four areas located in Rio Branco: two peri-urban areas in recently occupied settlements contiguous to the Amazon rainforest (Area 1: 9°57′33.0″S 67°52′23.3″W; Area 2: 9°57′51.4″S 67°52′14.9″W), and two rural areas, farms located 15 km from the city (Area 3: 10°00′39.7″S 67°56′14.9″W; Area 4: 9°56′49.7″S 67°44′09.4″W). One of the peri-urban study locations was a University campus that had forest areas, dams and both domestic and wild animals (Area 1). The other location refers to a housing complex with several dams and green areas (Area 2). The farms are occupied by pastures and dams, used mainly for cattle and horse breeding and fish farming, interspersed with forest areas upholding wild animals.

The climate in Rio Branco is hot and humid, with a dry season extending from May to October and a rainy season extending from November to April. The average annual temperature varies between 22 °C and 26 °C (Acre, 2000).

2.2. Animal capture

During the period from March to November 2015, capybaras were captured in all four previously chosen study sites (Area 1, Area 2, Area 3 and Area 4), using corral-traps with food offered daily (mineral salt, banana leaves, sugarcane and grass). Upon entry, the trap closed and the animals were mechanically contained in dip nets. Captured animals were identified by microchips (AnimalTAG®) and anesthetized with azaperone (1.0 mg/kg), ketamine (12 mg/kg) and diazepam (0.1 mg/kg) applied intramuscularly, following King et al. (2010). All captured animals were examined by veterinarians and biologists for health status assessment, measurement, weighing, sexing and collection of blood samples.

2.3. Blood samples and tick collection

Blood was collected by venipuncture of the femoral vein (Vacutainer®, BD, Franklin Lakes, NJ, USA). The samples were transported to the laboratory and centrifuged. Serum samples were labeled and stored in 1.5 mL microtubes (Eppendorf®, São Paulo, SP, Brazil) at −20 °C to be tested. Each capybara was carefully examined by at least two researchers for the presence of ticks for 20 min., and all the ticks found were collected manually and immediately preserved in tubes with 70% ethanol for further analysis.

Between November 2015 and April 2016, ticks were collected from the environment in the same study areas (Area 1, Area 2, Area 3 and Area 4) by the flag-dragging method (Oliveira et al., 2000). Collection was always done in the morning and sampling was carried out in a radius of 150 m from the center of the point where traps for capybaras were mounted. The flannel was checked for ticks every 10 m, for a total of eight collections in each study area. All specimens collected were stored in properly identified plastic tubes containing 70% ethanol.

2.4. Ethical approval

All procedures were authorized by the Ethics Committee on the Use of Animals of Federal University of Acre (No. 23107.016723 / 2014-41) and by the Chico Mendes Institute for Biodiversity Conservation - ICMBio (SISBIO No. 44791-100).

2.5. Tick identification

In the laboratory, the adult and nymph stages of ticks were identified to species level through morphological examination following dichotomous keys (Onofrio et al., 2006; Martins et al., 2010). Identification of larvae was performed by molecular analysis, as previously described (Blanco et al., 2017). For this purpose, representative specimens of each tick species were submitted to DNA extraction using the QIAamp® DNA Blood Mini Kit (Qiagen, Valencia, CA, USA) and tested by a PCR assay targeting a portion of the tick mitochondrial 16S rRNA gene, as previously described (Mangold et al., 1998). Amplicons (˜460 bp) were visualized on 1% agarose gels stained with Gel Red Nucleic Acid Gel Stain™ 10,000× in DMSO (Biotium, Hayward, CA, USA). PCR products of the expected size were purified with ExoSAP-IT® (Affymetrix, Cleveland, OH, USA), and sequenced in a 96-capillary 3730xl DNA Analyzer® (Applied Biosystems, Foster City, CA, USA) according to the protocols developed by Otto et al. (2008), using the same primers (forward and reverse) used for the PCR. Partial sequences obtained were submitted to BLAST analysis (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and were aligned with corresponding 16S rRNA sequences of different tick species available in the GenBank. Furthermore, some nymphs and adults of *Amblyomma* were also submitted to the same method to confirm taxonomic identification.

2.6. Serological analyses

The serological test of choice was the indirect immunofluorescence assay (IFA) for detection of anti - Spotted Fever Group - *Rickettsia* spp. antibodies (*R. rickettsii* antigen). Each capybara serum sample was

individually tested using *R. rickettsii* antigen (IgG test kit Focus Diagnostics®, CA, USA) following manufacturer's protocol. For this purpose, a 2-fold dilution series in phosphate-buffered saline was prepared from the serum, starting from the 1:64 dilution. A fluorescein isothiocyanate -labeled sheep anti-capybara IgG (CCZ, São Paulo/SP, Brazil) was used as conjugate, diluted to 1:100. In each slide, negative and a positive serum sample were used as negative and positive controls, respectively (Center for Zoonosis Control, CCZ, São Paulo, SP, Brazil).

2.7. Molecular analysis of the ticks

Some of the ticks were individually tested by PCR assays targeting bacteria of the genera *Rickettsia, Borrelia* and family Anaplasmataceae. The DNA extraction was performed as described above. Blank tubes containing water were always included for contamination control during DNA extraction. PCR for *Rickettsia* detection was performed with the genus-specific CS-78 primers (5′-GCAAGTATCGGTGAGGATGT AAT-3′) and CS-323 (5′-GCTTCCTTAAAATTCAATAAATCAGGAT-3), designed to amplify the 401-pb fragment of the citrate synthase gene (*gltA*), present in *Rickettsia* spp., as previously described (Labruna et al., 2004). For samples with positive PCR results for the *gltA* gene, another PCR of the sample was performed using primers 190.70 (5′-ATGGCG AATATTTCTCCAAAA-3′) and 190.701 (5′-GTTCCGTTAATGGCAGCA TCT-3′), with the target of the 632-pb fragment of the 190-kDa (*ompA*) outer membrane protein gene, present only in the rickettsiae of the spotted fever group (Fournier et al., 1998) and˜800 bp fragment of the *ompB* gene with primers 120-M59 (5′-CCGCAGGGTTGGTAACTGC-3′) and 120–807 (5'CCTTTTAGATTACCGCCTAA-3′) (Roux and Raoult, 2000).

To screen ticks for the *Borrelia* spp., the primers BorFlaF1 (5′-TAC ATCAGCTATTAATGCTTCAAGAA-3′) and BorFlaR1 (5′-GCAATCATW-GCCATTGCRGATTG-3′) were used to amplify the fragment of the *fla* gene of the 729 bp in the first reaction and BorFlaF2 (5′ CTGATGAT-GCTGCTGGWATGG-3′) and BorFlaR2 (5'TCATCTGTCATTRTWGCAT-CTT-3′) to amplify the fragment of 410 bp in the second reaction (nested PCR), according to Blanco et al. (2017). The primers used in the PCR for Anaplasmataceae were EHR16SD (5′-GGTACCYACAGAAGAA-GTCC-3′) andEHR16SR (5'-TGCACTCATCGTTTACAG-3), targeting a fragment of 345 bp of the gene 16S rRNA (Inokuma et al., 2000). All reactions were performed with 25 µl per reaction, 12.5 µl of DreamTaq TM Green PCR Master Mix, 8.0 µl of nuclease free water (Thermo Fischer Scientific Inc., Waltham, MA, USA), 1 µl of each primer at 10 µM (Invitrogen, Carlsbad, CA, USA) and 2.5 µl of DNA from the sample tested. In each PCR assay, negative controls (water) and an appropriate positive control sample (*R. rickettsii*, *Borrelia burgdorferi* sensu stricto and *Anaplasma phagocytophilum* DNA) were included in conjunction with the tick samples.

The PCR products considered positive were purified with ExoSAP-IT® (Affymetrix, Cleveland, OH, USA), and sequenced in 96-capillary 3730xl DNA Analyzer® (Applied Biosystems, Foster City, CA, USA) according to the protocols developed by Otto et al. (2008), using the same primers (forward and reverse) used in the PCR. Partial sequences obtained were analyzed by the Basic Local Alignment Search Tool (BLAST) (https://goo.gl/cgevl7) to determine the identity of the sequences obtained for other rickettsiae.

3. Results

Of the 672 ticks, 410 were collected from the capybaras and 262 from the environment. The species found on animals were *A. dubitatum* (82.4%), *Amblyomma naponense* Packard, 1869 (14.3%), *A. humerale* (0.7%), *Amblyomma pacae* Aragão, 1911 (0.4%) and *A. rotundatum* (0.2%). Some of larvae were only identified to the genus level (1.7%) (Table 1).

The species found in the environment were *R. microplus* (88.9%), *Dermacentor nitens* Neumann, 1897 (9.9%), *Amblyomma varium* Koch, 1844 (0.7%) and *A. rotundatum* (0.3%) (Table 2).

The fragments of 16S rDNA gene obtained were 98–100% identical with sequences of corresponding tick species available in the GenBank and the accession numbers of the sequences are: KY020985 (1 F and 1 N *A. pacae*), KY020986 (1 L and 1 N *A. rotundatum*), KY020987 (1 N *A. humerale*), KY020988 (1 N *A. varium*), KY020989-91 (2 N and 2 F *A. naponense*), KY020992 (25 L, 2 N *A. dubitatum*), KY020993 (7 L *R. microplus*), KY020994 (10 L *D. nitens*).

Some of the identified ticks (N = 317) were tested by PCR for the presence of tick-borne pathogens. By obtaining the partial nucleotide sequences of the positive PCR samples, it was possible to detect *Rickettsia bellii* in 2.4% of *A. dubitatum* (6/246) and 50% of *A. rotundatum* (1/2). The *gltA* fragment sequence identity with the corresponding complete *R. bellii* genome (CP000087) was 98.7% (385/390bp).

Rickettsia amblyommatis was detected in 33.3% (1/3) of *A. humerale*. The similarities with the corresponding sequences of the complete genome of *R. amblyommatis* (CP015012) were 99.7% (377/378bp), 99.6% (605/607 bp) and 99.4% (822/827 bp) for *gltA, ompA* and *ompB* genes respectively.

Rickettsia spp. were detected in 11.8% (4/34) of *A. naponense* with 99.7% (365/366 bp) identity of the sequence of *gltA* gene to *Rickettsia* sp. clone Tapirapé (KM042857). No products of PCR targeting *ompA* or *ompB* genes were obtained.

The GenBank nucleotide sequence accession numbers for the partial sequences generated in the present study are: KY053883 (*R. bellii, gltA*), KY053884 (*R. amblyommatis, gltA*), KY053885 (*R. amblyommatis, ompA*), and KY053882 (*Rickettsia* sp. Tapirapé, *gltA*).

No *Rickettsia* DNA was detected in *A. pacae* (N = 2), *A. varium* (N = 1), *D. nitens* (N = 13) and *R. microplus* (N = 15). DNA of *Borrelia* or Anaplasmatacae was not detected in any (N = 317) of the tested ticks.

In total, were captured 43 capybaras (10 animals in areas 1, 2 and 4,

Table 1
Species of ticks collected from free-living capybaras in the municipality of Rio Branco, Acre, Western Amazon. L: Larvae; N: Nymphs; M: Males; F: Females.

Tick species	LOCATION																TOTAL
	PERI-URBAN AREAS								RURAL AREAS								
	Area 1				*Area 2*				*Area 3*				*Area 4*				
	L	N	M	F	L	N	M	F	L	N	M	F	L	N	M	F	
A. dubitatum	–	–	–	–	1	8	71	36	–	1	75	43	22	65	7	9	338
A. naponense	–	4	20	9	–	2	2	2	–	–	2	3	–	1	8	6	59
A. humerale	–	–	–	–	–	3	–	–	–	–	–	–	–	–	–	–	3
A. pacae	–	1	–	1	–	–	–	–	–	–	–	–	–	–	–	–	2
A. rotundatum	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	1
***Amblyomma* spp.**	–	1	–	–	–	–	–	–	–	–	–	–	6	–	–	–	7
TOTAL	–	6	20	10	1	13	73	38	–	1	76	46	28	67	15	16	410

Table 2
Species of ticks collected from vegetation in the municipality of Rio Branco, Acre, Western Amazon. L: Larvae; N: Nymphs; M: Males, F: Females.

Tickspecies	LOCATION																TOTAL
	PERI-URBAN AREAS								RURAL AREAS								
	Area 1				*Area 2*				*Area 3*				*Area 4*				
	L	N	M	F	L	N	M	F	L	N	M	F	L	N	M	F	
A. rotundatum	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1
A. varium	–	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2
D. nitens	–	–	–	–	–	–	–	–	17	–	–	–	9	–	–	–	26
R. microplus	–	–	–	–	–	–	–	–	218	–	1	–	14	–	–	–	233
TOTAL	1	2	–	–	–	–	–	–	235	–	1	–	23	–	–	–	262

and 13 animals in area 3). All the capybara was non-reactive by IFA for *R. rickettsii*.

4. Discussion

The most abundant tick species found in capybaras was *A. dubitatum*, followed by *A. naponense*. The species *A. dubitatum* is commonly known as the capybara tick and has as primary hosts all stages of animals belonging to the family Caviidae (Rodentia), among which the main one is the capybara (Guglielmone et al., 2014; Martins et al., 2014). In the state of Acre, this is the first report of the species found in urban or rural areas, demonstrating that it is possibly established in this region.

Amblyomma naponense ticks are found most frequently parasitizing Tayassuidae (Guglielmone et al., 2014). However, they may parasitize various other mammals (Soares et al., 2015) and wild birds (Guglielmone et al., 2014).

In this study, we found a greater number of parasitized animals inhabiting the peri-urban areas, with a large flow of people. This is of great importance considering that *A. naponense* ticks were previously reported parasitizing humans (Guglielmone et al., 2006).

Others species were found in smaller number parasitizing the capybaras: *A. humerale, A. rotundatum* and *A. pacae. Amblyomma humerale* has been reported in several states, including those in the Amazon region. It is commonly found parasitizing reptiles, mainly tortoises (*Geochelone* sp.) (Testudinidae), but immature stages have lower parasitic specificity, so they can also parasitize small mammals and birds (Labruna et al., 2005; Ogrzewalska et al., 2010; Lima et al., 2018).

Amblyomma rotundatum is a species with a wide geographic range, occurring in the Neotropical and Nearctic regions (Guglielmone et al., 2014), commonly found on cold-blooded vertebrates such as amphibians, especially toads (Anura: Bufonidae) and reptiles (Squamata) (Guglielmone et al., 2014). In this study, only one nymph was found parasitizing a capybara, emphasizing the fact that other hosts such as Mammalia, are considered exceptional for this species (Guglielmone et al., 2010).

Amblyomma pacae, commonly known as the paca brown tick, is found in the Neotropical region and is mainly found on animals of the family Cuniculidae (Rodentia), but can be found parasitizing several other mammals (Aragão, 1936; Guglielmone et al., 2014), including humans (Guglielmone et al., 2006). This is the first record of the species parasitizing capybaras.

On vegetation, we found the species *R. microplus* in highest numbers, followed by *D. nitens*, *A.varium* and *A. rotundatum. Amblyomma varium* was recorded only in vegetation (nymph stage), collected in the peri-urban area. It is known in Brazil as the giant sloth tick and can be found in mammals of the Bradypodidae and Magalonychidae (Xenarthra) families. It has been reported in several countries and in different states of Brazil (Guglielmone et al., 2014). This is the first report of its occurrence in Acre.

Rhipicephalus microplus ticks preferably parasitize cattle, but other animals can serve as hosts. When high infestation occurs on cattle, this tick can also parasitize humans during handling (Barros-Battesti et al., 2006). *D. nitens* is one of the main species of ticks of equidae and there are few reports of parasitism in humans (Guglielmone et al., 2006). It has also been reported to parasitize other mammalian species (Guglielmone et al., 2014). This is the first report of *D. nitens* in Acre.

No serological evidence of spotted fever group - *Rickettsia* sp. exposure was recovered from the capybara samples, on the contrary to a field study in a southeastern endemic spotted fever area in Brazil, where 48.3% (N = 172) of animals were seroreactive for *R. rickettsii* (Krawczak et al., 2014). The negative results of serology tests obtained in the present study are in agreement with the present knowledge of epidemiology of spotted fever in Brazil, since there are no reports of human cases or proof of *R. rickettsii* presence in arthropod vectors anywhere in the Western Amazon region (Sinan, 2018). However, it is possible that these animals are infected with other rickettsiae, similar to the results obtained by Pacheco et al. (2007), who sampled animals from non-endemic areas and found animals seroreactive to *R. bellii* and *R. parkeri* but not to *R. rickettsii*. More specific research, using specific antigens for other species of rickettsiae should be performed to evaluate the participation of capybaras in the transmission cycle of other bacteria of the genus *Rickettsia* in the state. In ticks, we report three rickettsial agents; *R. bellii*, in *A. dubitatum* and *A. rotundatum, Rickettsia* sp. strain Tapirapé infecting *A. naponense* and *R. amblyommatis* in *A. humerale.*

Rickettsia bellii was found in all the areas investigated in this study. It has been reported on several tick species throughout several Brazilian states (Labruna et al., 2011; Ogrzewalska and Pinter, 2016), including *A. dubitatum* (Labruna et al., 2004; Brites-Neto et al., 2013) and *A. rotundatum* (Horta et al., 2015; Santos da Silva et al., 2016; Costa et al., 2017). It is considered non-pathogenic to humans (Labruna et al., 2011) and animals (Horta et al., 2007; Pinter et al., 2008), but serological evidence has been reported in capybaras (Pacheco et al., 2007).

Rickettsia sp. strain Tapirapé was found in two areas (urban and rural), parasitizing *A. naponense*. This is a new species of *Rickettsia* sp., according to its phylogenetic analysis. The agent found prior to this belongs to the *canadensis* group (CG) and is closest to the *R. monteiroi* species (Pacheco et al., 2011; Soares et al., 2015). No bacterium belonging to the *canadensis* group was isolated on humans or animals. However, in the USA serological evidence has been found that a bacterium of the *R. canadensis* (belonging to the *canadensis* group) species can cause spotted fever in humans (Parola et al., 2009). Our study contains the second report *Rickettsia* sp. strain Tapirapé; its pathogenicity to humans or animals is not known. However, *A. naponense* ticks are associated with parasitism in humans (Guglielmone et al., 2006), increasing the risk of transmission of possible pathogens.

Rickettsia amblyommatis was found in only one urban area, parasitizing *A. humerale*. It was previously reported in Acre, infecting *A. longirostre* and *A. geayi* (Lima et al., 2018). Despite having unknown

pathogenicity (Labruna et al., 2011), serological evidence has been obtained in the Amazon region in dogs (Labruna et al., 2007; Spolidorio et al., 2013; Costa et al., 2017). In the USA, it is suggested that some rickettsioses reported as Rocky Mountain spotted fever were caused by *R. amblyommatis* (Apperson et al., 2008).

No *Borrelia* or Anaplasmataceae was found. Studies reported *Borrelia* spp. pathogen can be transmitted by *Ixodes* ticks too (Radolf et al., 2012; Dall'Agnola et al., 2017). In Brazil, there is no evidence yet of *Amblyomma* infection with these pathogens.

5. Conclusions

This study increases the knowledge about the species of ticks that occur in the region and provides important data about rickettsia found in them. We report for the first time in Acre the species *A. dubitatum*, *A. naponense*, *A. pacae*, *A. varium* and *D. nitens* and report three *Rickettsia* species, one of them belonging to the spotted fever group, found in urban and rural areas with large movement of people and domestic animals. Due to these findings, periodic investigations and epidemiological surveillance by researchers and public health agencies are recommended to detect evaluate the appearance of other rickettsiae or the occurrence of increased infection rates of the species already registered in this study.

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