Ticks and Tick-borne Diseases 10 (2019) 540–545

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

journal homepage: www.elsevier.com/locate/ttbdis

Original article

Ticks and *Rickettsia* on anteaters from Southeast and Central-West Brazil

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ARTICLE INFO

Keywords:
Ticks
Anteater
Brazil
Rickettsia

ABSTRACT

The giant anteater (*Myrmecophaga tridactyla*) and the collared anteater (*Tamandua tetradactyla*) are widespread in Brazil and found in all Brazilian biomes. These hosts frequently use domestic animal environments such as pastures, where tick and related microorganism interchange may occur between hosts. Reports of tick infestations of these animals are scattered and refer to small samples and/or are geographically restricted. We herein present data on a wide geographic distribution of ticks and their *Rickettsia* collected from 72 giant and 30 collared anteaters, mostly road killed, over a period of 18 years, from Southeast and Central-West Brazil encompassing four States and 46 Municipalities. Overall nine tick species (*Amblyomma auricularium*, *A. calcaratum*, *A. nodosum*, *A. ovale*, *A. parvum*, *A. sculptum*, *A. triste*, *Rhipicephalus microplus* and *R. sanguineus* sensu lato) were collected from anteaters. *Amblyomma sculptum*, *A. nodosum*, and *A. calcaratum* were the most prevalent corresponding to, respectively, 48.8%, 39.3% and 2.7% of all ticks (n = 1775). However, *A. nodosum* tick numbers on collared anteaters were significantly higher ($P < 0.001$) than those on giant anteaters. At the same time, an abundance of *A. sculptum* adults on giant anteaters was significantly higher ($Z = 2.875$; $P = 0.004$) than that of *A. nodosum* and only eight *A. sculptum* nymphs were found on collared anteaters. DNA samples from 20 ticks from nine different animals yielded a visible amplicon in PCR targeting *gltA*. The PCR products targeting spotted-fever *Rickettsia* gene (*ompA*) from five adults of *A. nodosum* were sequenced and were shown to be 100% identical to *Rickettsia parkeri* strain NOD (MF737635.1). The product of one nymph and one adult of *A. sculptum* yielded a sequence 99% identical to *R. parkeri* strain NOD. Further, *Rickettsia bellii* genes were found in three *A. nodosum* adults. Ecological, behavioral and anatomical traits of anteaters are discussed to explain reported tick infestations and *Rickettsia* DNA found.

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https://doi.org/10.1016/j.ttbdis.2019.01.008
Received 2 June 2018; Received in revised form 12 January 2019; Accepted 23 January 2019
Available online 24 January 2019
1877-959X/

1. Introduction

Anteaters (Pilosa: Myrmecophagidae) are neotropical mammals with a diet based on termites and/or ants and are most of the time solitary (Medri et al., 2006). Brazil has seven from the nine known anteater species and from these, the giant anteater (*Myrmecophaga tridactyla*) and the collared anteater (*Tamandua tetradactyla*) are found in all Brazilian biomes (Medri et al., 2006; Miranda et al., 2017).

Anteaters are the principal hosts for adults of *Amblyomma calcaratum* and *A. nodosum* ticks, whereas immature stages of these species rather feed on passerine birds (Nava et al., 2017; Ogrzewalska and Pinter, 2016). *Amblyomma sculptum* (from *A. cajennense* complex, see Nava et al., 2014) is a third species regularly found on anteaters (Bechara et al., 2002; Martins et al., 2004; Pereira et al., 2000) but all stages of this tick have a broader array of hosts (Nava et al., 2014; Pereira et al., 2000). Another regular observation is the infection of *A. nodosum* and *A. calcaratum* collected from birds by *Rickettsia parkeri* strain NOD with unknown pathogenicity (Nieri-Bastos et al., 2018; Ogrzewalska and Pinter, 2016). Meanwhile, *A. sculptum* from *Amblyomma cajennense* complex, is in Brazil the main vector of *Rickettsia rickettsii*. This is the most lethal *Rickettsia* for humans with a high fatality rate (Angerami et al., 2012) however, no relationship of the human disease and anteaters has been detected so far.

In addition to their widespread distribution in Brazil, both giant and collared anteaters are recurrently found at anthropogenic sites. In fact, they are among the most frequently road-killed animals in the country (Cunha et al., 2010; Freitas et al., 2014). At the same time studies of tick infestations of anteaters refer most often to isolated reports, small sample sizes and/or were geographically restricted (Almeida et al., 2013; Bechara et al., 2002; Cutolo et al., 2000; Labruna et al., 2002; Martins et al., 2004; Oliveira et al., 2017; Pereira et al., 2000) and association of their ticks with *Rickettsia* is rare in literature (Almeida et al., 2013; Moerbeck et al., 2018). We herein present data on the geographic distribution of their ticks infected with *Rickettsia* collected from 72 giant and 30 collared anteaters, mostly road killed, over a period of 18 years, from Southeast and Central-West Brazil encompassing four States and 46 Municipalities. Differences in the infestation patterns between the two species are highlighted.

2. Material and methods

2.1. Tick sampling

Ticks were collected from 1999 until 2016 from dead giant anteaters (*M. tridactyla*) and collared anteaters (*T. tetradactyla*) hit by cars on roads. Lesser samples were also obtained from animals examined at the Veterinary Hospital of the Federal University of Uberlândia, Uberlândia, Minas Gerais State. The animal surface of both sides was examined and collected ticks stored in absolute ethanol in individual vials for each host. In a few instances, high infestation levels or decaying of animals precluded collection of all ticks.

2.2. Tick identification

Ticks were identified according Barros-Battesti et al. (2006) and Martins et al. (2010). Ticks from the *Amblyomma cajennense* species complex were identified according to Nava et al. (2014) and Martins et al. (2016). Damage to a few ticks prevented species identification and were retained as *Amblyomma* sp. Voucher tick specimens from the present study are deposited at the CC-FAMEV/UFU Tick Collection, Federal University of Uberlândia.

2.3. Rickettsia in ticks

For the detection of *Rickettsia*, DNA was extracted from a tick sample using the guanidine isothiocyanate phenol technique (Sangioni et al., 2005). Ticks were pooled (two or three ticks) for analysis whenever a host exhibited high infestation levels and each pool consisted of ticks from the same species, stage and host. DNA was then tested for *Rickettsia* by three consecutive PCR protocols. Initially, all samples were tested with primers targeting a 401-bp fragment of the citrate synthase gene (*gltA*) that occurs in all *Rickettsia* species (Labruna et al., 2004). A sample yielding a visible amplicon of the expected size was then tested by a second PCR protocol with primers targeting a 632-bp fragment of the 190-kDa outer membrane protein gene (*omp*A) (Roux et al., 1996) that is present in the spotted fever group *Rickettsia* species. Those samples that yielded no product on the second PCR for *Rickettsia* were further submitted to a third protocol using primers specific for a 338-bp fragment of the *R. bellii glt*A gene (Szabó et al., 2013). Each PCR run included a negative control (ultra-pure sterile water) and a positive control (*Rickettsia rickettsii* DNA) (Pinter and Labruna, 2006). All samples lacking a visible product in any PCR targeting *Rickettsia* genes were tested for tick mitochondrial 16S rDNA gene to control for DNA extraction failure. *Rickettsia* PCR products were sequenced and submitted to BLAST analysis to determine their similarities to the relevant sequences from identified *Rickettsia* species (Altschul et al., 1990).

Data analysis: Quantitative descriptors of anteater tick infestations (mean infestation intensity and infestation prevalence of tick populations) were used according to Bush et al. (1997). Abundance of *A. nodosum* ticks were compared to that of *A. sculptum* on giant anteaters using *Wilcoxon* test ($\alpha = 0.05$) and abundance of *A. nodosum* on collared anteaters compared to that of giant anteaters by *Mann- Whitney* (U) test ($\alpha = 0.05$). Statistical data evaluation was performed with the BioEstat 5.3 Software (BioEstat Software, Belém, Brazil) according to Ayres et al. (2007).

3. Results

The dominating landscape of sampling was of Cerrado Biome patches intermingled with agricultural lands (mostly soy, sugar cane and bovine pastures). Overall 72 giant and 30 collared anteaters from Central-West and Southeast Brazil were examined for ticks. Examinations of giant and collared anteaters occurred respectively, in 32 and 20 municipalities from four States (Minas Gerais, São Paulo, Goiás and Mato Grosso do Sul) and the Federal District (Brasília) (Fig. 1). Overall 1775 ticks from nine species, namely *Amblyomma auricularium*, *A. calcaratum*, *A. nodosum*, *A. ovale*, *A. parvum*, *A. sculptum*, *A. triste, Rhipicephalus microplus* and *R. sanguineus* sensu lato (s.l), were collected from both species of anteaters (Table 1). Eight damaged specimens were unsuitable for identification and were retained as *Amblyomma* sp. Several *A. sculptum*, *A. calcaratum*, *A. nodosum* and *R. microplus* adults were partially or fully engorged. Seven *Amblyomma* larvae from one giant anteater were engorged and molted to *A. sculptum* nymphs in the laboratory. Adults were the most numerous tick stage collected of all species, except for *R. microplus* whose nymphs were more numerous than adults in the samples (Table 1).

Overall, 1374 ticks from eight species were collected from giant anteaters and tick infestation prevalence was 91.7% (Table 2). Infestation range on giant anteaters was wide and high infestation intensity was common. In a few instances over 100 ticks were found on a single host (Table 2). The most prevalent tick species were *A. sculptum* (72.2% infestation prevalence) and *A. nodosum* (66.7% infestation prevalence). Abundance of *A. sculptum* adults on giant anteaters was significantly higher than that of *A. nodosum* ($Z = 2.875$; $P = 0.004$). In fact, mean infestation intensity of *A. sculptum* was 2.5 times higher than that of *A. nodosum*.

Amblyomma calcaratum, the third most prevalent tick species on giant anteaters (9.7% of prevalence), was collected in four municipalities close to each other and two distant and isolated ones (Fig. 1). *Rhipicephalus microplus* was collected from five animals and from these, one host from Patrocínio, Minas Gerais state, displayed a high

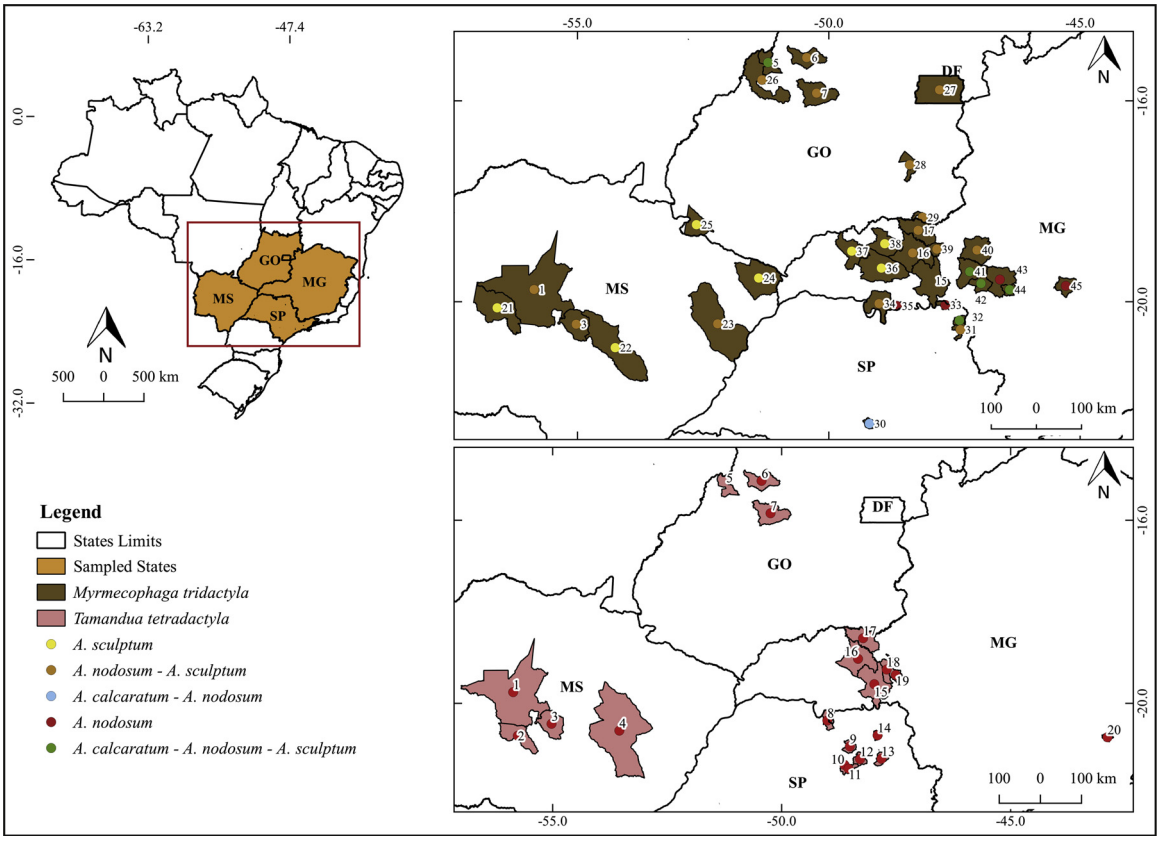

Fig. 1. Three main tick species found on anteaters from several municipalities of four Brazilian States and the Federal District (MS- Mato Grosso do Sul; GO- Goiás; MG- Minas Gerais; SP- São Paulo, and DF- Distrito Federal). Municipalities of giant anteater (*Myrmecophaga tridactyla*) sampling in brown (upper map), and that of collared anteater (*Tamandua tetradactyla*) in rose (lower map). Municipalities are indicated by numbers. 1- Aquidauana; 2- Anastácio; 3- Terenos; 4- Ribas do Rio Pardo; 5- Britânia; 6- Araguapaz; 7- Goiás; 8- Guaraci; 9- Bebedouro; 10- Cândido Rodrigues; 11- Taquaritinga; 12- Jaboticabal; 13- Ribeirão Preto; 14- Orlândia; 15- Uberaba; 16- Uberlândia; 17- Araguari; 18- Nova Ponte; 19- Santa Juliana; 20- Viçosa; 21- Miranda; 22- Campo Grande; 23- Três Lagoas; 24- Paranaíba; 25- Chapadão do Céu; 26- Jussara; 27- Brasília; 28- Pires do Rio; 29- Cumari; 30- Piratininga; 31- Franca; 32- Cristais Paulistas; 33- Igarapava; 34- Frutal; 35- Planura; 36- Prata; 37- Ituiutaba; 38- Monte Alegre de Minas; 39- Indianópolis; 40- Patrocínio; 41- Perdizes; 42- Araxá; 43- Ibiá; 44- Pratinha; 45- Bom Despacho. Data source: Instituto Brasileiro de Geografia e Estatística (IBGE), 2010.

infestation intensity (17 males, 8 females, 94 nymphs and 3 larvae). Solely one adult each of *A. auricularium*, *A. ovale*, *A. parvum* and *A. triste* was found on giant anteaters, respectively in Araguaína, Goiás State, Perdizes, Minas Gerais State, Britânia, Goiás State, and Miranda, Mato Grosso do Sul State. Thirty-seven giant anteaters were infested with more than one ticks species and mixed *A. sculptum* and *A. nodosum* infestations were the most common (n = 26) followed by animals with simultaneous *A. sculptum*, *A. nodosum* and *A. calcaratum* infestations (n = 5).

Collared anteaters were infested, overwhelmingly with *A. nodosum* adult ticks (infestation prevalence of 96.7% and mean infestation intensity of 13.3 ticks/host - Table 3) and significantly outnumbered the same tick species on giant anteaters (U = 414.000; P < 0.001) (Table 2). Two *R. sanguineus* s.l. adults (one male and one female) along with four males and two females of *A. nodosum* were collected from one collared anteater. This specific animal was rescued from a food factory and kept in the Veterinary Hospital of the Federal University of Uberlândia before sampling. In addition to this animal, two collared

Table 1
Ticks collected from giant anteaters (*Myrmecophaga tridactyla*) and collared anteaters (*Tamandua tetradactyla*) according to species and stages in Central-West and Southeast Brazil, 1999 to 2016.

Tick species	Giant anteater (n = 72)				Collared anteater (n = 30)		
	adults		nymphs	larvae	adults		nymphs
	M	F			M	F	
A. auricularium	1	–	–	–	–	–	–
A. calcaratum	39	9	–	–	–	–	–
A. nodosum	236	75	–	–	315	72	–
A. ovale	–	1	–	–	–	–	–
A. parvum	1	–	–	–	–	–	–
A. sculptum	574	197	80	7	–	–	8
A. triste	1	–	–	–	–	–	–
R. microplus	21	28	97	3	–	–	–
R. sanguineus s.l.	–	–	–	–	1	1	–
Amblyomma spp.	–	–	4	–	–	–	4

Table 2
Tick infestation parameters of giant anteaters (*Myrmecophaga tridactyla*) (n = 72), Central-West and Southeast Brazil.2002–2016.

Tick species	No of infested hosts	Prevalence (%)	Mean intensity (%) and range	No of municipalities
A. auricularium	1	1.4	1 (1)	1
A. calcaratum	7	9.7	6.9 (1-20)	6
A. nodosum	48	66.7	6.5 (1-39)	27
A. ovale	1	1.4	1 (1)	1
A. parvum	1	1.4	1 (1)	1
A. sculptum	52	72.2	16.5 (1-168)	28
A. triste	1	1.4	1 (1)	1
R. microplus	5	6.9	29.8 (1-122)	5
Total	66	91.7	20.8 (1-175)	33

Table 3
Tick infestation parameters of collared anteaters (*Tamandua tetradactyla*) (n = 30), Central-West and Southeast Brazil.1999–2014.

Tick species	No of infested hosts	Prevalence (%)	Mean intensity (%) and range	No of municipalities
A. nodosum	29	96.7	13.3 (1-52)	19
A. sculptum	3	10	2.7 (2-3)	3
R. sanguineus s.l.	1	3.3	2 (2)	1
Total	30	100	13.4 (1-52)	20

anteaters exhibited mixed tick infestations, both with *A. sculptum* and *A.nodosum*.

A total of 162 ticks were screened for the presence of *Rickettsia*. Samples of 22 giant anteaters from 12 municipalities comprised 43 *A. nodosum* adults, 81 *A. sculptum* (63 adults and 18 nymphs), three *A. calcaratum* adults, one *A. auricularium* adult and nine *R. microplus* (four adults and five nymphs). Samples from twelve collared anteaters from 10 municipalities comprised 22 *A. nodosum* adults and three *A. sculptum* nymphs. Good quality *Rickettsia* DNA was obtained from ten samples (11 ticks) of eight anteaters (Table 4); a pool of two *A. nodosum* adult ticks generated a *gltA* partial sequence 100% identical to *Rickettsia bellii* isolate AdMG (GenBank accession no. KX020409.1) and isolate LIC4327 (GenBank accession no. KT153043.1). The *ompA* products from five samples of *A. nodosum* (five adults) were sequenced and were shown to be identical to each other and 100% identical to *Rickettsia parkeri* strain NOD (GenBank accession no. MF737635.1). The product of one *A. sculptum* nymph yielded a sequence 99% (339/341) identical to *Rickettsia parkeri* strain NOD (GenBank accession no. MF737635.1). The product of another *A. sculptum* tick (an adult) also yielded a sequence 99% identical to *Rickettsia parkeri* strain NOD. Voucher GenBank nucleotide sequence accession numbers generated in this study are MH392532, MH392534 and MH351082 for the *ompA* partial sequences of *Rickettsia parkeri,* and MH392533 for the *gltA* partial sequence of *R. bellii*.

4. Discussion

Tick collection on road-killed anteaters, the majority of animals examined in this study, is associated with limitations due to exposure of sun (death/dehydration of ticks), decaying of hosts and utmost care taken during sample collection to avoid accidents by road sides. Thus, despite tick number underestimation by collection-associated limits, particularly of immature stages, giant anteaters exhibited both a high prevalence and high mean tick infestation rate.

Among tick species, *A. sculptum* was the most prevalent and associated with the highest mean infestations of giant-anteaters. Adults and immature stages of *A. sculptum* parasitizes large wild and domestic mammals of different orders (particularly horses, tapirs, pigs and capybaras), and larvae and nymphs are also parasites of small and medium-sized mammals (Queirogas et al., 2012; Martins et al., 2016; Nava et al., 2017). Several factors come together to favor giant anteater-tick relationship as observed in our study. Collections on anteaters occurred principally in the savannah Biome (cerrado) and anthropized sites where this tick species thrives (Martins et al., 2016; Queirogas et al., 2012; Szabó et al., 2007). Giant anteaters have a relatively large home range that may overcome several square kilometers (Bertassoni et al., 2017). This animal species also has a large body surface (weight over 45 kg and body length of 1.2 m) and a long tail sometimes as long as 90 cm (Medri et al., 2006) with long hair that brushes vegetation during its movement. *A. sculptum* is known to display ambush behavior and to seek for hosts on vegetation at heights that vary mainly from 15 to 50 cm (Ramos et al., 2017). These features altogether, enhance contact of giant anteaters and host seeking ticks on vegetation. It is important to note that many *A. sculptum* females found in this study were engorging or almost fully engorged indicating an adequate host for this tick species. Nevertheless, by being solitary and living over an extant home range, its densities in nature are low, estimated in one study at 0.15 individual/km^2 (Desbiez and Medri, 2010). Therefore, contribution of giant anteaters for the maintenance of *A. sculptum* populations in the environment is questionable and should be further evaluated.

Amblyomma nodosum infestation prevalence and geographic distribution on giant anteaters was inferior but close to that of *A. sculptum*. Anteaters and passerine birds are the main hosts for adults and immature stages of *A. nodosum*, respectively (Nava et al., 2017). In fact, many females of *A. nodosum* were found engorging or almost fully engorged reinforcing that anteaters are principal hosts for adults of this tick. Since *A. nodosum* is seldom found during environmental samplings in the Brazilian savannah (Ramos et al., 2014; Szabó et al., 2007, 2013; Veronez et al., 2010), the host-seeking site of adult ticks for giant

Table 4
Rickettsia species in anteater ticks according to date and municipality of collection, Brazil.2009–2016.

Tick collection		Anteater species	Tick		PCR target		*Rickettsia* species
Date	Municipality (State)		species	stage	*omp*A	*R. bellii*	
21/12/09	Perdizes (MG)	giant	*A. nodosum*	Adult	+	–	*R. parkeri* NOD
22/03/10	Uberlândia (MG)	giant	*A. nodosum*	Adult	–	+	NS (*R. bellii*)
26/04/10	Uberlândia (MG)	giant	*A. nodosum*	Adult	–	+	NS (*R. bellii*)
28/05/10	Uberlândia (MG)	giant	*A. nodosum*	Adult	+	–	*R. parkeri* NOD*
			A. nodosum	Adult	+	–	*R. parkeri* NOD*
23/02/11	Patrocínio (MG)	giant	*A. sculptum*	Adult	+	–	*R. parkeri* NOD
			A. nodosum	2 Adults		+	*R. bellii* *
01/07/16	Patrocínio (MG)	giant	*A. sculptum*	Nymph	+	–	*R. parkeri*.NOD*
04/10/09	Bebedouro (SP)	collared	*A. nodosum*	Adult	+	–	*R. parkeri* NOD
03/08/11	Anastácio (MS)	collared	*A. nodosum*	Adult	+	–	*R. parkeri* NOD

NS non-sequenced; *GenBank deposited. MG (Minas Gerais); SP (São Paulo); MS (Mato Grosso do Sul).

anteaters in the environment is unknown.

Amblyomma calcaratum, the third tick species on giant anteaters, was much less prevalent and presented an erratic geographic distribution that precludes speculations about patterns. *A. calcaratum* is phylogenetically close (Nava et al., 2017) and specially nymphs, are morphologically similar to *A. nodosum* (Martins et al., 2010). Therefore, these tick species may have been confused several times. *Amblyomma calcaratum* adults, just as, *A. nodosum* do, feed chiefly on anteaters as adults and on passerine birds as immatures over a wide geographic area that includes Cerrado (savannah), Atlantic rainforest and Amazon Biomes in Brazil and several other countries in South and Central America (Cutolo et al., 2000; Luz et al., 2016; Nava et al., 2017; Ogrzewalska et al., 2010, 2013). The reasons for restricted distribution of *A. calcaratum* in our sampling is unknown and ecological traits should be further investigated to highlight this issue.

Even though at low prevalence, *Rhipicephalus microplus* was responsible for the highest mean infestation intensity among all tick species. *R. microplus* is the main cattle tick in Brazil and was brought to the country with colonization (Pereira et al., 2008). It thrives on bovine pastures (Pereira et al., 2008) and accidental infestations occur on several domestic and wild animals that access such environment (Szabó et al., 2001, 2007). At the same time epigeous termite mounds are frequently observed on pastures (Oliveira et al., 2011) and giant anteaters must have been infested during search for termites on pastures.

Only one adult of each remaining tick species was found on giant anteaters and may be rather seen as an accidental infestation. *Amblyomma auricularium* immatures and adults feed on armadillos and *A. parvum* adults feed on a large array of both wild and domestic mammals. Carnivores are the preferred hosts for adults and rodents for immatures of *A. ovale*. Finally, marsh-deer is the primeval host for adults of *A. triste* and rodents for its immature stages (reviewed by Nava et al., 2017).

Collared anteaters were chiefly infested with *Amblyomma nodosum* whereas *A. sculptum* was restricted to eight nymphs on two animals. Since most anteaters from both species were found in similar environments, behavioral and anatomical traits (Araújo, 2013; Desbiez and Medri, 2010; Medri et al., 2006) may be responsible for a decreased infestation of collared anteaters with *A. sculptum*. Compared to the giant anteater, this host is much smaller (an adult weights ˜7 kg), its thinner prehensile tail has shorter hair, it has a smaller home range (estimated at 1 km^2 by Araújo, 2013) and exhibits scansorial behavior (Medri et al., 2006) that altogether diminishes contact with host seeking *A. sculptum* on vegetation. Likewise on giant anteaters, several *A. nodosum* females were engorging on collared anteaters reinforcing its suitability as host for this tick species.

Two *R. sanguineus* s.l. adults together with five *A. nodosum* were found on a single collared anteater. *R. sanguineus* s.l. is a tick species associated with dogs in Brazil and anthropized environment such as kennels (Szabó et al., 2001) and is not reported in tick samplings from natural environments in the savannah Biome (Ramos et al., 2014; Szabó et al., 2007; Veronez et al., 2010). In fact, this anteater was found inside a ration factory and kept for two days in a Veterinary Hospital before examined for ticks. Therefore, infestation with *R. sanguineus* s.l. in one of these human dwellings cannot be ruled out.

Just as with tick infestation *Rickettsia* infection of ticks may be underestimated in our study. Most of the ticks remained for several years in alcohol vials and many were collected dehydrated and/or dead after being exposed for hours or days to the sun on the road-killed animals. Under such conditions, DNA deterioration may have occurred, hampering *Rickettsia* detection in some of the ticks. Nonetheless, two *Rickettsia* species were found in ticks from anteaters, *Rickettsia bellii* and *Rickettsia parkeri* strain NOD. *Rickettsia bellii* has been described from several tick species, including *A. nodosum*, all over the Americas (Krawczak et al., 2018; Ogrzewalska and Pinter, 2016). It is considered a non-pathogenic *Rickettsia* species, although its interference in the ecology and epidemiology of other *Rickettsia* species cannot be disregarded (Krawczak et al., 2018).

Rickettsia parkeri strain NOD DNA was found in adult *A. nodosum* ticks over a wide geographic distribution and on several different hosts. These results reinforce previous ones that show a strong association between this specific *Rickettsia* and this tick species (Nieri-Bastos et al., 2018; Ogrzewalska et al., 2009; Ogrzewalska and Pinter, 2016). Nonetheless, these studies described *R. parkeri* strain NOD mainly from nymphs of *A. nodosum* collected from passerine birds, meanwhile reports from adult ticks are rare (Almeida et al., 2013; Moerbeck et al., 2018). In this work, *R. parkeri* strain NOD DNA was also found in *A. sculptum* ticks, to our knowledge for the first time. Nonetheless, *A. sculptum* and *R. parkeri* strain NOD relationship is unknown. Since simultaneous parasitism of *A. sculptum* and *A. nodosum* on giant anteaters is common, *A. sculptum* may have ingested the *Rickettsia* during cofeeding on the same host as already described for other tick and *Rickettsia* species (Moraes-Filho et al., 2018). In our study DNA of *R. parkeri* strain NOD was not found in an *A. nodosum* cofeeding with positive *A. sculptum,* thus infection source as well as bacteria viability in this tick species remain unknown.

Whatever the infection source of *R. parkeri* strain NOD for tick is, the sanitary importance of this bacteria is unknown. It was recently considered a strain of *Rickettsia parkeri* (Nieri-Bastos et al., 2018) belonging to the spotted-fever group which comprises several human pathogens. In addition, it was described from *A. nodosum* tick in an endemic area of spotted fever in Brazil (Moerbeck et al., 2018). In this regard, simultaneous infestation of hosts with *A. nodosum* and *A. sculptum* might pose a risk of infection of the latter and, considering the aggressiveness of *A. sculptum* towards humans, a bridge of such *Rickettsia* species to humans. Therefore, despite the uncertainty of source and likelihood of *A. sculptum* as vector, human infections with *R. parkeri* strain NOD should be monitored.

In conclusion, our observations reinforced previous observations on anteater tick infestations. Our findings also displayed important infestation differences between both host species as well as a preliminary geographic pattern that will be fulfilled with additional and concatenated data. Furthermore, we clearly show that *R. parkeri* strain NOD is also frequently associated with adult *A. nodosum* ticks and that it might infect *A. sculptum* ticks as well. Last but not the least, it is important to consider that many sampling sites were under strong anthropogenic influence and anteater infestations described herein reflect both natural and human altered conditions.

Acknowledgements

Authors acknowledge Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq (Academic Career Research Fellowship) to M.P.J. Szabó and Labruna, M.B. and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) for financial support.

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