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Ticks (Acari: Ixodidae) in the Serra da Canastra National Park in Minas Gerais, Brazil: species, abundance, ecological and seasonal aspects with notes on rickettsial infection

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Abstract
The Cerrado Biome is the second largest in Brazil covering roughly 2 million km^2, with varying features throughout its area. The Biome is endangered but it is also source of animal species for rural, green urban and degraded rainforest areas. Ticks are among Cerrado species that establish at anthropogenic sites and although information about them is steadily increasing, several features are unknown. We herein report tick species, abundance and some ecological relationships within natural areas of the Cerrado at higher altitudes (800–1500 m) within and around Serra da Canastra National Park, in Minas Gerais State Brazil. In total of 1196 ticks were collected in the environment along 10 campaigns held in 3 years (2007–2009). *Amblyomma sculptum* was the most numerous species followed by *Amblyomma dubitatum* and *Amblyomma brasiliense*. Distribution of these species was very uneven and an established population of *A. brasiliense* in the Cerrado is reported for the first time. Other tick species (*Amblyomma ovale*, *Amblyomma nodosum*, *Amblyomma parvum*, *Ixodes schulzei* and *Haemaphysalis leporispalustris*) were found in lesser numbers. Domestic animals displayed tick infestations of both rural and urban origin as well as from natural areas (*Rhipicephalus sanguineus* sensu lato, *Rhipicephalus microplus*, *Dermacentor nitens*, *A. sculptum*, *A. ovale*, *Amblyomma tigrinum*, *Argas miniatus*). *Amblyomma sculptum* had the widest domestic host spectrum among all tick species. DNA of only one *Rickettsia* species, *R. bellii,* was found in an *A. dubitatum* tick. Several biological and ecological features of ticks of the studied areas are discussed.

Keywords Ticks · Environment · Savannah · Brazil · Ecology

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Introduction

The Cerrado Biome is roughly located in southeast and central Brazil and covers about 2 million km^2, representing ca. 22% of the territory of Brazil with small areas extending into Bolivia and Paraguay (Oliveira-Filho and Ratter 2002). The Biome exhibits a mosaic of physiognomies, from grasslands to forests. Between these two extremes lies a continuum of savanna formations spanning the entire range of woody plant density, referred to collectively as the *cerrados* (Oliveira-Filho and Marquis 2002). At several sites the Cerrado is intermingled with neighboring Biomes such as Atlantic rainforest, Amazon, and Caatinga (dry lands with unevenly distributed rainfalls). The Cerrado is very adequate for agro-industrial activities and the whole Biome is endangered by human activities. At the same time, reinstatement of rainforests and riparian forests and establishment, within urban settings, of green leisure and sport areas create an environment similar in several aspects to the Cerrado. These new areas are prone to colonization by several species that live in the Cerrado. An extreme example of a Cerrado animal flourishing within urban and peri-urban settings is the tick *Amblyomma sculptum*, a member of the *Amblyomma cajennense* species complex (Nava et al. 2014). This tick species is commonly found in relatively small green areas at anthropogenic sites (Queirogas et al. 2012) and in a few locations is the vector of *Rickettsia rickettsii*, the highly lethal Brazilian spotted-fever agent (Labruna 2009; Szabó et al. 2013a).

Recognition of species and their abundance, natural ecological relationships and patterns in original settings may be of great value to understand unbalanced situations within anthropized areas. Despite the several studies of tick populations within the Cerrado Biome during the last two decades (Bechara et al. 2002; Campos Pereira et al. 2000; Knight 1992; Martins et al. 2011, 2015; Szabó et al. 2007a; Veronez et al. 2010), information is still scarce, and many features of the Biome were not addressed. The huge extension of the Cerrado and the variable influence of the neighboring Biomes certainly allows for a certain amount of variations within the Biome. Considering the importance of ticks as hazards for both public and animal health and lack of knowledge on several aspects of species distribution and ecological relationships we herein report tick species and features of this parasite's abundance, ecological relationships and seasonality within natural areas of the Serra da Canastra National Park as well as neighboring private owned natural areas. This park has as characteristic features sloping plateaus, ranging from 800 to 1500 m of elevation, rocky open grasslands, occasional forest patches and a rich biodiversity. Previous reports from the Park are restricted to ticks collected from Xenarthra and Carnivora (Arrais 2013; Botelho et al. 1989; Martins et al. 2015) none of which addressed ticks free living in the environment, ecological or seasonal aspects.

Materials and methods

Study site

This study was conducted from April 2007 to November 2009 in the Serra da Canastra National Park and in natural areas from neighboring properties. Serra da Canastra National Park is within the Cerrado domain, in the southwestern part of the Minas Gerais State in Brazil (20°20′S–46°40′W). It has an area of 198,000 ha, and is characterized by sloping plateaus, from 800 to 1500 m high. According to Köppen's system the climate of the region is Cwb, with a dry and cool season from April to September, with (mean temperature of 17 °C), and a wet and hot season from October to March (mean temperature of 22 °C). Annual precipitation ranges from 1300 to 1700 mm. Tick sampling occurred at eight locations of diverse phytophysiognomies, four within the park and at higher altitude (above 1300 m) and four in natural areas in neighboring private properties (Table 1) with altitudes ranging from 823 to 912 m.

Tick sampling in the environment

Ten tick collection campaigns were led in locations 1–7 from April 2007 to November 2009 (Table 1). Location eight refers to a farm where only animal sampling was conducted. To evaluate tick seasonal activity, campaigns occurred at each of eight consecutive seasons from March 2008 onwards. Cloth dragging, CO_2 traps and visual search on vegetation were used for tick sampling as described before (Terassini et al. 2010; Veronez et al. 2010). Briefly, ticks were collected in each location and sampling campaign using 20 CO_2 traps and dragging for 60 min with white 2-m-long and 1-m-wide flannels. Visual search was performed at distances of approximately 400 m just before dragging and only in forestall phytophysiognomies exhibiting evident trails (sites 5 and 6). Adult and nymphal ticks were counted individually whereas each larval cluster was considered a unit.

Domestic animal sampling

Domestic animals from four private properties (farms/ranches) bordering the park (sites 5–8) and occasionally stray dogs were examined for ticks by convenience at each campaign. A few animals, mainly dogs, were examined repeatedly along the several campaigns but never twice in the same campaign. Each animal was examined by two researchers for 3–5 min. Whenever infestations were too high (usually bovines and horses), only a sample of ticks was collected.

Tick identification

Collected ticks were stored in alcohol but engorged larvae and nymphs were kept alive in laboratory at 27 °C and 85% UR until molting to the next stage and identified based on reference bibliography (Martins et al. 2010; Nava et al. 2014; Onofrio et al. 2006) and comparison with reference laboratory specimens. Lack of keys for Neotropical *Amblyomma* larvae as well as damaged samples precluded identification of some tick samples which were retained as *Amblyomma* sp. Seven larval batches were identified to species level by molecular methods. For this purpose, a sample from the larval batch was processed by DNA extraction, PCR, and partial DNA sequencing of the tick mitochondrial 16S rDNA gene, as previously described (Ogrzewalska et al. 2009a). Voucher tick specimens collected in the present study have been deposited at the CC-FAMEV/UFU Tick Collection, Federal University of Uberlândia (accession numbers: 259–263; 411–412; 440–443; 449–450; 471–476).

Table 1 Locations, coordinates, altitude and phytophysiognomies for tick sampling in the Serra da Canastra Nacional Park (SCNP), Minas Gerais State, Brazil 2007–2009

Location	Ownership	Coordinates	Altitude (m)	Phytophysiognomies
1[a]	SCNP	20°15′28.9″S and 46°25′18.6″W	1326	Mosaic of campo rupestre[1], campo limpo[2] and small riparian forest
2	SCNP	20°15′34.3″S and 46°25′11.5″W	1316	Cerradão[3]
3	SCNP	20°15′35.8″S and 46°25′72.2″W	1406	Campo limpo
4	SCNP	20°14′60.9″S and 46°26′79.6″W	1321	Source of the São Francisco river: gallery forest patch
5	Private ranch	20°18′50.5″S and 46°32′03.8″W	893	Gallery forest and montane forest
6	Private ranch	20°21′35.8″S and 46°29′04.4″W	912	Gallery forest and montane forest
7	Private ranch	20°19′05.9″S and 46°31′14.8″W	823	Riparian forest
8	Private ranch	20°15′09.1″S and 46°23′32.4″W	827	Farm

[a] 1—low shrub savannah on bare rockeries; 2—prairies; 3—almost closed woodland with crown cover of 50–90%, made up of trees, often of 8–12 m or even taller, casting a considerable shade so that the ground vegetation layer is much reduced

Rickettsia in ticks

DNA was extracted from a sample of 48 ticks (1 female *Amblyomma dubitatum*; 1 male *Amblyomma brasiliense*; 23 female and 15 male *A. sculptum*; 5 female *Rhipicephalus microplus*; 2 female and 2 male *Rhipicephalus sanguineus* mostly from dogs, equines but also from bovines and environment) using the guanidine isothiocyanate phenol technique (Sangioni et al. 2005). DNA was then tested for *Rickettsia* by three consecutive PCR protocols. Firstly, all samples were tested with primers CS-78 and CS-323, 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 Rr190.70F and Rr190.701R, targeting a 632-bp fragment of the 190-kDa outer membrane protein gene (*ompA*) (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 gltA* gene (Szabó et al. 2013b). All samples lacking visible product in any PCR targeting *Rickettsia* genes were tested for tick mitochondrial 16S rDNA gene to control for DNA extraction failure. The only PCR *Rickettsia* product was sequenced and submitted to BLAST analysis, to determine its similarities to the relevant sequences from identified *Rickettsia* species (Altschul et al. 1990).

Data analysis

Quantitative descriptors of tick populations were used according to Bush et al. (1997).

Ethics and permits

All procedures were approved by the Ethics Committee on Animal Use/CEUA of the Federal University of Uberlândia (Protocol No 007/08) and Brazilian Environment Institute—IBAMA (Permit No 11398-1).

Results

Tick numbers and species

Ticks from the environment: A total of 1196 ticks belonging to three genera and eight species were collected along 10 campaigns held in 3 years (2007–2009). *Amblyomma sculptum* was the most numerous species (576 adults, 272 nymphs and 2 larval batches, 71.6% of the total) followed by *A. dubitatum* (140 adults and 33 nymphs, 14.5% of the total) and *A. brasiliense* (131 adults, 30 nymphs and 2 larval batches, 13.6% of the total). Other tick species were much less numerous; *Amblyomma ovale* (4 adults, 0.3%); *Amblyomma nodosum* (2 adults and 1 nymph, 0.3%); *Amblyomma parvum* (1 nymph, 0.1%); *Ixodes schulzei* (1 larval batch, 0.1%), and *Haemaphysalis leporispalustris* (1 larval batch, 0.1%). Additionally, 64 nymphs and 20 larval batches were retained as *Amblyomma* spp.

The *I. schulzei* larval batch was identified by morphological comparison with laboratory-reared larvae derived from an engorged female collected in the state of São Paulo, as reported elsewhere (Labruna et al. 2003). The remaining larval pools were identified to species through molecular analysis. In these cases, 2 larval pools generated 16S rDNA identical fragments that were 99.7% (371/372-bp) identical to the GenBank sequence of *A. sculptum* (KY172626); 3 larval pools generated 16S rDNA identical fragments that were 99.8% (417/418-bp) identical to the GenBank sequence of *A. brasiliense* (KM519941); and 1 larval pool generated a 16S rDNA fragment that was 100% (382/382-bp) identical to the GenBank sequence of *H. leporispalustris* (KU096986). These 16S rDNA sequences generated in the present study have been submitted to GenBank under accession numbers MH114024 (*A. sculptum*), MH114023 (*A. brasiliense*) and MH114025 (*H. leporispalustris*).

Domestic animals

Out of 96 dog examinations, 51 yielded ticks (prevalence of 53.1%, mean infestation intensity of 3.3 ticks). Overall 169 ticks of five species were found; *A. sculptum* (6 adults, 73 nymphs and 1 larva, 47.3% of the total), *R. sanguineus* sensu lato (72 adults and 1 nymph, 43.2%), *A. ovale* (8 adults, 4.7%); *R. microplus* (6 adults and 1 nymph, 4.1%) and *Amblyomma tigrinum* (1 adult, 0.6%). Additionally, 55 nymphs and 21 larvae were retained as *Amblyomma* spp. On 17 bovine examinations, all animals were infested (prevalence of 100%) and two tick species found; *R. microplus* (185 adults and 15 nymphs) and *A. sculptum* (11 adults and 3 nymphs) as well as two *Amblyomma* sp. nymphs. Every examined horse (n = 16) was infested (prevalence of 100%) and three tick species were found; *A. sculptum* (136 adults, 11 nymphs and 8 larvae), *Dermacentor nitens* (29 adults and 9 nymphs) and *R. microplus* (48 adults and 3 nymphs) as well as four nymphs and eleven larvae retained as *Amblyomma* sp. On three pigs kept unrestrained in one of the properties 26 adult *A. sculptum* were found. Solely one cat was examined and one *A. sculptum* and four *Amblyomma* sp. larvae were found. Two chicken pens were also examined and in one of them 23 adults, 25 nymphs and 14 larvae of *Argas miniatus* were found.

Humans

During the field work accidental tick-bites of researchers were recorded as follows: four female, five male and two nymphs of *A. sculptum* as well as four *Amblyomma* spp. nymphs.

Seasonality

Analysis of seasonal occurrence of ticks in the environment is restricted to *A. sculptum*, *A. brasiliense* and *A. dubitatum* (Fig. 1), species found in numbers high enough for this purpose. *A. sculptum* adults peaked in both summers but with the peak in the second summer more prominent. Two *A. sculptum* nymphal peaks were observed, both in winter, the first being much more prominent. It is noteworthy that the biggest nymphal peak was followed by biggest adult peak. All four larval batches identified as *A. sculptum* were found in autumn; three in 2008 and one in 2009. No clear-cut seasonal activity could be depicted for both *A. brasiliense* and *A. dubitatum* nymphs and adults. *A. brasiliense* adults had a small peak in 2008 autumn and a bigger one in winter 2009 meanwhile nymphs peaked in winter 2008. *A. dubitatum* adults peaked in the winter of 2009 whereas nymphs remained in constantly low numbers along all studied period. Tick sampling of domestic animals set by convenience was improper for seasonal analysis; it is noteworthy, however, that all *A.*

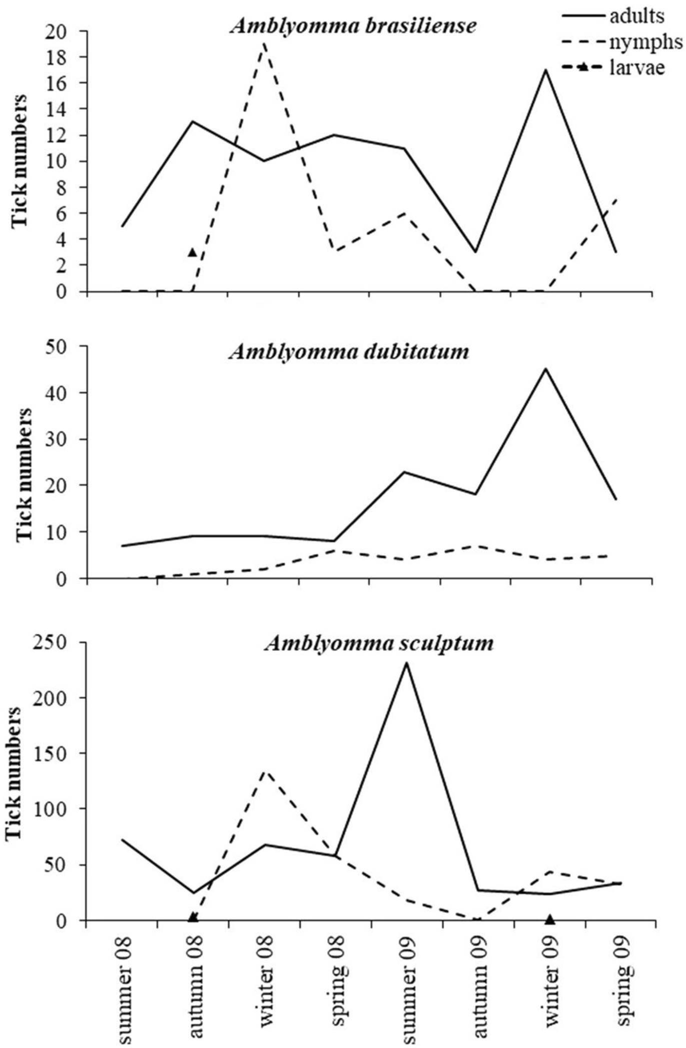

Fig. 1 Seasonal distribution of *Amblyomma brasiliense*, *A. dubitatum* and *A. sculptum* in the Serra da Canastra Nacional Park, Minas Gerais State, Brazil 2007–2009

sculptum nymphs on dogs were found in winter (eight in 2007, 23 in 2008 and 42 in 2009) whereas *R. sanguineus* adults were collected from dogs in all 10 campaigns without a peak in infestations (data not shown).

Ecological relationships

A quite uneven distribution among the several locations was observed in tick numbers and species (Table 2). Among the three most numerous species in the environment *A. sculptum* was found in all locations; however, 51.5% were from location 6 and only 0.5% and 0.1% from locations 2 and 3. Overall *A. sculptum* represented 80.4% of all ticks collected outside

Table 2 Percentage of tick species found in the environment according to seven sampled locations in the Serra da Canastra Nacional Park, Minas Gerais State, Brazil 2007–2009

Location	*Amblyomma sculptum* (n=850)	*Amblyomma dubitatum* (n=173)	*Amblyomma brasiliense* (n=163)	*Amblyomma ovale* (n=4)	*Amblyomma nodosum* (n=3)	*Amblyomma parvum* (n=1)	*Ixodes schulzei* (n=1)	*Haemaphysalis leporispalustris* (n=1)
1	11.9	3.5	1.2	0	0	0	0	0
2	0.1	0	1.2	0	0	0	100	0
3	0.5	0	0	0	0	0	0	0
4	4.5	93.1	0.6	0	0	100	0	0
5	12.1	2.9	85.9	25	0	0	0	100
6	51.5	0	0	75	66.7	0	0	0
7	19.4	0.6	11	0	33.3	0	0	0

Total number of ticks from each species is between parentheses the park at lower altitudes and 45.2% inside the park, at altitudes 404–583 m higher. Distribution of the other two more prevalent species, *A. brasiliense* and *A. dubitatum* were utterly uneven. The former was found mainly in location 5 (85.9% of all ticks from this species) and location 7 (11%) whereas the latter species was found overwhelmingly in location 4 with 93.1% of the ticks from this species. Locations 2 and 3 yielded the lowest number of ticks, with six and four ticks, respectively, found along the 3 years of research. One *A. ovale* and many of the *A. brasiliense* and *A. sculptum* adults but not a single *A. dubitatum* were found by visual search at the tip of leaves facing trails and always within forestall formations.

Rickettsia

One DNA sample from an *A. dubitatum* adult female collected as nymph in location 4 yielded visible amplicon in PCR targeting *gltA*, no product in the PCR for *ompA*, and visible product in the third protocol using primers specific of the *R. bellii gltA* gene. PCR product from the first *gltA* PCR was DNA sequenced, generating a 190-bp sequence that was 100% identical to a fragment of the *gltA* gene of *R. bellii* from GenBank (CP000087).

Discussion

Amblyomma sculptum was by far the most prevalent and widespread species in the environment as already observed at lower altitudes elsewhere in the Cerrado (Szabó et al. 2007a, b; Veronez et al. 2010). This taxon was recently reinstated as a valid species and separated from five others that all together compose the *A. cajennense* species complex (Nava et al. 2014). In fact, each species from the complex, even though very similar morphologically, seem to have distinct ecological preferences (Beati et al. 2013; Labruna 2018). *Amblyomma sculptum* but not the other species from the complex, has a strong association with the Brazilian Cerrado (Martins et al. 2016; Nava et al. 2014). Thus, unless otherwise stated, *A. cajennense* ticks collected in this Biome before the recognition of *A. cajennense* complex (Knight 1992; Szabó et al. 2007a; Veronez et al. 2010) should be regarded as *A. sculptum*.

Amblyomma sculptum was found in all sampled locations but its distribution was uneven with most individuals collected within gallery and montane forests at lower altitudes and under stronger anthropogenic pressure whereas it was almost absent from prairies and savannah on bare rockaries. This observation has no straightforward explanation because altitude and lower temperatures, phytophysiognomy, host species and abundance and anthropogenic factors may all have an influence on its distribution. In fact, up to our knowledge this is the first systematic study of this tick species at higher altitudes of the Cerrado and information should be compared to forthcoming information. Nevertheless, association of *A. sculptum* with forestall phytophysiognomies was observed before in both natural and anthropogenic sites (Ramos et al. 2014a; Souza et al. 2006; Veronez et al. 2010) and shows the importance of shadowed area within the Cerrado for this tick species. By being attracted to CO_2 traps and questing on leaves along trails within forests *A. sculptum* exhibited both hunting and ambush host locating strategies as already noted (Ramos et al. 2017). This dual behavior certainly contributes to greater host finding success.

Two other tick species, *A. dubitatum* and *A. brasiliense* were found routinely along the study period. *Amblyomma dubitatum* was collected only in locations beside water fonts and among these, overwhelmingly at the source of the São Francisco River. Truly, this tick species was already associated with environment prone to flooding (Queirogas et al. 2012; Szabó et al. 2007b). Not coincidently, *A. dubitatum* has capybaras (*Hydrochoerys hydrochaeris*), a rodent with semi-aquatic habits as main hosts for all stages, although small mammals may also feed immatures (Coelho et al. 2016; Nava et al. 2010). Capybara scats and visual observation of one animal at the gallery forest patch by the São Francisco River source indicates that this host was maintaining the local tick population. At this location, several *A. sculptum* ticks were found as well, albeit in lesser numbers. Actually, association of these tick species and capybara populations close to water sources is common at anthropogenic sites (Brites-Neto et al. 2013; Queirogas et al. 2012; Souza et al. 2006). Nonetheless, proportion of each species may vary with *A. dubitatum* prevailing in flood-prone areas besides the water sources and *A. sculptum* a little further away, at drier areas (Queirogas et al. 2012; Szabó et al. 2007b). Under experimental settings both tick species may transmit *R. rickettsii* (Sakai et al. 2014; Soares et al. 2012) but *A. dubitatum* seems to be less aggressive to humans (Pajuaba Neto et al. 2018) and thus Brazilian spotted-fever is usually linked to *A. sculptum* bites.

Amblyomma brasiliense is a Neotropical tick associated with tropical and subtropical moist broadleaf forests (Guglielmone et al. 2014) found in Argentina, Brazil and Paraguay (Nava et al. 2007). It is rather associated with Atlantic rainforests in Brazil (Pinheiro et al. 2014; Sabatini et al. 2010; Silveira and Fonseca 2013; Szabó et al. 2009, 2013b). Records outside this Biome such as Amazon (Aragão 1936) and South Brazil (Corrêa 1954) are rare, usually restricted to one individual and thus must be viewed with caution. At the same time, several systematic tick studies in the Cerrado and Pantanal Biomes (Campos Pereira et al. 2000; Knight 1992; Martins et al. 2011, 2015; Ramos et al. 2014a, b; Saraiva et al. 2012; Sponchiado et al. 2015; Szabó et al. 2007a, b; Veronez et al. 2010) failed to find *A. brasiliense*. Therefore, our work is the first to record an established population in this Biome. It is noteworthy, however that *A. brasiliense* in our environmental sampling was found overwhelmingly in the more humid phytophysignomies within the Cerrado and those that closest resemble humid rainforests. This tick species has several hosts among Perissodactyla and Artiodactyla and is frequently associated to Tayassuidae (Aragão 1936). In fact, peccary group was reported in the location of higher incidence of the tick. Importantly, *A. brasiliense* is among the most aggressive tick species towards humans (Aragão 1936; Guglielmone et al. 2006a; Szabó et al. 2006) although pathogen transmission has not been recorded. It was a tick species that was commonly found at the tip of leaves along trails within forests and thus exhibited ambush strategy to access hosts.

Only four *A. ovale* adults were found in environment, all at lower altitudes and within gallery and montane forests. This tick is also a common species within lower altitude Atlantic rainforests among southeast Brazil. In such areas, it may be the most prevalent adult tick species questing for hosts on leaves and on dogs with access to the forest (Sabatini et al. 2010; Szabó et al. 2009, 2013b). Observations of Barbieri et al. (2015) showed that in Atlantic Rainforests of southeast Brazil *A. ovale* tends to be more numerous at altitudes below 700 m. Thus, forest fragments with altitudes around 900 m of the Cerrado in southeast Brazil seems to provide a borderline environment for this tick species and explains, at least partially, decreased tick numbers. Nonetheless public health importance of this tick in the Cerrado should be deeply investigated. In the Atlantic rainforest, *A. ovale* was shown to harbor and transmit *Rickettsia parkeri* strain Atlantic rainforest, a recognized human pathogen (Barbieri et al. 2014; Nieri-Bastos et al. 2016, 2018; Sabatini et al. 2010; Spolidorio et al. 2010; Szabó et al. 2013b).

Although only three *A. nodosum* adults were found in the environment, it is a common parasite species in the Cerrado. Adults parasitizes anteaters (Bechara et al. 2002; Campos

Pereira et al. 2000; Martins et al. 2004, 2011) and nymphs are frequently the most prevalent species on passerine birds within this Biome (Luz et al. 2012; Pascoal et al. 2013; Ramos et al. 2015; Torga et al. 2013). Very likely this species exhibits a nidicolous or other host questing behavior for which our sampling methodology was inadequate. Immature stages of this species collected on wild birds have been found infected with *R. parkeri* strain NOD, yet of unknown pathogenicity for people (Nieri-Bastos et al. 2018; Ogrzewalska et al. 2009b).

Remaining tick species were represented by only one individual or single larval cluster, hampering ecological associations. In this regard finding a single specimen of *A. parvum* was surprising. Apart from its potential wide host array (Olegário et al. 2011), a broad analysis of its geographic distribution showed that this tick has a strong association with dry areas belonging to Chaco in Argentina and Paraguay, and Cerrado and Caatinga in Brazil (Nava et al. 2016). Since our sampling methodology was proven adequate for the species (Ramos et al. 2014a), higher numbers were expected. Thus, factors such as higher altitude and other undetermined ones may have interfered with the establishment of more numerous *A. parvum* populations in the study site.

Finding ticks from *Ixodes* genus solely once along several years of study was predictable. The richness and abundance of species of the *Ixodes* genus are higher in the Atlantic Forest and Amazon while in Cerrado, Chaco and Caatinga Biomes species of this genus are rarely recorded (Sponchiado et al. 2015). On the other hand, finding *I. schulzei* in the woodland location indicates the tropism of this tick to habitats with more dense vegetation cover, where it is likely to be associated with its main host species, the water rat *Nectomys squamipes* (Labruna et al. 2003; Saraiva et al. 2012). Collection of a larval cluster of *H. leporispalustris* in the environment also seems an incidental finding and it is usually collected on its habitual host, the wild rabbit *Sylvilagus brasiliensis* in Brazil (Labruna et al. 2000; Saraiva et al. 2012) but not during environmental samplings. It is a species of public health interest; it was shown to harbor under natural conditions and transmit under experimental settings, the bacterium *R. rickettsii* (Freitas et al. 2009; Fuentes et al. 1985; Parker et al. 1951).

Description of seasonal activity, attempted only for the three most abundant tick species, did not display a clear pattern for either *A. brasiliense* or *A. dubitatum*. This lack of pattern can be attributed to the overall low numbers and patchy distribution of ticks that is prone to stochastic variations. *Amblyomma sculptum* tick numbers varied intensely from 1 year to the other, nonetheless, grossly within a seasonal frame already described for the species (Labruna et al. 2002; Veronez et al. 2010) with adults prevailing around summer, nymphs in winter and larvae in autumn.

Domestic animal tick infestation did not present surprising features. Many animals harbored ticks from both wild and anthropized environment reflecting a dual exposure pattern. In this context, *R. microplus*, *R. sanguineus* lato sensu, *D. nitens* and *A. miniatus* are species strongly associated with, respectively, cattle, dogs, horses and chicken and their environment. Infestation of other animals with these species occurs when using environment infested by the aforementioned hosts (review by Guglielmone et al. 2006b).

Amblyomma sculptum was the species with broader distribution being found on all domestic animal species except in chickens. In a few instances, such infestations were reflecting merely exposure to a species highly prevalent in the environment, in others the domestic host may have contributed to *A. sculptum* tick populations. Observations under experimental and/or natural settings indicate that among domestic animals, horses and pigs are primary hosts for this tick species as well by feeding adults that will display adequate reproductive performance (Castagnolli et al. 2003; Labruna et al. 2001; Osava et al. 2016; Ramos et al. 2014b). Nymphs of *A. sculptum* seem to have an even larger array of adequate domestic hosts which includes horses, pigs, dogs and bovines (Castagnolli et al. 2003; Labruna et al. 2001; Osava et al. 2016; Ramos et al. 2014b, 2016; Szabó et al. 2001, 2007b). In fact, many engorged nymphs collected from all domestic animals in our survey successfully molted to adults in the laboratory. Clearly this tick species in the Cerrado is the one that poses the widest wild-domestic interface.

Other two tick species found, both on dogs, albeit in very low numbers display features of interest. *Amblyomma ovale* is a tick from wild carnivores (Labruna et al. 2005) and, as mentioned above, host seeks in humid forestall areas. Thus, dogs will be infested during access to wild areas/forests, a behavior that represents an important aspect for the Atlantic rainforest rickettsiosis (Szabó et al. 2013b) yet unknown in the Cerrado. *Amblyomma tigrinum* found once on a dog, is also a tick species that parasitizes carnivores during the adult stages (Labruna et al. 2005) whereas immature stages are found on small rodents and ground feeding birds (Nava et al. 2006). In fact, *A. tigrinum* was found on several carnivores captured in the same reserve of our survey (Arrais 2013; Martins et al. 2015) and was found in high prevalence and as the main tick species on manned wolves (Arrais 2013). In this regard, it is surprising that not a single tick from these species was collected during our environmental sampling along ten seasons, many times at locations known to be used by manned wolves.

Survey for *Rickettsia* yielded only one species but should be viewed as preliminary since analyzed tick sample was small. *Rickettsia bellii,* the only species found, is of unknown pathogenicity and should probably be viewed rather as a tick endosimbiont due to its high prevalence within tick populations and the wide array of species it colonizes without a perceptible damaging effect upon ticks (Labruna et al. 2007; Pacheco et al. 2009; Sakai et al. 2014). In this sense finding in *A. dubitatum* only expands its geographic range because it has been described from this tick species multiple times (Pacheco et al. 2009; Sakai et al. 2014).

In conclusion tick samplings, herein reported show that tick species distribution in the Cerrado is uneven, patchy, reflecting ecological preferences, host availability and other unknown factors. In this context, *A. brasiliense*, a tick rather related to Atlantic rainforest, is capable to establish in the Cerrado under peculiar conditions, probably with necessity for sites with enhanced humidity. *A. sculptum* on the other hand is a tick species that seems to be well adapted to an array of the Cerrado environments/phytophysignomies and thus it is more widespread within the Biome. Such favorable conditions seem to be replicated within green anthropized areas, allowing for the establishment of *A. sculptum* populations. Furthermore, its wide host array provides nourishment throughout several phytophysiognomies. Thus, it is not surprising that it is the main tick species to bite humans within this Biome both in natural but also in anthropized areas. Nonetheless, environment at higher altitudes, above 1300 m, seem to impact negatively upon this tick species and further observations should pinpoint factors that are responsible for such observation.

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